**Philippine Journal of Science**
151 (1): 139-152, February 2022
ISSN 0031 - 7683
Date Received: 31 Aug 2021

# Fatty Acid Composition and Nutritional Indices/Ratios of Colostrum and Milk from Murrah and "Murrah × Carabao" Crossbred Buffaloes

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**This study aims to determine the fatty acid (FA) composition and compare FA-based nutritional indices/ratios of colostrum and milk obtained on the 30$^{th}$, 60$^{th}$, and 90$^{th}$ day of lactation from 27 Murrah and 18 "Murrah × Carabao" crossbred buffaloes. The major saturated FA (SFA) in colostrum and milk is palmitic acid (C16:0), comprising 32–33% by weight of total FAs. Other important SFAs – myristic acid (C14:0), stearic acid (C18:0), and lauric acid (C12:0) – were lower ($P < 0.05$) in colostrum than in milk. Oleic acid (C18:1-n9c), a monounsaturated fatty acid (MUFA), is the second most abundant FA in colostrum (28.5%) and milk (18.0–18.8%). Arachidonic acid (C20:4-n6, AA) is the dominant polyunsaturated fatty acid (PUFA) in colostrum (0.92%) and milk (0.42–0.45%). Conjugated linoleic acid (C18:2-c9t11, CLA) was higher in colostrum (0.64%) than in milk (0.14–0.16%). Colostrum and milk had a very low PUFA/SFA ratio (0.02–0.06: 1). The linoleic acid (C18:2-n6, LA) to α-linolenic acid (C18:3-n3, ALA) ratio was higher in colostrum (3.21: 1) than in milk (0.62–1.55: 1). The omega-6 (LA and AA) to omega-3 [ALA and docosahexaenoic acid (C22:6-n3, DHA)] or n-6/n-3 ratio was more balanced for milk (1.76-2.34: 1) than colostrum (3.37: 1). Colostrum had lower atherogenicity (2.53 *vs.* 4.50–4.66), lower thrombogenicity (2.68 *vs.* 4.48–4.59), and higher health-promoting index (0.39 *vs.* 0.21–0.22) than milk. The hypocholesterolemic/hypercholesterolemic (h/H) ratio was higher for colostrum (0.64: 1) than milk (0.34–0.36: 1). Except for AA, the FA composition of colostrum and milk were not significantly different between Murrah and "Murrah × Carabao" crosses ($P > 0.05$).**

Keywords: colostrum, fatty acids, milk, Murrah, "Murrah × Carabao" buffaloes, nutritional indices

## INTRODUCTION

Milk FAs are important nutritional components that may have positive effects on human health (Cichosz *et al.* 2020). On the other hand, milk and some dairy products (butter) have also been criticized for the unfavorable FA profile in milk fat (Hanus *et al.* 2018). The FA composition is an important measure of the quality of raw milk or colostrum used in the manufacture of dairy products with improved nutritional quality and health benefits available to consumers (Timlin *et al*. 2021). Recently, FA in colostrum has attracted interest as a potentially beneficial food ingredient or functional food (Mehra *et al.* 2021; Silva Galdino *et al.* 2021). Colostrum yield, however, is only 0.5% of the cow's annual milk production. Surplus colostrum was even previously thought of as unmarketable since the focus was more on its role in the development of the calf (O'Callaghan *et al.* 2020).

While studies on FAs in buffalo colostrum are scarce, several studies have reported the milk FA composition of milk from buffaloes belonging to different breeds

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such as the Anatolian water buffalo in Turkey (Cinar *et al.* 2019), Bhadawari buffaloes in India (Kushwaha *et al.* 2018), Bulgarian Murrah (Penchev *et al.* 2016; Ilieva *et al.* 2020), Kundi in Pakistan (Talpur *et al.* 2007), Mediterranean buffaloes in Italy (Varricchio *et al.* 2007; Pegolo *et al.* 2017) and in France (Menard *et al.* 2010) – plus Murrah buffaloes in Brazil (Fernandes *et al.* 2007) and Nili-Ravi in Pakistan (Talpur *et al.* 2007; Qureshi *et al.* 2015). However, the reports listed above vary in the number of individual FAs involving different or no measures of nutritional quality that are indicative of the effect of FAs on human health and disease (Chen and Liu 2020).

In the Philippines, buffalo milk is the second most-produced milk after cow's (bovine) milk, contributing about one-third of the 2019 dairy output of 24.4 million liters (PSA 2020). Unfortunately, very limited work has been carried out to investigate the FA profile of buffalo colostrum and milk. In this regard, the objective of this study was to compare the FA composition and FA-based nutritional indices of colostrum and milk obtained on the 30$^{th}$, 60$^{th}$, and 90$^{th}$ day of lactation from Murrah and "Murrah × Carabao" crossbred buffaloes.

## MATERIALS AND METHODS

This study was conducted in compliance with the requirements of the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños (UPLB), Laguna, Philippines.

### Experimental Animals and Data

Forty-five (45) buffaloes (*i.e.* 27 Murrah and 18 "Murrah × Carabao" crosses) that calved between 04 Apr 2019 and 27 Jul 2020 at the Philippine Carabao Center (PCC) dairy farm in UPLB, Laguna, Philippines were used in the study. The crossbred buffaloes consisted of 3 "50% Murrah-50% Carabao," 3 "75% Murrah-25% Carabao," 9 "87.5% Murrah-12.5% Carabao," and 3 "93.75% Murrah-6.25% Carabao" (preliminary analyses of colostrum and milk showed no significant differences in milk composition among the buffalo crosses; data not shown). The age at calving and parity of the experimental animals was 7.83 ± 3.08 yr and 4.07 ± 2.44 calving, respectively. Buffaloes were equally managed to fulfill all welfare requirements and were kept in individual parturition pens about 2 wk before calving. In addition to forage, the buffaloes were fed with commercial lactating feed concentrates containing 15.52% crude protein, 5.87% crude fat, 11.99% crude fiber, 7.90% moisture, 8.74% ash, 1.02% calcium, and 0.58% phosphorus.

Four milk samples were collected from each buffalo consisting of colostrum taken on the day of calving, and subsequent milk on the 30$^{th}$, 60$^{th,}$ and 90$^{th}$ day post parturition, placed in plastic bottles, and immediately frozen at –20 °C until further analysis. The number and distribution of buffaloes and colostrum/milk samples collected by breed are shown in Table 1. Other data were also collected including age at calving, parity (number of calving), test-day milk yield, and fat percentage corresponding with each colostrum or milk sample.

A total of 19 medium- to very long-chained FAs were each analyzed as a percentage (g/ 100 g) of total FAs in colostrum and milk samples, *i.e.* eight SFAs [C12:0 (lauric acid), C14:0 (myristic acid), C15:0 (pentadecylic acid), C16:0 (palmitic acid), C17:0 (margaric acid), C18:0 (stearic acid), C20:0 (arachidic acid), and C22:0 (behenic acid)], six MUFAs [C14:1 n-5 (myristoleic acid), C16:1 n-7 (palmitoleic acid), C18:1 n-9 (oleic acid), C18:1 n-7 (trans-vaccenic acid), C20:1 n-11 (eicosenoic acid), and C22:1 n-9 (erucic acid)], and five PUFAs [C18:2 c9tll (CLA), C18:2 n-6 (LA), C18:3 n-3 (ALA), C20:4 n-6 (AA), and C22:6 n-3 (DHA)].

The FA composition of feed concentrates was also analyzed and found to contain 64.32% SFA [C12:0 (37.64%), C14:0 (13.60%), C16:0 (10.02%), C18:0 (2.48%), C20:0 (0.50%), and C22:0 (0.08%)], 14.24% MUFA [C16:1 n-7 (0.07%) and C18:1n9c (14.17%)], and 8.12% PUFA [C18:2 n-6 (8.00%) and C18:3 n-3 (0.12%)].

Following Chen and Liu (2020), six FA groups (*i.e.* SFA, MUFA, PUFA, UFA = MUFA + PUFA, omega-3 FA = C18:3 n-3 and C22:6 n-3, and omega-6 FA = C18:2 n-6 + C20:4 n-6) and seven FA-based nutritional indices/ratios with health implications were also determined. The FA-based nutritional indices/ratios were: 1) PUFA/SFA ratio, 2) n-6/n-3 ratio, 3) LA/ALA ratio, 4) atherogenicity index (IA), 5) thrombogenicity index (IT), 6) health-promoting index (HPI), and 7) h/H ratio.

The IA and IT were calculated using the equations of Ulbricht and Southgate (1991) as follows:

$$IA = [C12{:}0 + (4 \times C14{:}0) + C16{:}0]/\Sigma\, UFA \quad (1)$$

$$IT = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5 \times MUFA) + (0.5 \times n-6\ PUFA) + (3 \times n-3) + (n-3/n-6)] \quad (2)$$

The HPI used by Chen et al. (2004) was:

$$HPI = UFA/[C12{:}0 + (4 \times C14{:}0) + C16{:}0] \quad (3)$$

The h/H ratio used by Mierlita (2018) was:

**Table 1.** Number of samples and least squares means of colostrum or test-day milk yield, fat content, and daily fat yield for different breeds and different milk types.

| | Murrah | "Murrah × Carabao" crosses | Total |
|---|---|---|---|
| **No. of buffaloes** | 27 | 18 | 45 |
| **No. of samples** | | | |
| - Colostrum | 27 | 18 | 45 |
| - Milk 30-d | 26 | 17 | 43 |
| - Milk 60-d | 25 | 17 | 42 |
| - Milk 90-d | 22 | 16 | 38 |
| Total no. of samples | 100 | 68 | 168 |
| **Yield, kg** | | | |
| - Colostrum | $3.97 \pm 0.37^{b}$ | $5.91 \pm 0.47^{a}$ | $4.94 \pm 0.30^{y}$ |
| - Milk 30-d | $6.12 \pm 0.37^{b}$ | $7.41 \pm 0.46^{a}$ | $6.76 \pm 0.29^{x}$ |
| - Milk 60-d | $5.86 \pm 0.38^{b}$ | $7.22 \pm 0.46^{a}$ | $6.54 \pm 0.30^{x}$ |
| - Milk 90-d | $6.01 \pm 0.40^{a}$ | $6.88 \pm 0.47^{a}$ | $6.45 \pm 0.30^{x}$ |
| **Fat content, %** | | | |
| - Colostrum | $4.38 \pm 0.46^{a}$ | $5.31 \pm 0.56^{a}$ | $4.85 \pm 0.36^{x}$ |
| - Milk 30-d | $4.73 \pm 0.47^{a}$ | $4.85 \pm 0.58^{a}$ | $4.79 \pm 0.37^{x}$ |
| - Milk 60-d | $5.36 \pm 0.48^{a}$ | $5.27 \pm 0.58^{a}$ | $5.32 \pm 0.38^{x}$ |
| - Milk 90-d | $4.81 \pm 0.52^{a}$ | $5.79 \pm 0.60^{a}$ | $5.30 \pm 0.40^{x}$ |
| **Daily fat yield, g** | | | |
| - Colostrum | $16.18 \pm 3.32^{b}$ | $30.51 \pm 4.24^{a}$ | $23.67 \pm 2.69^{y}$ |
| - Milk 30-d | $28.92 \pm 3.32^{a}$ | $36.13 \pm 4.11^{a}$ | $32.52 \pm 2.64^{x}$ |
| - Milk 60-d | $30.96 \pm 3.39^{b}$ | $39.11 \pm 4.11^{a}$ | $35.04 \pm 2.66^{x}$ |
| - Milk 90-d | $29.50 \pm 3.70^{a}$ | $37.38 \pm 4.24^{a}$ | $33.44 \pm 2.81^{x}$ |

Milk 30-d, Milk 60-d, and Milk 90-d refer to milk collected on the 30$^{th}$, 60$^{th}$, and 90$^{th}$ day of lactation, respectively.
Least squares means with different superscript letters (a, b) within a row are significantly different between Murrah and "Murrah × Carabao" crosses ($P < 0.05$).
Least squares means with different superscript letters (x, y) within the "total" column are significantly different between milk types ($P < 0.05$).

$$h/H = (C18{:}1\ n-9 + PUFA)/[C12{:}0 + C14{:}0 + C16{:}0] \quad (4)$$

## Fat Content Analysis

The fat content of each colostrum and milk sample was analyzed using the MilkoScan Mars (FOSS Analytical A/S, Hillerod, Denmark) that is based on Fourier-transformed infrared spectroscopy technology.

## FA Analysis

Fat was extracted from colostrum and milk samples, following the method presented by Folch *et al.* (1957). Ten (10) mL of chloroform/methanol (2:1, v/v) was added to 1 mL of homogenized colostrum or milk sample and mixed for 1 min. Two mL of 1% NaCl solution was then added to induce phase separation and was remixed for about 30 s. Centrifugation was done at 10,000 rpm for 10 min. The lower organic phase was carefully collected using a pipettor that was inserted through the protein disk interphase. The organic phase was finally transferred in a screw-capped glass test tube (16.5 × 105 mm).

The fatty acid methyl esters (FAMEs) were prepared, following the rapid methanolysis/ methylation procedure (Ichihara and Fukubayashi 2010), using concentrated HCl. The lipid extract (organic phase) was placed in a screw-capped glass test tube sealed with a Teflon-lined cap. It was added with 3 mL of 8% methanolic HCl solution. Methylation was done in a water bath at 100 ˚C for 1 h. After cooling to room temperature, 1 mL of n-hexane and 3 mL of distilled water were added and mixed by shaking for 30 s. The sample in the glass tube was centrifuged for 5 min at 8000 rpm. The aqueous layer was removed while the hexane layer was transferred in 2 mL amber gas chromatography (GC) vials and purged with ultra-pure nitrogen gas for 20 s. The samples were stored in the refrigerator (–20 °C) prior to FA analysis by GC.

The FAMEs were then quantified using a Shimadzu GC 2010 Plus capillary GC system (Shimadzu Corporation, Kyoto, Japan) that is equipped with a flame ionization detector 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 chromatographic conditions were as follows: the split ratio was 50:1, and the injection volume is 1 µL. The oven temperature was set to 125 °C and then increased to 240 °C at a rate of 3 °C/min, where it was held for 5 min. The injection port was set to 230 °C while the detector temperature was set to 250 °C. Hydrogen was used as a carrier gas at a flow rate of 40 mL/min, and nitrogen was used as makeup gas at a flow rate of 30 mL/min.

Quantitation of individual FAMEs was based on their correction factors against the external standard. The FAME standard mix for 19 long-chained FAs was purchased from Sigma Aldrich and consisted of the grain FAME mix (CRM47801), AA (A3611), DHA (D2534), trans-vaccenic acid (V1131), and CLA (I6413). The FAME mix was analyzed at the start of each weekly batch of samples prepared for GC analysis. Accuracy was monitored by comparing the measured concentration of this FAME mix against its true concentration. Data (in triplicates) were processed using LabSolutions software. The FAs were identified by comparing their retention times with known FAME standards.

**Statistical Analysis**

Differences in colostrum or test-day milk yield (kg), fat content (%), and daily fat yield (g/d, *i.e.* test-day yield multiplied by fat content) among the milk types and between breeds (within the type of milk) were determined using ANOVA.

Pearson product-moment correlation coefficients among the individual FAs and their relationships with age at calving, parity, colostrum and test-day milk yield, and fat percentage were determined separately for colostrum and milk samples using the CORR procedure (SAS Institute Inc., Cary, NC).

The general least squares procedures for unbalanced data were used to examine the principal sources of variation affecting each FA in colostrum and milk. Statistical significance was set at $P < 0.05$. Only those significant fixed effects and covariates were included in the final linear models. Hence, the non-significant fixed effects of breed (*i.e.* Murrah and "Murrah × Carabao" crosses) and covariate effect of age at calving (yr) were removed in the final model. The final mathematical model for all FAs was as follows: $y_{ijklm} = \mu + MType_i + Parity_j + Yield_k + PFat_l + e_{ijklm}$, where $y_{ijklm}$ is the percent area or the proportion of FA (g/ 100 g of total identified FAs) in buffalo colostrum or milk, µ is the overall mean, $MType_i$ is the fixed effect of the $i^{th}$ type of milk (*i.e.* colostrum and milk collected on the 30th, 60th, and 90th day of lactation), $Parity_j$ is the $j^{th}$ covariate effect of parity, $Yield_k$ is the $k^{th}$ covariate effect of test-day yield (kg), $PFat_l$ is the $l^{th}$ covariate effect of fat percentage, and $e_{ijklm}$ is the error term assumed to be normally distributed with the variance of errors as constant across observations.

The least-square means for each FA were used to compare the FA-based nutritional indices/ratios between milk types. Comparison of FA-based nutritional indices/ratios between breeds was based on the least-square means from the same mathematical model that included the effect of breed (*i.e.* Murrah and "Murrah × Carabao" crosses) nested within the type of milk.

## RESULTS

**Amount of Fat Produced in Colostrum and Milk**

Colostrum yield (4.9 kg) was significantly lower ($P < 0.05$) than daily milk yield (*i.e.* 6.4–6.8 kg obtained on the 30th, 60th, and 90th day of lactation, respectively) (see Table 1). While the fat percentage was similar ($P > 0.05$) between colostrum and milk, the total amount of milk fat produced per day was significantly lower ($P < 0.05$) for buffalo colostrum (23.7 g) than for milk (32.5–35.0 g). In practice, however, the processing of buffalo colostrum into high-value products in commercial quantities will be constrained by the small amount of colostrum that may be produced per calving, even in a year-round calving system that ensures a consistent supply of colostrum.

"Murrah × Carabao" crosses had higher colostrum yield (5.9 kg *vs.* 4.0 kg) and daily milk yield (6.9–7.4 kg *vs.* 5.9–6.1 kg) than Murrah. "Murrah × Carabao" crosses also produced more colostrum fat (30.5 g *vs.* 16.2 g) and more milk fat per day (36.1–39.1 g *vs.* 28.9–31.0 g) than purebred Murrah. However, the advantage of producing more milk fat in "Murrah × Carabao" crosses over purebred Murrah, perhaps due to heterosis, will be of little importance in many local dairy farms that commonly raise a mix of purebred and crossbred buffaloes.

**Correlations among Selected FAs**

Among the major colostrum FAs with highest proportions and importance, oleic acid – a MUFA – was negatively correlated with SFAs [C12:0 (r= –0.66), C14:0 (r= –0.74), and C16:0 (r = -0.43)] and positively correlated with PUFAs [LA (r = 0.37) and AA (r = 0.66)]. Among the PUFAs, LA was positively correlated with ALA (r = 0.37) (see Table 2). Except for C20:4 n-6, all major FAs were not correlated with colostrum fat percentage ($P > 0.05$).

For milk FAs, oleic acid – a MUFA – was negatively correlated with SFAs [C12:0 (r = –0.47) and C14:0 (r = –0.62)] but positively correlated with C16:0 (r = 0.20) and C18:0 (r = 0.18). Oleic acid was positively correlated with PUFAs – LA (r = 0.18), ALA (r = 0.32), and AA (r = 0.21) (see Table 2).

**Correlations of Selected FAs with Age at Calving, Parity, Colostrum or Test-day Milk Yield, and Fat Percentage**

For the major FAs in colostrum, oleic acid was negatively correlated with age at calving (r = –0.52) and parity (r = –0.63) (see Table 3). Both C14:0 and C16:0 were not correlated with age at calving, parity colostrum yield, and fat percentage. In the case of omega-3 and omega-6 FAs, ALA was negatively correlated with parity (r = –0.30), while AA was positively correlated with age at calving (r = 0.33). The CLA, LA, and DHA were not correlated with age at calving, parity, colostrum yield, and fat percentage.

For milk FAs, oleic acid was negatively correlated with age at calving (r = –0.18) (see Table 3). C14:0 was positively correlated with age at calving (r = 0.20), parity (r = 0.18), and test-day yield (r = 0.29). C16:0 was positively correlated with age at calving (r = 0.19) and parity (r = 0.19). C18:0 was negatively correlated with test-day yield (r = –0.29). C12:0 was not correlated with age at calving, parity, and test-day yield. All major FAs were not correlated with milk fat percentage ($P > 0.05$). All PUFAs (CLA, LA, ALA, AA, and DHA) were negatively correlated with test-day yield (r = –0.21, –0.26, –0.22, –0.18, and –0.18, respectively). ALA was also negatively correlated with age at calving (r = –0.27) and parity (r = –0.27).

**Factors Affecting FA Composition**

C12:0 was the most variable among the major SFAs with a coefficient variation (CV) of 27.50% – followed by C18:0 (CV = 19.47%), C14:0 (CV = 14.01%), and C16:0 (CV = 8.54%). By comparison, the CV for UFAs – oleic acid, LA, ALA, and CLA – were 23.19, 42.34, 20.07, and 70.06%, respectively (see Table 4).

All 19 FAs in the study were significantly affected ($P < 0.01$) by the type of milk. Higher parity was significantly associated ($P < 0.01$) with a decrease in the proportion of C14:0, C18:0, and ALA. The higher test-day yield was associated with an increase in C14:0 and a decrease in C18:0, LA, and CLA. A higher fat percentage was associated with an increase in C16:0.

**Major FAs in Buffalo Colostrum**

The three major FAs in buffalo colostrum – representing about 74% of total FAs – were C16:0 (32.3%), C18:1 n-9 (28.5%), and C14:0 (12.8%). These were followed by C18:0 and C12:0 with lower proportions of 5.4 and 4.4%, respectively (see Table 5). Colostrum contained smaller amounts of omega-6 FAs [*i.e.* LA (C18:2 n-6) and AA (C20:4 n-6) at 1.14% and 0.92%, respectively] and omega-3 FAs [*i.e.* ALA (C18:3 n-3) and DHA (C22:6 n-3 or DHA) at 0.36% and 0.26%, respectively]. Colostrum contained 0.64% CLA (C18:2 c9t11).

**Major FAs in Milk Obtained on the 30$^{th}$, 60$^{th}$, and 90$^{th}$ Day of Lactation**

The five major FAs in buffalo milk – representing about 82 to 84% of total FAs – were C16:0 (32.0–33.0%), C18:1 n-9 (18.0–18.8%), C14:0 (15.1–15.6%), C18:0 (8.7–9.7%), and C12:0 (7.8–8.2%) (see Table 5). Buffalo milk contained very small amounts of omega-6 FAs [*i.e.* LA and AA at 0.18–0.48% and 0.42–0.45%, respectively] and omega-3 FAs [*i.e.* ALA and DHA at 0.29–0.31% and 0.06–0.18%, respectively]. Milk contained 0.14–0.16% CLA. Except for myristoleic acid (C14:1 n-5) and palmitoleic acid (C16:1 n-7), all FAs in this study were not significantly different for milk obtained on the 30$^{th}$, 60$^{th}$, and 90$^{th}$ day of Lactation. Both C14:1 and C16:1 in milk collected on the 90$^{th}$ day of lactation (0.77 and 1.60%, respectively) was higher than on the 60$^{th}$ day (0.65 and 1.43%, respectively) and 30$^{th}$ day of lactation (0.64 and 1.41%, respectively).

## DISCUSSION

**Comparison of FAs between Milk Types**

The SFAs were about 0.85 times lower in buffalo colostrum than in milk obtained on the 30$^{th}$, 60$^{th}$, and 90$^{th}$ day of lactation. On the other hand, the MUFAs were about 1.48–1.55 times higher in colostrum than in milk. The PUFAs were also 2.12–2.96 times higher in colostrum than in milk. Colostrum seems to provide a higher concentrated UFA diet for calves, although the metabolic needs in FAs of buffalo calves in the first days of life are unknown and a correlation with FA titers in colostrum is impossible (Coroian *et al.* 2013).

For the three major FAs with the highest proportions, C16:0 was similar in colostrum and milk. C14:0 was 0.82–0.85 times lower in colostrum than in milk. Oleic acid – the major MUFA, however – was 1.52–1.59 times higher in colostrum than in milk.

Omega-3 FAs (ALA and DHA) were 1.27–1.74 times higher in colostrum than in milk. Omega-6 FAs (LA and AA) were 2.22–3.38 times higher in colostrum than in milk.

**Table 2.** Pearson correlation coefficients among fatty acids in buffalo colostrum (upper right off-diagonals) and milk (lower left off-diagonals).

| FAs | SFA | | | | | | | | MUFA | | | | | | | PUFA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12:0 | 14:0 | 15:0 | 16:0 | 17:0 | 18:0 | 20:0 | 22:0 | 14:1 n-5 | 16:1 n-7 | 18:1 n-9 | 18:1 n-7 | 20:1 n-11 | 22:1 n-9 | 18:2 c9t11 | 18:2 n-6 | 18:3 n-3 | 20:4 n-6 | 22:6 n-3 |
| **SFA** | | | | | | | | | | | | | | | | | | | |
| C12:0 | – | **0.79** | ns | ns | –0.36 | ns | ns | ns | 0.33 | ns | **–0.66** | ns | ns | ns | ns | –0.35 | ns | **–0.39** | ns |
| C14:0 | **0.77** | – | **0.43** | ns | **–0.48** | –0.31 | **0.66** | ns | **0.66** | ns | **–0.74** | ns | ns | ns | ns | ns | ns | **–0.46** | ns |
| C15:0 | ns | ns | – | 0.37 | ns | 0.36 | 0.31 | **0.60** | ns | 0.37 | **–0.53** | **0.49** | **0.66** | 0.48 | ns | ns | **0.46** | **–0.60** | ns |
| C16:0 | **–0.37** | ns | ns | – | **–0.41** | ns | ns | ns | **0.42** | ns | **–0.43** | ns | ns | ns | ns | ns | ns | ns | ns |
| C17:0 | **–0.60** | **–0.71** | **0.26** | –0.19 | – | **0.49** | **0.50** | ns | **–0.54** | ns | **0.36** | 0.38 | **0.41** | ns | ns | **0.40** | ns | ns | ns |
| C18:0 | **–0.34** | **–0.42** | 0.20 | **–0.38** | **0.56** | – | ns | **0.42** | **–0.76** | **–0.57** | ns | **0.41** | **0.60** | ns | ns | ns | ns | ns | ns |
| C20:0 | **–0.50** | **–0.62** | ns | ns | **0.68** | **0.35** | – | **0.55** | ns | ns | ns | **0.43** | 0.36 | ns | 0.34 | **0.50** | **0.44** | ns | ns |
| C22:0 | **–0.23** | **–0.24** | **0.40** | ns | **0.33** | **0.31** | **0.28** | – | ns | ns | ns | **0.49** | **0.79** | ns | ns | ns | **0.45** | ns | ns |
| **MUFA** | | | | | | | | | | | | | | | | | | | |
| C14:1 n-5 | **0.44** | **0.45** | **–0.30** | ns | **–0.54** | **–0.74** | **–0.40** | **–0.28** | – | **0.48** | **–0.39** | ns | ns | ns | ns | ns | ns | ns | ns |
| C16:1 n-7 | –0.18 | –0.20 | **–0.32** | **0.34** | ns | **–0.59** | ns | ns | 0.21 | – | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| C18:1 n-9 | **–0.47** | **–0.62** | ns | 0.20 | **0.43** | 0.18 | **0.51** | ns | **–0.52** | ns | – | ns | ns | ns | ns | 0.37 | ns | **0.66** | ns |
| C18:1 n-7 | ns | ns | ns | ns | ns | ns | ns | 0.21 | ns | ns | **–0.24** | – | **0.64** | ns | ns | **0.59** | **0.63** | ns | ns |
| C20:1n-11 | **–0.34** | **–0.42** | **0.45** | ns | **0.42** | **0.49** | **0.45** | **0.82** | **–0.31** | **–0.25** | ns | 0.20 | – | ns | ns | 0.37 | **0.55** | ns | ns |
| C22:1 n-9 | ns | ns | ns | ns | ns | ns | ns | 0.27 | **–0.31** | ns | 0.26 | ns | 0.26 | – | ns | ns | ns | ns | ns |
| **PUFA** | | | | | | | | | | | | | | | | | | | |
| C18:2 c9t11 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | – | ns | ns | ns | ns |
| C18:2 n-6 | **–0.58** | **–0.23** | ns | ns | ns | **0.23** | **0.25** | ns | **–0.44** | ns | 0.18 | 0.18 | 0.23 | ns | **0.60** | – | 0.37 | ns | ns |
| C18:3 n-3 | **–0.29** | **–0.43** | 0.20 | ns | **0.31** | ns | **0.42** | **0.36** | **–0.32** | ns | **0.35** | ns | **0.42** | **0.39** | **0.59** | **0.32** | – | ns | ns |
| C20:4 n-6 | ns | **–0.19** | ns | **–0.30** | ns | ns | ns | **–0.28** | –0.21 | ns | **0.53** | ns | ns | ns | 0.23 | **0.36** | ns | – | ns |
| C22:6 n-3 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | – |

[ns] correlation coefficient is not significantly different from zero, $P > 0.05$
[Not in **bold** font] correlation coefficient is significantly different from zero, $P < 0.05$
[In bold font] correlation coefficient is highly significantly different from zero, $P < 0.01$

**Table 3.** Pearson correlation coefficients between individual FAs and age at calving, parity, colostrum/test-day milk yield, and fat percentage in buffalo colostrum and milk.

| FA | Colostrum | | | | Milk | | | |
|---|---|---|---|---|---|---|---|---|
| | Age at calving | Parity | Colostrum yield | % Fat | Age at calving | Parity | Test-day milk yield | % Fat |
| **SFA** | | | | | | | | |
| C12:0 | –0.31* | ns | ns | ns | ns | ns | ns | ns |
| C14:0 | ns | ns | ns | ns | 0.20* | 0.18* | 0.29** | ns |
| C15:0 | –0.51** | –0.49** | ns | ns | –0.25** | –0.27** | –0.24** | ns |
| C16:0 | ns | ns | ns | ns | 0.19* | 0.19* | ns | ns |
| C17:0 | ns | –0.31* | ns | ns | –0.29** | –0.31** | –0.18* | ns |
| C18:0 | –0.41** | –0.43** | ns | ns | ns | ns | –0.29** | ns |
| C20:0 | ns | ns | ns | ns | –0.19* | –0.19* | –0.19* | ns |
| C22:0 | –0.39** | –0.41** | –0.46** | ns | ns | ns | –0.24** | ns |
| **MUFA** | | | | | | | | |
| C14:1 n-5 | ns | ns | ns | ns | ns | ns | 0.23** | ns |
| C16:1 n-7 | 0.36* | 0.43** | ns | ns | ns | ns | ns | ns |
| C18:1 n-9 | –0.52** | –0.53** | ns | ns | –0.18* | ns | ns | ns |
| C18:1 n-7 | –0.40** | –0.44** | ns | 0.38* | ns | ns | ns | ns |
| C20:1 n-11 | ns | ns | ns | ns | –0.21* | –0.18* | –0.38** | ns |
| C22:1 n-9 | ns | ns | ns | ns | –0.28* | –0.30* | ns | ns |
| **PUFA** | | | | | | | | |
| C18:2 c9t11 | ns | ns | ns | ns | ns | ns | –0.21* | ns |
| C18:2 n-6 | ns | ns | ns | ns | ns | ns | –0.26** | ns |
| C18:3 n-3 | ns | –0.30** | ns | ns | –0.27** | –0.27** | –0.22* | ns |
| C20:4 n-6 | 0.33* | ns | ns | 0.35* | ns | ns | –0.18* | ns |
| C22:6 n-3 | ns | ns | ns | ns | ns | ns | –0.18* | ns |

[ns] correlation coefficient is not significantly different from zero, $P > 0.05$
[*] correlation coefficient is significantly different from zero, $P < 0.05$
[**] correlation coefficient is highly significantly different from zero, $P < 0.01$

**Table 4.** Mean square F tests for the effects of milk type and covariate effects of parity, test-day milk yield, and percent fat on the proportion of FAs (g/ 100 g of total identified FAs) in buffalo colostrum and milk.

| | Milk type | Parity | Test-day yield | Percent fat | Coefficient of variation (%) |
|---|---|---|---|---|---|
| **SFA** | | | | | |
| C12:0, lauric acid | ** | ns | ns | ns | 27.50 |
| C14:0, myristic acid | ** | ns | *0.174 ± 0.082 | ns | 14.01 |
| C15:0, pentadecylic acid | ** | **–0.020 ± 0.004 | **–0.016 ± 0.005 | ns | 15.48 |
| C16:0, palmitic acid | ** | ns | ns | *0.188 ± 0.092 | 8.54 |
| C17:0, margaric acid | ** | **–0.016 ± 0.004 | ns | ns | 23.07 |
| C18:0, stearic acid | ** | **–0.147 ± 0.053 | **–0.218 ± 0.064 | ns | 19.47 |
| C20:0, arachidic acid | ** | ns | ns | ns | 57.10 |
| C22:0, behenic acid | ** | *–0.002 ± 0.001 | **–0.005 ± 0.001 | ns | 27.38 |
| **MUFA** | | | | | |
| C14:1 n-5, myristoleic acid | ** | ns | *0.016 ± 0.008 | ns | 26.42 |
| C16:1 n-7, palmitoleic acid | ** | *0.028 ± 0.011 | ns | ns | 20.78 |

| | Milk type | Parity | Test-day yield | Percent fat | Coefficient of variation (%) |
|---|---|---|---|---|---|
| C18:1 n-9, oleic acid | ** | ns | ns | ns | 23.19 |
| C18:1 n-7, trans-vaccenic acid | ** | ns | ns | ns | 26.86 |
| C20:1 n-11, eicosenoic acid | ** | **–0.004 ± 0.001 | **–0.007 ± 0.001 | ns | 21.59 |
| C22:1 n-9, erucic acid | * | ns | ns | ns | > 100 |
| **PUFA** | | | | | |
| C18:2 c9 t11, CLA | ** | ns | *–0.019 ± 0.008 | ns | 70.06 |
| C18:2 n-6, LA | ** | ns | *–0.028 ± 0.010 | ns | 42.34 |
| C18:3 n-3, ALA | ** | **–0.007 ± 0.002 | ns | ns | 20.07 |
| C20:4 n-6, AA | ** | ns | ns | ns | 33.81 |
| C22:6 n-3, DHA | ** | ns | ns | ns | > 100 |

[ns] no significant differences ($P > 0.05$); [*] significant differences ($P < 0.05$); [**] highly significant differences ($P < 0.01$)
Numbers in covariate columns are the regression coefficients and corresponding standard errors

**Table 5.** Least squares means of the proportion of FA (g/ 100 g of total identified FAs) and calculated FA groups plus FA-based nutritional indices/ratios for different milk types.

| | Colostrum | Milk 30-d | Milk 60-d | Milk 90-d |
|---|---|---|---|---|
| C12:0 | $4.42 \pm 0.32^{b}$ | $8.22 \pm 0.30^{a}$ | $7.82 \pm 0.31^{a}$ | $8.24 \pm 0.33^{a}$ |
| C14:0 | $12.84 \pm 0.34^{b}$ | $15.12 \pm 0.32^{a}$ | $15.27 \pm 0.32^{a}$ | $15.58 \pm 0.34^{a}$ |
| C15:0 | $0.81 \pm 0.02^{b}$ | $0.89 \pm 0.02^{a}$ | $0.89 \pm 0.02^{a}$ | $0.89 \pm 0.02^{a}$ |
| C16:0 | $32.29 \pm 0.45^{ab}$ | $32.03 \pm 0.43^{b}$ | $32.78 \pm 0.43^{ab}$ | $33.00 \pm 0.46^{a}$ |
| C17:0 | $0.66 \pm 0.02^{a}$ | $0.50 \pm 0.02^{b}$ | $0.49 \pm 0.02^{b}$ | $0.45 \pm 0.02^{b}$ |
| C18:0 | $5.42 \pm 0.26^{c}$ | $9.44 \pm 0.25^{a}$ | $9.67 \pm 0.25^{a}$ | $8.68 \pm 0.27^{b}$ |
| C20:0 | $0.21 \pm 0.01^{a}$ | $0.08 \pm 0.01^{b}$ | $0.09 \pm 0.01^{b}$ | $0.08 \pm 0.01^{b}$ |
| C22:0 | $0.09 \pm 0.01^{b}$ | $0.12 \pm 0.00^{a}$ | $0.11 \pm 0.00^{a}$ | $0.11 \pm 0.01^{a}$ |
| C14:1 n-5 | $0.80 \pm 0.03^{a}$ | $0.64 \pm 0.03^{b}$ | $0.65 \pm 0.03^{b}$ | $0.77 \pm 0.03^{ab}$ |
| C16:1 n-7 | $1.88 \pm 0.05^{a}$ | $1.41 \pm 0.05^{c}$ | $1.43 \pm 0.05^{c}$ | $1.60 \pm 0.05^{b}$ |
| C18:1 n-9 | $28.49 \pm 0.80^{a}$ | $17.96 \pm 0.75^{b}$ | $18.80 \pm 0.76^{b}$ | $18.37 \pm 0.80^{b}$ |
| C18:1 n-7 | $0.11 \pm 0.01^{b}$ | $0.15 \pm 0.01^{a}$ | $0.14 \pm 0.01^{a}$ | $0.16 \pm 0.01^{a}$ |
| C20:1 n-11 | $0.13 \pm 0.32^{b}$ | $0.19 \pm 0.01^{a}$ | $0.18 \pm 0.01^{a}$ | $0.17 \pm 0.01^{a}$ |
| C22:1 n-9 | $0.06 \pm 0.02^{a}$ | $0.02 \pm 0.02^{b}$ | $0.02 \pm 0.02^{b}$ | $0.02 \pm 0.02^{b}$ |
| C18:2 c9 t11, CLA | $0.64 \pm 0.03^{a}$ | $0.15 \pm 0.03^{b}$ | $0.14 \pm 0.03^{b}$ | $0.16 \pm 0.03^{b}$ |
| C18:2 n-6, LA | $1.14 \pm 0.04^{a}$ | $0.48 \pm 0.04^{b}$ | $0.46 \pm 0.04^{b}$ | $0.18 \pm 0.04^{c}$ |
| C18:3 n-3, ALA | $0.36 \pm 0.01^{a}$ | $0.31 \pm 0.01^{b}$ | $0.31 \pm 0.01^{b}$ | $0.29 \pm 0.01^{b}$ |
| C20:4 n-6, AA | $0.92 \pm 0.32^{a}$ | $0.45 \pm 0.03^{b}$ | $0.42 \pm 0.03^{b}$ | $0.43 \pm 0.03^{b}$ |
| C22:6 n-3, DHA | $0.26 \pm 0.05^{a}$ | $0.18 \pm 0.05^{ab}$ | $0.07 \pm 0.04^{b}$ | $0.06 \pm 0.05^{b}$ |
| SFA | 56.74 | 66.39 | 67.11 | 67.03 |
| UFA | 34.78 | 21.93 | 22.60 | 22.20 |
| MUFA | 31.47 | 20.37 | 21.21 | 21.09 |
| PUFA | 3.31 | 1.56 | 1.39 | 1.12 |
| n-3 (ALA + DHA) | 0.61 | 0.48 | 0.38 | 0.35 |
| n-6 (LA + AA) | 2.06 | 0.93 | 0.88 | 0.61 |

| | Colostrum | Milk 30-d | Milk 60-d | Milk 90-d |
|---|---|---|---|---|
| PUFA/SFA ratio | 0.06 | 0.02 | 0.02 | 0.02 |
| LA/ALA ratio | 3.21 | 1.55 | 1.48 | 0.62 |
| n-6/n-3 ratio | 3.37 | 1.93 | 2.34 | 1.76 |
| IA | 2.53 | 4.59 | 4.50 | 4.66 |
| IT | 2.68 | 4.48 | 4.58 | 4.59 |
| HPI | 0.39 | 0.22 | 0.22 | 0.21 |
| h/H ratio | 0.64 | 0.35 | 0.36 | 0.34 |

Least squares means with different superscript letters within a row are significantly different ($P < 0.05$).

[Milk 30-d, Milk 60-d, and Milk 90-d] milk collected on the 30th, 60th and 90th day of lactation, respectively; [SFA] saturated fatty acids; [UFA] unsaturated fatty acids; [MUFA] monounsaturated fatty acids; [PUFA] polyunsaturated fatty acids; [LA] linoleic acid (C18:2 n-6); [ALA] α-linolenic acid (C18:3 n-3); [AA] arachidonic acid (C20:4 n-6); [DHA] docosahexaenoic acid (C22:6 n-3); [n-3] omega-3 fatty acids; [n-6] omega-6 fatty acids, [h/H ratio] hypocholesterolemic/ hypercholesterolemic ratio

The CLA was also 4.00–4.57 times higher in colostrum than in milk. The CLA is the best-known ruminant trans fats found in dairy fat and is widely recognized as an anticarcinogenic, antiatherogenic, anti-inflammatory, and weight-reducing substance (Rodriguez-Alcala *et al.* 2013; Agarwal and Gupta 2016; Whigham *et al.* 2007).

**Comparison of Individual FAs with Other Breeds**

***FAs in buffalo colostrum.*** Except for AA, all FAs in this study were not significantly different between Murrah and "Murrah × Carabao" crosses ($P > 0.05$). AA in colostrum and milk fat was higher for Murrah (0.97 and 0.45–0.48%, respectively) than in "Murrah × Carabao" crosses (0.87 and 0.33–0.39%, respectively) (data not shown in Table 5). The AA is an essential omega-3 FA that helps keep cell membranes, especially those of nerve cells, healthy and permeable leading to stable nutrient supply in the brain and other parts of the body (Silva *et al.* 2019).

Colostrum from both Murrah and "Murrah × Carabao" crosses had lower SFAs, higher MUFAs, and higher PUFAs than colostrum from Romanian buffaloes reported by Coroian *et al.* (2013). In their study involving 10 SFAs and six UFAs, Coroian *et al.* (2013) also showed that some FAs in colostrum were affected by calving season, with higher concentrations of FAs possibly influenced by pasture nutrition during the summer season. They suggested that buffaloes can be milked starting with day four as the calves already benefit from a sufficient amount of colostrum and the surplus until Day 7 could easily be used for human nutrition. In terms of the major SFAs, colostrum from Murrah and "Murrah × Carabao" crosses contained lower C16:0 and C18:0, but higher C12:0 and C14:0 than those from Romanian buffaloes. Furthermore, Murrah and "Murrah × Carabao" crosses had higher oleic acid but lower LA than in the colostrum of Romanian buffaloes.

***FAs in buffalo milk.*** The SFAs in milk from both Murrah and "Murrah × Carabao" crosses were similar to the SFAs reported for Kundi buffaloes in Pakistan (Talpur *et al.* 2007). The SFAs in milk in this study were however lower than those reported for Bulgarian Murrah (Ilieva *et al.* 2020), Nili-Ravi in Pakistan (Qureshi *et al.* 2015), Mediterranean in Italy (Pegolo *et al.* 2017), Anatolian water buffaloes in Turkey (Cinar *et al.* 2019), the Mediterranean in France (Menard *et al.* 2010), Bulgarian Murrah (Penchev *et al.* 2016), and Bhadawari in India (Kushwaha *et al.* 2018). The SFAs in milk in this study were higher than those reported for Murrah in Brazil (Fernandes *et al.* 2007) and the Mediterranean in Italy (Varricchio *et al.* 2007).

The MUFAs in milk from both Murrah and "Murrah × Carabao" crosses were similar to the MUFAs reported for Bhadawari in India (Kushwaha *et al.* 2018). The MUFAs in milk in this study were however lower than those reported for Nili-Ravi in Pakistan (Qureshi *et al.* 2015), Bulgarian Murrah (Penchev *et al.* 2016), Mediterranean in Italy (Pegolo *et al.* 2017), Mediterranean in Italy (Varricchio *et al.* (2007), the Mediterranean in France (Menard *et al.* 2010), Bulgarian Murrah (Ilieva *et al.* 2020), Kundi in Pakistan (Talpur *et al.* 2007), Anatolian water buffaloes in Turkey (Cinar *et al.* 2019), and Murrah in Brazil (Fernandes *et al.* 2007).

The PUFAs in milk from both Murrah and "Murrah × Carabao" crosses were similar to the PUFAs reported for Bhadawari in India (Kushwaha *et al.* 2018) and the PUFAs reported for the Mediterranean breed in France (Menard *et al.* 2010). The PUFAs in milk fat in this study were higher than the PUFAs reported for Anatolian water buffaloes in Turkey (Cinar *et al.* 2019). The PUFAs in milk in this study were however lower than those reported for Bulgarian Murrah (Penchev *et al.* 2016), Kundi in Pakistan (Talpur *et al.* 2007), Bulgarian Murrah (Ilieva *et al.* 2020), the Mediterranean in Italy (Pegolo *et al.* 2017),

Nili-Ravi in Pakistan (Qureshi *et al.* 2015), Murrah in Brazil (Fernandes *et al.* 2007), and the Mediterranean in Italy (Varricchio *et al.* 2007).

The oleic acid in milk from Murrah and "Murrah × Carabao" crosses was similar to the oleic acid reported for the Mediterranean in Italy (Pegolo *et al.* 2017). The oleic acid in milk in this study was higher than the oleic acid reported for Bhadawari in India in Turkey (Kushwaha *et al.* 2018). The oleic acid in milk fat in this study was, however, lower than those reported for the Mediterranean in France (Menard *et al.* 2010), Nili-Ravi in Pakistan (Qureshi *et al.* 2015), Bulgarian Murrah (Ilieva *et al.* 2020), Bulgarian Murrah (Penchev *et al.* 2016), Kundi in Pakistan (Talpur *et al.* 2007), Anatolian water buffaloes in Turkey (Cinar *et al.* 2019), and the Mediterranean in Italy (Varricchio *et al.* 2007).

The ALA in milk from Murrah and "Murrah × Carabao" crosses were similar to the ALA for the Anatolian water buffaloes in Turkey (Cinar *et al.* 2019) and the ALA reported for Bulgarian Murrah (Ilieva *et al.* 2020). The ALA in milk in this study were however slightly lower than those reported for Bhadawari in India (Kushwaha *et al.* 2018), Mediterranean in France (Menard *et al.* 2010), Kundi in Pakistan (Talpur *et al.* 2007), Nili-Ravi in Pakistan (Qureshi *et al.* 2015), Bulgarian Murrah (Penchev *et al.* 2016), Mediterranean in Italy (Pegolo *et al.* 2017), and the Mediterranean in Italy (Varricchio *et al.* 2007).

The LA in milk from Murrah and "Murrah × Carabao" crosses were similar to the LA reported for Bulgarian Murrah (Ilieva *et al.* 2020), Bhadawari in India (Kushwaha *et al.* 2018), and Nili-Ravi in Pakistan (Qureshi *et al.* 2015). The LA in milk in this study was, however, slightly lower than those reported for the Mediterranean in Italy (Pegolo *et al.* 2017), Bulgarian Murrah (Penchev *et al.* 2016), the Mediterranean in France (Menard *et al.* 2010), Kundi in Pakistan (Talpur *et al.* 2007), and the Mediterranean in Italy (Varricchio *et al.* 2007).

The CLA in milk from Murrah and "Murrah × Carabao" crosses were similar to those reported for Nili-Ravi in Pakistan (Qureshi *et al.* 2015) but slightly lower compared to the Mediterranean in Italy (Pegolo *et al.* 2017; Varricchio *et al.* 2007), Bulgarian Murrah (Ilieva *et al.* 2020), Kundi in Pakistan (Talpur *et al.* 2007), Mediterranean in France (Menard *et al.* 2010), Anatolian water buffaloes in Turkey (Cinar *et al.* 2019), and Murrah in Brazil (Fernandes *et al.* 2007).

### FA-based Nutritional Indices/Ratios

The comparison of nutritional indices/ratios (related to cardiovascular health) among milk types and between breeds are shown in Tables 5 and 6, respectively.

***PUFA/SFA ratio.*** The PUFA/SFA ratio is the commonly used index to assess the impact of diet on cardiovascular health (Chen and Liu 2020). The higher this ratio, the more positive the effect. This is because all PUFAs in the diet are known to depress low-density lipoprotein cholesterol

**Table 6.** Calculated values for FA groups and FA-based nutritional indices/ratios of colostrum and milk from Murrah and "Murrah × Carabao" (M×C) crosses.

| | Colostrum | | Milk 30-d | | Milk 60-d | | Milk 90-d | |
|---|---|---|---|---|---|---|---|---|
| | Murrah | (M×C) cross | Murrah | (M×C) cross | Murrah | (M×C) cross | Murrah | (M×C) cross |
| SFA | 56.51 | 56.87 | 65.83 | 67.34 | 66.66 | 67.84 | 67.13 | 66.96 |
| UFA | 34.94 | 34.89 | 22.81 | 20.45 | 23.25 | 21.55 | 22.35 | 21.84 |
| MUFA | 31.66 | 31.50 | 22.15 | 19.05 | 21.75 | 20.34 | 21.16 | 20.84 |
| PUFA | 3.28 | 3.39 | 1.66 | 1.40 | 1.50 | 1.22 | 1.19 | 1.00 |
| n-3 (ALA + DHA) | 0.58 | 0.66 | 0.53 | 0.41 | 0.38 | 0.36 | 0.35 | 0.35 |
| n-6 (LA + AA) | 2.09 | 2.06 | 0.98 | 0.85 | 0.97 | 0.73 | 0.67 | 0.52 |
| PUFA/SFA ratio | 0.06 | 0.06 | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 |
| LA/ALA ratio | 3.14 | 3.28 | 1.57 | 1.52 | 1.54 | 1.38 | 0.52 | 0.47 |
| n-6/n-3 ratio | 3.61 | 3.12 | 1.83 | 2.06 | 2.52 | 2.02 | 1.93 | 1.48 |
| IA | 2.48 | 2.55 | 4.31 | 5.13 | 4.29 | 4.87 | 4.63 | 4.76 |
| IT | 2.69 | 2.63 | 4.27 | 4.87 | 4.47 | 4.76 | 4.60 | 4.60 |
| HPI | 0.40 | 0.39 | 0.23 | 0.19 | 0.23 | 0.21 | 0.22 | 0.21 |
| h/H ratio | 0.65 | 0.64 | 0.38 | 0.31 | 0.38 | 0.34 | 0.34 | 0.34 |

[Milk 30-d, Milk 60-d, and Milk 90-d] milk collected on the 30$^{th}$, 60$^{th}$ and 90$^{th}$ day of lactation, respectively; [SFA] saturated fatty acids; [UFA] unsaturated fatty acids; [MUFA] monounsaturated fatty acids; [PUFA] polyunsaturated fatty acids; [LA] linoleic acid (C18:2 n-6); [ALA] α-linolenic acid (C18:3 n-3); [AA] arachidonic acid (C20:4 n-6); [DHA] docosahexaenoic acid (C22:6 n-3); [n-3] omega-3 fatty acids; [n-6] omega-6 fatty acids, [h/H ratio] hypocholesterolemic/ hypercholesterolemic ratio

and lower levels of serum cholesterol, whereas all SFAs contribute to high levels of serum cholesterol. However, not all PUFAs may positively affect the prevention of cardiovascular disease just as not all SFAs may contribute equally to serum cholesterol. For example, C20:4 n-6 (the major PUFA found in buffalo colostrum and milk) is known to increase high-density lipoprotein cholesterol level – which would neutralize some SFAs such as C12:0, C14:0, and C16:0 (Hanus *et al.* 2018). On the other hand, C14:0 and C16:0 – the major SFAs found in buffalo colostrum and milk – may increase total blood cholesterol levels (Zock *et al.* 1994) and, hence, widely thought to be one of the main causes of cardiovascular disease. In particular, the long-term consumption of SFAs in higher proportion to other healthy fats may increase the risk for cardiovascular disease (German and Dillard 2010). However, recent systematic reviews show a lack of association between saturated fat intake and cardiovascular disease (Siri-Tarino *et al.* 2010; de Souza *et al.* 2015). The SFAs, although not essential, also play numerous beneficial roles within the body. They are involved in lipogenesis, fat deposition, PUFAs bioavailability, and apoptosis (Legrand and Rioux 2010). Saturated fat also has protective effects against alcoholic liver disease (Kirpich *et al.* 2016).

In this study, the PUFA/SFA ratio was very low, *i.e.* 0.06: 1 for colostrum and 0.02: 1 for milk fat. The PUFA/SFA ratio in milk was similar for purebred Murrah (0.02–0.03: 1) and "Murrah × Carabao" crosses (0.01–0.02: 1). This suggests the very low amounts of PUFAs in relation to SFAs, and that healthier dairy products derived from either colostrum or milk regardless of the breed will need supplementation with PUFAs. For example, DHA (C22:6 n-3) may be added to help protect the cardiovascular system from the unhealthy effects of atherosclerotic lesions (Naeini *et al.* 2020).

***LA/ALA ratio.*** LA and ALA are considered essential FAs because they cannot be synthesized by humans. Originally developed in guiding the manufacture of infant formula, the LA/ALA ratio describes the balance between LA (C18:2 n-6) and ALA (C18:3 n-3) as these PUFAs compete for the same desaturase and elongase enzymes, which they use to synthesize long-chain unsaturated FAs. The balance may be the most important factor, especially when long-chain PUFAs are not present in infant formulas (Chen and Liu 2020). When judging the nutritional value of baby food and infant formula, the LA/ALA ratio has a higher minimum reference value (within 5–15: 1). Tissues of adults have a lower rate of synthesis of n-3 long-chain PUFAs than those of infants, so the LA/ALA ratio in the diet does not have too much of an impact on adults.

In this study, LA/ALA ratio was low in colostrum (3.21: 1) and decreasingly lower in milk (0.62–1.55: 1) when obtained from the 30$^{th}$, 60$^{th}$, until the 90$^{th}$ day of lactation. The LA/ALA ratio in milk was similar for purebred Murrah (0.52–1.57: 1) and "Murrah × Carabao" crosses (0.47–1.52: 1). Nonetheless, the low LA/ALA ratio implies the restricted use of buffalo colostrum or milk – regardless of breed – for infants.

***n-6/n-3 ratio.*** The n-6/Omega-3 ratio is an important determinant of PUFAs and their coinciding effects on inflammatory diseases. The n-6 PUFAs (*i.e.* LA and AA) and n-3 PUFAs (*i.e.* ALA and DHA) are precursors to eicosanoids, which have important roles in the regulation of inflammation.

In general, eicosanoids derived from n-6 PUFA are pro-inflammatory, while eicosanoids derived from n-3 PUFA are anti-inflammatory. Increases in the n-6/n-3 ratio (as high as ~ 15:1 in the Western diet) are associated with greater metabolism of the n-6 PUFA compared with n-3 PUFA. This increase in the n-6/n-3 ratio coincides with increases in chronic inflammatory diseases such as nonalcoholic fatty liver disease, cardiovascular disease, obesity, inflammatory bowel disease, rheumatoid arthritis, and Alzheimer's disease. Optimal dietary intakes of the n-6/n-3 ratio should be around 1–4: 1 (Patterson *et al.* 2012). A lower n-6/n-3 ratio (1–2: 1) may reduce the risk of many chronic diseases, although the optimal ratio may vary with the disease under consideration depending on the degree of severity of disease resulting from the genetic predisposition (Simopoulos 2002).

In this study, the n-6/n-3 ratio was lower (*i.e.* more balanced) for buffalo milk (1.8–2.3: 1) and highest (*i.e.* least balanced) for colostrum (3.4: 1). The n-6/n-3 ratio in milk obtained on the 30$^{th}$, 60$^{th}$, and 90$^{th}$ day of lactation was similar for Murrah (1.8–2.5: 1) and "Murrah × Carabao" crosses (1.5–2.1: 1).

***IA.*** The IA originally proposed by Ulbricht and Southgate (1991), is a measure of the dietary contribution of some SFAs (C12:0, C14:0, and C16:0 except C18:0) that are pro-atherogenic (*i.e.* they favor the adhesion of lipids to cells of the circulatory and immunological systems). This is expressed in relation to all MUFAs and PUFAs that are anti-atherogenic (*i.e.* they inhibit the accumulation of fatty plaque and reduce the levels of phospholipids, cholesterol, and esterified FAs). Dietary fat with lower IA values, therefore, has greater health benefits (*i.e.* lower tendency to form fatty plaques in the arteries), as its consumption may reduce the risk of coronary heart disease (Chen and Liu 2020).

In this study, the IA was lower (*i.e.* greater health benefits) in colostrum (2.53) than in milk (4.50–4.66). The atherogenicity potential of milk from purebred Murrah (4.29–4.63) was slightly lower than in "Murrah × Carabao" crosses (4.76–5.13).

***IT.*** The IT, developed by Ulbricht and Southgate (1991), is used to determine the dietary contribution of pro-thrombogenic SFAs (*i.e.* C12:0, C14:0, and C16:0) in relation to the anti-thrombogenic MUFAs and PUFAs. Consumption of foods or products with lower IT values (*i.e.* lower tendency to form clots in blood vessels) is beneficial for cardiovascular health (Chen and Liu 2020).

In this study, the IT was lower (*i.e.* more beneficial for cardiovascular health) in colostrum (2.68) than in milk (4.48–4.59). The differences in IT for milk of Murrah (4.27–4.60) and "Murrah × Carabao" crosses (4.60–4.87) were small.

***HPI.*** The HPI as proposed by Chen *et al.* (2004) is the inverse of the IA. It is mainly used in research on dairy products such as milk and cheese with values commonly ranging from 0.16–0.68. Dairy products with a high HPI value are considered more beneficial to human health (Chen and Liu 2020).

In this study, the HPI was higher in buffalo colostrum (0.39) than in milk (0.21–0.22). The HPI values of milk from purebred Murrah (0.22–0.23) were slightly higher than in "Murrah × Carabao" crosses (0.19–0.21).

***h/H ratio.*** The h/H ratio is a new index to assess the effect of dietary FA composition on cholesterol. First used by Mierlita (2018) in sheep milk, the h/H ratio characterizes the relationship between hypocholesterolemic FA (oleic acid and PUFA) and hypercholesterolemic FA (C12:0, C14:0, and C16:0). Compared with the PUFA/SFA ratio, the h/H ratio may more accurately reflect the effect of the FA composition on cardiovascular disease. Dietary fat with lower h/H values has greater health benefits. For dairy products, the range is 0.32–1.29: 1 (Chen and Liu 2020).

In this study, the h/H ratio was higher in buffalo colostrum (0.64: 1) than in milk (0.34–0.36: 1), suggesting that the use of colostrum may result in higher blood cholesterol levels. The h/H ratio milk from purebred Murrah (0.34–0.38: 1) was slightly higher than in "Murrah × Carabao" crosses (0.31–0.34: 1).

## CONCLUSION

Buffalo colostrum seems to be more beneficial for cardiovascular health because of its lower IA and IT values and higher HPI than milk. However, raw milk had a more balanced n-6/omega-3 ratio and lower h/H ratio (*i.e.* related to lower blood cholesterol levels) than colostrum.

Milk from the Murrah breed may have slight advantages in terms of lower atherogenicity values and higher HPI values than milk from "Murrah × Carabao" crosses. On the other hand, milk from "Murrah × Carabao" crosses had a slightly lower h/H ratio than milk from Murrah. The "Murrah × Carabao" crosses also produced more milk fat per day than Murrah.

## ACKNOWLEDGMENTS

This research was supported by the Philippine Agriculture and Fisheries Biotechnology Program under the Department of Agriculture Biotechnology Program (DA-BIOTECH R1807). The authors acknowledge Thelma Almendral-Saludes and Abraham G. Tandang of the PCC at UPLB, as well as Aldrin O. Ebron, and Alyssa T. Odiner for their assistance in the collection and storage of colostrum and milk samples.

## STATEMENT ON NO CONFLICT OF INTEREST

There is no conflict of interest between the authors and other people and organizations.

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