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The effects of genetic groups, and age on the chemical composition, physical properties, and somatic cell count of raw buffalo milk

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Abstract The genetic groups of the buffaloes influenced all the chemical compositional traits ($P<0.05$), and some physical properties, such as the percentage of citric acid content, and the milk urea nitrogen concentration ($P<0.01$). Milk from Swamp buffaloes had the highest percentage of fat content (11.1%) compared to purebred Murrah (7.9%) and 75%Murrah crossbreeds (8.2%), but it was not statistically different from the 50% Murrah crossbreeds (9.5%). Similar results were found in the percentages of protein, solid-not-fat, and total solids content. The purebred Murrah showed the highest average lactose content (4.9%), but it did not statistically differ from those of the 50% Murrah crossbreeds (4.6%) and Swamp buffalo (4.5%). The highest citric acid content was found in the Swamp buffaloes (0.20%), while the lowest of the trait was observed in the 100%Murrah. The Swamp buffaloes and the 50% Murrah crossbreeds had higher averages of urea concentration than the 100% Murrah and the 75% Murrah crossbreeds. The age of the animals in different genetic groups did not affect most of the studied traits ($P>0.05$), except the percentage of milk fat content, the percentage of citric acid and the percentage of urea concentration ($P<0.05$). The 50%Murrah crossbreeds and Swamp buffaloes which were older than 9 years old which had higher averages of fat content than the other groups. The highest citric acid content average was found in the 50%Murrah crossbreeds which were older than 9 years old, and the highest urea concentration average was detected in milk from the 50% Murrah crossbreeds which were younger than 7 years old.

Keywords: Raw milk properties, Citric acid, Milk urea nitrogen, Murrah, Swamp buffalo

Introduction

According to formal and behavioral criteria, the Asiantic water buffalo is classified into 2 types, which are the swamp buffalo (*Bubalus carabanesis*, with a chromosome number of 2n=48) and the river buffalo (*Bubalus bulalis* with a chromosome number of 2n=50) (Talib and Maytham, 2014). The swamp buffalo is a popular domestic animal in Thailand. In the year 2000, there were

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approximately 1.75 million buffaloes, while the number had decreased to 1.26 million by 2020 (IBIC, 2020). Most buffaloes are raised in the north-eastern part of Thailand. Not only are they used for labor in rice fields, but currently, they are also used for meat and in leather production, and or for genetic preservation. In 1996, fifty river buffaloes (Mehsana buffaloes) consisting of 5 males and 45 females, were imported. The National Dairy Council of India presented them to King Rama IX on account of The Fiftieth Anniversary (Golden Jubilee) Celebrations of His Majesty's Accession to the Throne. They were transported and raised at the Animal Breeding and Research Center Buriram Province under the Department of Livestock Development (DLD) in 2001 for study, research, and breeding. Currently, Mehsana buffaloes are distributed to farmers in the lowlands who have an interest in feeding the buffaloes via bidding. However, for the farmers who were members of the Royal Project Development in Mae Ta at Chiangmai province, the Highland Research and Development Institute Public Organization has supported the Mehsana buffalo from DLD since 2005 (HRDI, 2012).

The other famous river buffalo strain in Thailand is the Murrah, which have been raised in the Murrah Farm, a private commercial company, namely, Murrah Dairy Company Limited, since 2003. The farm is located in Plangyao District, Chachoengsao Province. Both Murrah buffalo and their crossbreeds (Murrah x Swamp buffalo) are fed to produce organic milk for Mozzarella Cheese (an expensive fresh cheese), and pasteurized milk production (Murrah Farm, 2018).

Since July 2021, Thai Agricultural Standard for raw buffalo milk (TAS 6007-2021) have been set up. Many criteria for good quality milk are established. The farmers must be concerned for producing quality milk in order to obtain more money. An alternative to help the farmers, the factors impact the milk quality traits are necessary to study.

Most published documents that are reported about buffaloes in Thailand deal with dietary and feeding, utility, and milk production performance are reported (Noppadon, 2009; DLD, 2013; Piyatida and Sasitorn, 2013; Thiamphop *et al.*, 2016). Academic studies focusing on intrinsic factors, such as genetic groups and how the age of the animals affect the buffalo raw milk quality, are still limited. The reasons are factors that recorded pedigree takes a long time to gathered data. Fortunately, the present study was supported such data from Murrah farm’s owner, hence, the factors affecting the common economically important constituent traits of the milk, such as fat, protein, lactose, solid-not-fat, and total solids nutrients, some physical properties, and somatic cell count were investigated.

Materials and methods

Animals

The Murrah farm raised both river buffalo, mostly Murrah, Thai Swamp buffalo, and their crossbreeds, which have 75%, or 50% Murrah blood. The animals are fed with concentrate, which consists of company feed and feed self-mixed in the farm, with a ratio of 30:70, respectively, as shown in Table 1. They are 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 are older than 4 years old. Milking is done twice daily, at 6 a.m., and 2 p.m., using machines. Before milking, buffalo's udders were cleaned and their teats were dried, the mastitis was then tested. Animals with negative test of mastitis were milked.

Table 1. Chemical composition in different concentrate formulas for buffaloes feeding at the 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 in the farm
2/: Bought from feed company
3/: Mixed between self-mixed at Murrah farm and bought from feed company with 3:1 ratio

Sample and data collection

Data consisted of 2 parts as primary data consisting of the chemical composition, physical properties, and somatic cell count of the raw milk and secondary data consisting of data concerning the animals (pedigree records and individual performances).

Fresh milk from fifty-seven buffaloes, which consisted of 26 River buffaloes (100%Murrah), 6 of 75%Murrah blood, 21 of 50%Murrah blood (crossbreeds of Murrah x Thai Native Swamp buffalo), and 4 Thai Native Swamp buffalos, was collected in March 2018. One hundred and fifty mililitres of milk from each animal was collected at 2 pm. The samples were put in 200 ml plastic bottles. All samples were maintained at 4°C in a storage box and were transported to University Laboratory, where the specific gravity (SG) of milk was immediately measured by using lactometer following the method of Davide (1977) protocol. After that, all the samples were frozen at -70°C until for further study.

To analyze the chemical composition, 30 ml of each thawed milk sample was put into a plastic bottle. They were then warmed up at 37°C for 15 minutes in a water bath. After that, the raw milk samples were analyzed using the CombiFoss^{TM} FT+ (FTIR (Fourier Transform InfraRed) Technology). Chemical composition such as fat, protein, lactose, solid-not-fat, and total solids were reported in term of percentages. The other milk properties, such as freezing point depression (FPD), citric acid content (%), milk urea nitrogen (MUN) or urea concentration (mg/dL), and somatic cell count (x10^{3}colony/ml) were also printed out. The FPD was reported in term of m°C unit, in the present study, however, the unit was described in term of °C, for example 554m°C to be -0.554°C. The milk compositional analysis and other properties, except specific gravity, were analyzed at Saraburi Artificial Insemination and Biotechnology Research Center, Saraburi Province, as follows CombiFoss FT+ protocol.

The secondary data were pedigree recorded which supported by the Murrah farm owner, such as age, lactation period, genetic group. Descriptive statistics were used to analyze and categorize the data as study factors.

Statistical analysis

The effects of genetic groups and age on the chemical compositional traits of buffalo milk were analyzed using a general linear model. Due to the independent variables of the categories, the age of the animals and the genetic groups were unequal, therefore, the nested model between age within genetic groups was performed as follows:

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

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, 3 and 4, where 1=100%Murrah, 2=75%Murrah blood, 3=50%Murrah blood, and 4=Swamp buffalo)
$Age(GG)_{ij}$ = 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 the studied trait data is shown in Table 2. For the chemical composition trait, the range of the total solids percentages was the highest (16.14%), while the lowest range was found in the percentage of lactose (3.47%). The averages of the physical properties of milk were 1.031, -0.532 °C, 0.14%, and 27.05 mg/dL for specific gravity, freezing pressure depression, citric acid content, and urea concentration, respectively. The somatic cell count had an average of 117 (x10^3 cells/ml). The range of the trait varied widely from 10 to 548 (x10^3 cells/ml).

Table 2. Descriptive statistics of the chemical composition, physical properties, and somatic cell count (n=57)

Studied traits	Mean	Std Dev	Minimum	Maximum
Chemical composition				
Fat (%)	8.81	2.36	5.14	17.01
Protein (%)	4.86	0.81	2.36	6.57
Lactose (%)	4.68	0.65	2.49	5.96
Solid-not-fat (%)	10.31	1.24	6.33	12.09
Total solids (%)	19.07	2.95	11.62	27.76
Properties				
Specific gravity	1.031	0.003	1.020	1.038
FPD (°C)[1/]	-0.532	0.061	-0.639	-0.319
Citric acid (%)	0.14	0.06	0.03	0.38
Urea (mg/dL)	27.05	6.06	12.30	39.10
SCC x10^3(cells/ml)[2/]	117.63	110.72	10.00	548.00

1/: Freezing pressure depression
2/: Somatic cell count

Factors affecting the studied traits

The effects of genetic groups and age within the different genetic group on the milk chemical compositional traits and some properties traits of buffalo milk are shown in Table 3. It was found that the genetic group factor had highly statistically significantly influenced all the studied traits, such as fat, protein, and total solids percentages (P<0.01), whereas lactose and solid-not-fat percentages were significantly affected by the factor ($P<0.05$). The factor of age within the genetic group affected only fat percentage ($P<0.01$), and it also affected the total solids percentage with the P-value equaling 0.064. However, the R-squares values of all study traits were low.

The genetic group factor had also highly statistically significantly affected the citric acid percentage and urea concentration ($P<0.01$), as well as both traits were influenced by the factor of age within the different genetic group ($P<0.05$). Whereas the studied factors did not impact the specific gravity, the freezing pressure depression, and the somatic cell count in the raw milk.

Table 3. P-values of factors, genetic group, and age within the genetic group, and R-squares affecting buffalo milk chemical composition and other properties

Traits	**P-values**		**R-squares**
	Genetic group	Age (Genetic group)	
Chemical Composition			
Fat (%)	0.0073	0.0060	0.396
Protein (%)	0.0022	0.6855	0.276
Lactose (%)	0.0248	0.1323	0.274
Solid-not-fat (%)	0.0189	0.4319	0.228
Total solids (%)	0.0027	0.0636	0.355
Properties			
SG [1/]	0.4056	0.0896	0.191
FPD (°C)[2/]	0.0518	0.2948	0.215
Citric acid (%)	0.0008	0.0136	0.440
Urea (mg/dL)	0.0010	0.0132	0.367
SCCsx10^3(cells/ml)[3/]	0.4628	0.6718	0.094

[1/] Specific gravity
[2/] Freezing pressure depression
[3/] Somatic cell count

The effect of genetic groups

Swamp buffaloes had the highest milk fat percentage (11.1%) but did not statistically differ from the 50%Murrah crossbreeds (9.5%). It was interesting that the higher the percentage of Murrah blood, the lower the percentage of fat (Table 4). Similarly, the results showed that the percentage of protein from the Swamp buffaloes was the highest (5.9%), and that it was not statistically different from the 50%Murrah crossbreeds (5.1%). Although the milk of the 75%Murrah crossbreeds showed the lowest percentage of protein content (4.2%), this did not statistically vary from the 100%Murrah (4.7%). The percentage of total solids in the milk from the Swamp buffaloes (22.4%) and the 50%Murrah crossbreeds (20.1%) were higher than those from the 100%Murrah (18.1%) and the 75%Murrah crossbreeds (17.2%).

In the case of lactose content, it was found that although the 100%Murrah showed a higher average of this trait (4.9%) than the 75%Murrah (4.1%), but this did not statistically differ from the 50%Murrah crossbreeds and the Swamp buffaloes (Table 4). The 75%Murrah crossbreeds had the lowest average of solid-not-fat percentage (9%).

Table 4. Least squares means and standard errors of milk chemical compositional traits affected by the genetic group

Chemical composition	**Genetic group**			
	100%Murrah (n=26) LSM±SE$^{/1}$	75%Murrah (n=6) LSM±SE$^{/1}$	50%Murrah (n=21) LSM±SE$^{/1}$	Swamp (n=4) LSM±SE$^{/1}$
Fat (%)	7.90±0.38^{b}	8.20±0.80^{b}	9.52±0.49^{ab}	11.10±0.98^{a}
Protein (%)	4.68±0.14^{bc}	4.17±0.30^{c}	5.12±0.18^{ab}	5.89±0.37^{a}
Lactose (%)	4.90±0.17^{a}	4.08±0.24^{b}	4.65±0.15^{a}	4.46±0.30^{ab}
Solid-not-fat (%)	10.27±0.23^{a}	9.05±0.48^{b}	10.58±0.29^{a}	11.34±0.58^{a}
Total solids (%)	18.13±0.50^{b}	17.16±1.03^{b}	20.09±0.63^{a}	22.36±1.27^{a}

[1/] Different letters in the same row had statistically significant differences (P<0.05).

The highest citric acid content was found in the Swamp buffaloes (0.20%), but this did not statistically differ from the 50% Murrah crossbreeds (Table 5). It was interesting that the studied trait from the 100% Murrah was the lowest (0.11 %), and this did not differ from the 75% Murrah crossbreeds (0.13%). The Swamp buffaloes and the 50% Murrah crossbreeds had urea concentration averages higher (33.65 and 30.61 mg per dL) than the 100% Murrah and the 75% Murrah crossbreeds (25.05 and 24.85 mg per dL, respectively).

Table 5. Least squares means and standard errors of the physical properties, and the somatic cell count affected by the genetic group

Studied traits	**Genetic group**			
	100%Murrah (n=26) LSM±SE	75%Murrah (n=6) LSM±SE	50%Murrah (n=21) LSM±SE	Swamp (n=4) LSM±SE
Specific gravity	1.031±0.001	1.032±0.001	1.031±0.001	1.034±0.002
FPD (°C)$^{/1,4}$	-0.544±0.011^{b}	-0.471±0.023^{a}	-0.538±0.014^{b}	-0.538±0.029^{b}
Citric acid (%)$^{/2}$	0.11±0.01^{c}	0.13±0.02^{bc}	0.17±0.01^{ab}	0.20±0.02^{a}
Urea (mg/dL)$^{/2}$	25.05±1.01^{b}	24.85±2.10^{b}	30.61±1.28^{a}	33.65±2.58^{a}
SCCx10^{3}(cells/ml)$^{/3}$	143.72±22.13	102.33±45.99	102.56±28.06	65.75±56.32

[1/] Freezing pressure depression

[2/] Different letters in the same row had statistically significant differences (P<0.01).

[3/] Somatic cell count

[4/] Different letters in the same row had statistically significant differences (P=0.0518).

In cast of the freezing pressure depression, the average of the trait showed the highest in the 75% Murrah crossbreeds (-0.471 °C) at the P-level of 0.0518. Whereas the averages of the trait in the other genetic groups equaled -0.54 °C.

The effect of age within different genetic groups

The results indicated that the averages of percentage of milk fat from the 50%Murrah crossbreeds and Swamp buffaloes which were older than 9 years old and higher than other groups (11.9 and 11.1% for the 50%Murrah crossbreeds and the Swamp buffaloes, respectively), as shown in Table 6. Even though the age within different genetic groups of the total solids content did not show statistically significantly differ (P = 0.063), the Swamp buffalo and 50% Murrah crossbreeds older than 9 years old had the averaged total solids content higher than the others (22.36 and 22.23 %, respectively).

Table 6. Least squares means and standard errors of the chemical composition in raw milk according to the factors of age within the different genetic groups

Age (yrs)	**GG**[1]	**n**	**% Fat** LSM ±SE[4]	**%Protein** LSM±SE	**%Lactose** LSM±SE	**%SNF**[2] LSM±SE	**%TS**[3] LSM±SE
<7	100%M	8	8.04±0.69^{c}	4.48±0.26	4.52±0.21	9.72±0.41	17.73±0.90
7-9	100%M	9	7.95±0.65^{c}	4.95±0.24	5.13±0.20	10.74±0.39	18.67±0.84
>9	100%M	9	7.72±0.66^{c}	4.62±0.24	5.04±0.20	10.35±0.39	17.99±0.84
<7	75%M	6	8.20±0.80^{c}	4.17±0.30	4.08±0.24	9.05±0.48	17.16±1.03
<7	50%M	3	8.42±1.13^{bc}	5.27±0.43	4.88±0.34	10.91±0.68	19.33±1.46
7-9	50%M	10	8.28±0.62^{c}	4.98±0.23	4.71±0.19	10.51±0.38	18.72±0.80
>9	50%M	8	11.87±0.69^{a}	5.10±0.26	4.37±0.21	10.31±0.41	22.23±0.90
>9	Swamp	4	11.10±0.98^{ab}	5.89±0.37	4.46±0.30	11.34±0.58	22.36±1.27

[1] Genetic groups

[2] %Solid-not-fat

[3] % Total solids

[4] Different letters in the same column had statistically significant differences (P<0.05).

The factors of age within the different genetic groups affecting to the physical properties and the somatic cell count are shown in Table 7. The highest average of the citric acid content was found in milk from the 50% Murrah crossbreeds which were older than 9 years old (0.22%), but it did not differ from the Swamp buffaloes with the same age (0.20%). While the lowest average of the trait was observed in the 100% Murrah and in the 75%Murrah crossbreeds with all the studied ages. The highest average of milk urea nitrogen or urea concentration was detected in milk from the 50% Murrah crossbreeds which were younger than 7 years old (35.93 mg/dL), but it was not statistical significantly different from the Swamp bullaloes (33.65 mg/dL) and from the 50% Murrah crossbreeds (29.84 mg/dL), which were older than 9 years old.

Within the same Murrah purebreeds, the lowest of average milk urea concentration was observed in the animals that were older than 9 years old (22.22 mg/dL), it was not statistical different from the animals which were younger than 7 years old (25.18 mg/dL).

Table 7. Least squares means and standards error of the physical properties and somatic cell count in raw milk according to the factor of age within the different genetic groups

Age (yrs)	**GG**[/1]	**n**	**SG**[/2] LSM ±SE[4/]	**FPD**[/3] LSM±SE	**%Citric**[/4] LSM±SE	**Urea**[/4,5] LSM±SE	**SCC**[/6] LSM±SE
<7	100%M	8	1.030±0.001	-0.518±0.020	0.12±0.02^{c}	25.18±1.82^{cd}	165.50±39.83
7-9	100%M	9	1.032±0.001	-0.567±0.019	0.11±0.02^{c}	27.76±1.72^{bc}	143.89±37.55
>9	100%M	9	1.031±0.001	-0.549±0.023	0.11±0.02^{c}	22.22±1.72^{d}	121.78±37.55
<7	75%M	6	1.032±0.001	-0.471±0.023	0.13±0.02^{c}	24.85±2.10^{d}	102.33±45.99
<7	50%M	3	1.032±0.002	-0.561±0.033	0.13±0.03^{bc}	35.93±2.98^{a}	97.67±65.04
7-9	50%M	10	1.033±0.001	-0.539±0.018	0.14±0.02^{bc}	26.05±1.63^{cd}	70.00±35.62
>9	50%M	8	1.029±0.001	-0.512±0.020	0.22±0.02^{a}	29.84±1.82^{abc}	140.00±39.83
>9	Swamp	4	1.034±0.002	-0.538±0.029	0.20±0.02^{ab}	33.65±2.58^{ab}	65.75±56.32

[1/] Genetic groups
[2/] Specific gravity
[3/] Freezing pressure depression (°C)
[4/] Different letters in the same column had statistically significant differences (P<0.05).
[5/] Urea concentration (mg/dL)
[6/] Somatic cell count x10^3(cells/ml)

Discussion

Buffalo milk is characterized as a high percentage of fat and protein (Aspilcueta-Borquis *et al*., 2010). The means of all the studied traits in this study were higher than those reported by Sarkar *et al.* (2006) and Piyatida and Sasitorn (2013). The percentages of fat, protein, lactose, SNF, and TS were 6.99, 3.78, 5.37, 10.01, and 16.99, respectively (Sarkar *et al*., 2006), while the traits reported by Piyatida and Sasitorn (2013) were 6.01, 3.61, 4.84, 9.64, and 15.34, respectively.

The averages of %fat, %protein, %SNF, and %TS contents from our study (8.81, 4.84, 10.31, and 19.07, respectively), were compliant with Thai Agricultural Standard of Raw Buffalo Milk (TAS 6007-2021), in which the contents of fat, protein, SNF, and TS should not be less than 5.0%, 3.5%, 9.0%, and 14.0%, respectively (TAS, 2021). The averages of SG, FPD, and SCC (1.031, -0.532°C, and 117.63 (x10^3/ml) in the present study were agreed with the TAS 6007-2021. However, the TAS 6007-2021 does not establish the properties of citric acid content and milk urea concentration as indicators for milk quality.

Intrinsic factors such as breed, species, age, and extrinsic factors such as season, dietary composition, had influence on the buffalo milk composition (Prasanta *et al*., 2018). In accordance with this study, our results found that genetic groups of milking buffaloes had an influence on all study traits. It was interesting that the least squares mean of %fat, %protein, and %TS in the milk of Swamp buffaloes from our results were higher than those in 100%Murrah, which agreed with Chuaychoo *et al*. (2013) who reported that the fat, protein, and TS of milk from swamp buffalo were higher than those of Murrah milk, except lactose, which was higher in Murrah milk. Although Misra *et al.* (2008) reported that Murrah buffaloes were the best performance breed for fat, total protein and casein percentages compared to Mehsana and Bhadawari.

Percentages of fat, SNF and TS in the milk of the Swamp buffaloes from this study were found to be 11.10±0.98, 11.34±0.58, and 22.36±1.27, which were higher than those of Swamp buffalo milk of Assam in India. Zaman *et al*. (2007) found the means of the above-mentioned traits were 8.48±0.07, 9.20±0.04, and 17.68±6.09 for %fat, %SNF, and %TS, respectively. However, Chantalakhana (1981) found that the fat percentage and TS content in Swamp buffaloes of Thailand ranged from 8.00 to 10.00 for %fat and 18.1 to 21.3% for %TS, respectively, which the values were close to our results.

The current study focused on the factor of age within the different genetic groups. Buffalo can be milked, when they are older than 4 years old, which is the first parity or the first lactation number. Sundaram and Harharan (2013) found that the lactation number had a highly significant effect ($P \leq 0.01$) on milk fat%, protein%, and TS%, in contrast to the current study, which observed that the age factor within the genetic groups affected only %milk fat ($P<0.01$). There was not a significant difference between animals with ages of less than 7, 7 to 9, and more than 9 years old for Murrah purebreeds or 50%Murrah crossbreeds, in milk fat percentage, while the trait of 50%Murrah crossbreeds and the Swamp buffaloes with older than 9 years old showed the highest percentage of the trait. Moreover, this study agreed with Thanisa (2019), who reported that age and genetic group did not influence most milk composition ($P>0.05$), except the genetic group had affected the crude protein content, and that the crossbred Murrah had a higher average of the trait than that of the purebred Murrah.

FAO (1993) reported that the specific gravity of milk measured at 15^{o}C or 20^{o}C is normally ranges from 1.028 to 1.033 g/ml. The specific gravity depends on the protein and fat content. The correlation between the SG and protein from our result was r=0.13, and between the SG and fat content was r=-0.49. Thus, higher fat content, but lower SG. However, if the SG is lower than 1.01 g/ml, this may indicate extraneous water content higher than the normal water content in milk (FAO, 1993). The average of the SG in this study (1.031) was close to Haggag *et al*. (1991) who reported that the value of 1.032 for the specific gravity of normal buffalo milk. No studied factors affected this trait.

For the FPD, this trait is dependent on salt and lactose in the milk (FAO,1993). The correlation between the FPD and lactose in the present study was r=0.88. This correlation coefficient strongly supported that the factor of genetic groups an effect on the percentage of lactose and especially the FPD (P=0.0518). The 75%Murrah crossbreeds had a lower average of lactose content (4.08%) than the others, and they also had a lower average FPD (471m°C). The average FPD (-0.532°C) from this study was different from Ceniti *et al.* (2019), in which the mean was -0.545 ℃, although both studies used a MilkoScan FT plus an infrared analyzer to analyze the FPD in buffalo milk. This may be due to there being many factors influence the trait, besides the milk chemical composition and breed, such as feed, time of milking, lactation period etc (Ceniti *et al.*, 2019).

The citric acid (CA) content is a parameter for milk quality. The metabolites of citric acid are intermediate in the tricarboxylic acid cycle for energy metabolism, and in glycolysis and in fatty acid synthesis. Ducháček *et al.* (2012) studied the relationship between milk fatty acid and citric acid content, and they found that the correlations ranged from r=0.66 to r=0.74 in the sixth week of lactation. They also reported that the content of CA in milk is a good indicator of energy balance in dairy cows. The CA content is higher in healthy animals than in sick animals (Khaled *et al*., 1999). Our results found that the correlation between fat content and CA content was r=0.83. The Swamp buffaloes had a higher milk fat content and a higher CA content than the 100%Murrah. It can be assumed that the Swamp buffaloes have energy balance and are healthier than the Murrah purebreds. The 50%Murrah crossbreeds and the Swamp buffaloes which were older than 9 years old had a higher fat and CA content than the 100%Murrah with across all the studied ages, and the 75% Murrah crossbreeds.

The milk urea (MU) concentration or the milk urea nitrogen (MUN) is an important parameter for dairy herd management. This indicator can be used for monitoring the utilization efficiency of protein in a ruminant diet (Selcuk, 2016). The MU is a by-product of the breakdown of dietary protein, and it is formed from the normal daily metabolism of nitrogen and protein. Results in this study found that the average of MU was 27.05 mg/dL, which it was lower than those reported by Di Francia *et al.* (2003) that it was 40.8 mg/dL. The correlation between MU and protein content was r=0.43. The genetic groups and age of buffaloes effected the MU level. Roy *et al.* (2011) reported that MU varied by season, month, parity group, stage of lactation and sample type.

An important parameter to indicate milk quality is somatic cell count (SCC). Somatic cells are white blood cells and cells from the udder secretory tissue which remove infections and repair tissue damage processed by bacteria (Mills, 2020). From the raw data, we found that only one animal (100%Murrah) had SCC of 548 (x10^3 cells/ml), two animals (one of the 100%Murrah, and one of the Swamp buffalo) had 477 and 415 (x10^3 cells/ml), respectively. Most animals, 32 heads, had a SCC less than 100 (x10^3 cells/ml), while the 22 animals had SCC 100 to 299 (x10^3 cells/ml). The SCC varied widely from 10 to 548 (x10^3 cells/ml), therefore, the studied factors did not affect this trait, because of the differences in standard errors. It was interesting that all the milk samples were collected from the negative mastitis test buffaloes, but the SSC of the samples which more than 400 (10^3 cells/ml) were found. TAS (2021) recommends the SSC should be less than 400,000 cells/ml. The reasons for this may be that the somatic cells were eliminated under non-pathological conditions, such as physiological processes during estrus or the advanced stage of lactation (Raynal-Ljutovac *et al.*, 2007). In addition, SCC is usually utilized as a sanitary control for milk, and the coagulating properties deteriorated when the SCC increased.

The R-squares of all the studied traits in the current study were low, especially in the SCC trait, the R-squares was very low. To increase the R-squares values, other factors, both intrinsic and extrinsic factors should be added to the statistical model.

It is concluded that the genetic group of the buffaloes played an important role in the chemical composition of milk, citric acid content, and milk urea concentration, as well as freezing point depression. The Swamp buffaloes and the 50%Murrah crossbreeds showed a higher performance than the others. Swamp buffalo, which are not commonly used for milking in Thailand, could be improved in milk yield. Furthermore, crossbreeds of River buffalo and Swamp buffalo should be developed. The property of citric acid content and milk urea concentration should be included as criteria in the TAS 6007-2021.

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