




# Fraud with the addition of cow's milk alters the lipid fraction of buffalo mozzarella

Mirelle Pignata VIANA<sup>1</sup>, Sergio Augusto de Albuquerque FERNANDES<sup>2\*</sup> , Andréa Gomes da SILVA<sup>3</sup>, Márcio dos Santos PEDREIRA<sup>4</sup>, Pablo Teixeira VIANA<sup>5</sup>, Valdirene Santana RODRIGUES<sup>6</sup>, Ellen Cristina Quirino LACERDA<sup>7</sup>

## Abstract

The shortage of milk at certain times of the year leads to adulteration of buffalo mozzarella, and these frauds alter the composition of milk and buffalo derivatives. This study describes the dynamics of the adulteration on the nutritional quality of mozzarella. Mozzarella was produced from buffalo milk incorporated with cow milk at 0, 10, 20, 30, 40 and 50% (v/v). The chemical composition, fatty acids profile and cholesterol content of the cheeses were evaluated. The results showed that the fat and protein contents of buffalo cheeses decreased with the addition of cow milk. Furthermore, C4:0, C16:0, C22:0 and C16:1 fatty acids decreased while C8:0 and C10:0 acids fatty acids increased. The most dramatic observation was the elevation of the cholesterol content when cow milk was added. The altered content of short-chain saturated fatty acids and cholesterol content, due to the addition of cow milk to buffalo milk for mozzarella production, modified the nutritional indices. The addition of cow milk to buffalo milk for mozzarella production altered the content of short-chain SFA and the cholesterol content, thereby modifying the nutritional indices.

**Keywords:** cholesterol; fatty acid; nutritional indices; seasonality.

**Practical Application:** Know the effects of the fraud on the buffalo's mozzarella cheese.

## 1 Introduction

As of 2018, the volume of cheese traded worldwide had increased, largely due to a rise in consumption prompted by the sensorial and nutritional characteristics of cheese (Food and Agriculture Organization, 2019). Due to its milk origin, cheese provides a broad spectrum of nutrients, such as vitamins, minerals, protein, fatty acids, and other bioactive substances, originating from the composition of milk and the microbiota (Lucey et al., 2017; Ottavian et al., 2012; Santiago-López et al., 2018). Factors such as species, race, stage and number of lactations, reproductive seasonality, feeding, mastitis and genetic polymorphism (Talpur et al., 2008; Nawaz et al., 2009) can influence milk composition. However, the species of origin is the main determinant of milk composition (Lopez et al., 2011; Salman et al., 2014; Boro et al., 2018).

Mozzarella is typically fresh Italian “pasta filata” buffalo milk cheese produced with a Protected Designation Origin (PDO), and it must have the denomination “Mozzarella di Bufala Campana (MBC)” on the seal (European Union 1996;

Italy, 2003; Dalmasso et al., 2011; Ilić et al., 2011). It is also a target of fraud, which is still observed despite the rigor of PDO (European Union, 2017; Gonçalves et al., 2017). Factors such as (i) the decrease in buffalo milk production due to the reproductive seasonality of the species (Phogat et al., 2016); (ii) the scarcity of buffalo mozzarella on the market (Locci et al., 2008; Penchev et al., 2016) and (iii) a consequent price increase have motivated fraud through addition of milk from different species to buffalo milk for mozzarella production (Czerwenka et al., 2010; Gunning et al., 2019). PDO mozzarella fraud in Italy is most often perpetrated by including buffalo milk from outside the PDO production zone, followed by adding cow's milk to mozzarella production (Bontempo et al., 2019), and brings dramatic consequences to allergic people.

The consumption of buffalo mozzarella adulterated with cow's milk by allergic people can cause them to develop health problems related to the allergenic compounds present in cow's milk, which is absent in buffalo's milk (Ramesha et al., 2016; Bontempo et al.,

Received 09 Aug., 2019

Accepted 11 Dec., 2019

<sup>1</sup> Grupo de Pesquisa em Biotecnologia do Semiárido, Centro Universitário FG - UniFG, Campus São Sebastião, Guanambi, BA, Brasil

<sup>2</sup> Grupo de Produção Animal Sustentável no Semiárido, Centro de Estudos em Leite, Programa de Pós-graduação em Engenharia e Ciência de Alimentos, Departamento de Tecnologia Rural e Animal, Universidade Estadual do Sudoeste da Bahia - UESB, Campus Juvino Oliveira, Itapetinga, BA, Brasil

<sup>3</sup> Laboratório de Tecnologia de Produtos de Origem Vegetal, Programa de Pós-graduação em Engenharia e Ciência de Alimentos, Departamento de Tecnologia Rural e Animal, Universidade Estadual do Sudoeste da Bahia - UESB, Campus Juvino Oliveira, Itapetinga, BA, Brasil

<sup>4</sup> Grupo de Pesquisa em Produção Animal no Semiárido, Laboratório de Nutrição Animal, Programa de Pós-graduação em Zootecnia, Departamento de Fitotecnia e Zootecnia, Universidade Estadual do Sudoeste da Bahia - UESB, Campus Vitória da Conquista, Vitória da Conquista, BA, Brasil

<sup>5</sup> Grupo de Pesquisa em Produção Animal e Forragicultura no Piauí, Programa de Desenvolvimento Científico e Tecnológico Regional do Piauí, Universidade Estadual do Piauí - UESPI, Campus Jesualdo Cavalcanti, Corrente, PI, Brasil

<sup>6</sup> Laboratório de Análises Microbiológicas, Departamento de Ensino, Instituto Federal de Educação Ciência e Tecnologia do Norte de Minas Gerais, Campus Salinas, Salinas, MG, Brasil

<sup>7</sup> Departamento de Nutrição Básica e Experimental, Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro - UFRJ, Rio de Janeiro, RJ, Brasil

2019). Cheese ingestion is a sensorial and nutritive experience enriched by these compounds (Santiago-López et al., 2018). Failure as a producer or researcher occurs when adulterated mozzarella is available as an original product.

Aiming to detect falsified buffalo mozzarella, researchers have been using various techniques, such as mass spectrometry (Cozzolino et al., 2002; Poonia et al., 2017; Bontempo et al., 2019), DNA (Feligini et al., 2005), electrophoresis (Pesic et al., 2011), liquid chromatography, coupled or not to mass spectrometry (Enne et al., 2005; Czerwenka et al., 2010; Russo et al., 2012), isoelectric focusing (Sakaridis et al. 2013) and infrared spectroscopy (Hansen & Holroyd, 2019), to detect fraud in milk and cheese. These techniques focus on the milk protein fraction, whereas others focus on the mineral fraction (Bontempo et al., 2019) and vitamins (Dal Bosco et al., 2018) as biomarkers. However, studies analysing the effect of fraudulent mozzarella composition are rare. One paper described the effects of cow's milk inclusion in buffalo milk on some fatty acids (Farag et al., 1984), but the conjugated linoleic acid (CLA) content and nutritional indices were not investigated. To address this, the current work studied the effect of cow milk inclusion on the lipid fraction of buffalo-milk-based mozzarella.

## 2 Materials and methods

### 2.1 Sampling

Morning milk samples were collected from 30 crossbred Jafarabadi/Murrah buffaloes and 30 Holstein/Zebu crossbred cows in the initial lactation phase (45 days on average), on day weekly for three consecutive weeks.

### 2.2 Experimental design

The design consisted of six treatments composed of cow milk inclusion levels (0, 10, 20, 30, 40 and 50%) in the processing of buffalo milk-based mozzarella cheese, where 0% corresponds to the control treatment (Table 1).

To prepare mozzarella, 30.L<sup>-1</sup> of pasteurised milk from each species (buffalo and cow) was used, with three repetitions. The Italian mozzarella cheese-making procedure (Calandrelli, 2007) was changed, and the fat content was corrected to approximately 4%.

### 2.3 Chemical and physical characteristics

Each method was conducted in triplicate on triplicate samples from every batch (3 batches × 3 repetitions × 3 samples = 27).

**Table 1.** Experimental design.

Cheeses	Milks (%)	
	Buffalo	Cow
Mozzarella (0)	100	0
10	90	10
20	80	20
30	70	30
40	60	40
50	50	50

### Milk

Physical analyses of pH (using a pH meter; Quimis, Diadema, São Paulo, Brazil), titratable acidity (acid lactic g.100 mL<sup>-1</sup>), and density (g.mL<sup>-1</sup>); measured using a Quevenne thermolactodensimeter (Incoterm, Porto Alegre, RS, Brazil) were performed at 15 °C. The fat percentage was determined by the Gerber method, and the total nitrogen was assayed by the Kjeldahl method using a conversion factor of 6.38 for the calculation of the total protein. Lactose was evaluated by the Fehling reduction test; the total solids (TS) were determined gravimetrically; the dry matter was calculated as the difference between the TS and the fat content, and moisture content was estimated by TS - 100%. All analysis are according Brasil (2018).

### Cheese composition

For the mozzarella cheese, gravimetric methods were used to measure the moisture content (oven-drying at 105 °C) and ash content (incineration of the sample at 550 ± 5 °C). The fat, total nitrogen, lactose, TS, dry matter and moisture contents were assayed, as described in section 2.3.1 (Brasil, 2018).

### 2.4 Lipid analysis

#### Total lipid extraction

Lipids were extracted using a chloroform/methanol/water solution, according to Bligh & Dyer (1959).

#### Fatty acid methyl esters (FAME)

The FAME were prepared from the lipids extracted from the cheese samples by adding 5.0 mL of 0.25 mol.L<sup>-1</sup> sodium methoxide solution in methanol/diethyl ether (1:1, v/v) to approximately 150 mg of lipids, with stirring for 3 min. Next, 2.0 mL *iso*-octane and 10.0 mL of saturated NaCl solution were added, and the tube was agitated again and allowed to stand for phase separation. The supernatant was transferred to duly identified Eppendorf flasks for further chromatographic analysis (Bannon et al., 1982).

The FAME were separated using a Trace-GC-Ultra gas chromatograph (Thermo Finnigan, Milan, Italy), equipped with a fused silica BPX-70 capillary column (120 m, 0.25 mm film thickness; Thermo Finnigan), a flame ionisation detector and an automatic injector (Thermo Finnigan). The gas flows (White Martins, São Paulo, Brazil) were 6.5 mL.min<sup>-1</sup> for the entrainment gas (H<sub>2</sub>); 30 mL.min<sup>-1</sup> for the auxiliary gas (N<sub>2</sub>); 30 mL.min<sup>-1</sup> for H<sub>2</sub> and 250 mL.min<sup>-1</sup> for the synthetic flame air. The sample split ratio was 90:10. The volumes of the injections were 1.2 µL. The peak areas of the FAME were determined using ChromQuest 4.1 software (Thermo Finnigan, Milan, Italy).

The FAME were identified after checking the equivalent chain length of the peaks, evaluating the flame ionisation detector response and comparing the retention times of methyl esters of fatty acids containing *cis*-9, *trans*-11 and *trans*-10, *cis*-12 linoleic acids (189-19, O-5632 and O-5626, Sigma-Aldrich, Saint Louis, USA).

The fatty acids (mg.g<sup>-1</sup> total lipids) were quantified using methyl tricosanoate (C23:0) (Sigma) as the internal standard. Before transesterification of the weighed lipid samples (≈150 mg), 1000 µL of the internal standard solution of known concentration (1.00 g.mL<sup>-1</sup>) was added. For quantification, the theoretical response factors were used, after verifying the agreement of these values with the experimental ones.

### Cholesterol content

The cholesterol content was analysed by direct saponification and hexane extraction (Bauer et al., 2014) using a high-performance liquid chromatograph model SPD-M20A (Shimadzu, Kyoto, Japan), with a quaternary solvent system, injection valve with 20 µL sampling loop, column furnace and diode arrangement detector. An analytical C18 column, 15 cm × 6 mm × 5 mm (Shimadzu, Kyoto, Japan) was used for the cholesterol quantification, which was done through external standardisation (Golay et al., 2016).

### Statistics

The results were interpreted using analysis of variance and regression analysis. Statistical models were chosen, according to the level of significance and determination coefficients (R<sup>2</sup>), using the F test and  $\alpha = 0.05$  (R Core Team, 2015).

## 3 Results and discussion

### 3.1 Chemical composition

The DOP standard of MBC was used as a guideline to classify the cheese manufactured in our study (European Union, 1996). The buffalo mozzarella presented fat and moisture contents within the standards established in Italy for MBC (Table 2), indicating that these characteristics are peculiar to buffalo mozzarella.

The crude protein content in the buffalo milk varies between 4.32-4.43% (Gagliostro et al., 2015) while cow milk varies between 3.00-3.28% (Feltus et al., 2016; Bondan et al., 2018). Considering these facts, the addition of milk of lower crude protein content to the cheese-making mixture should decrease the cheese crude protein content. The observed decrease in the cheese was close to 10.70%. The other factors evaluated, including the moisture, ash and TS, were not affected by the treatments (European Union, 1996; Italy, 2003). To the more information about fat and crude protein in milk and mozzarella cheese made from cow and buffalo milk see Pignata et al. (2015).

### 3.2 Fatty acid composition

Twenty-four fatty acids were identified and quantified in cheese fat (Table 3). In decreasing order, palmitic (C16:0), oleic (C18:1 *n-9cis*), myristic (C14:0) and stearic (C18:0) fatty acids were the most concentrated in all treatments, consistent with the trend observed by Romano et al. (2011) when evaluating MBC. Conversely, (Bergamo et al., 2003) and Martini et al. (2016) observed a different order; C16:0 > C18:1 > C18:0 > C14:0. Among the SFA, butyric (C4:0, P = 0.012), palmitic (C16:0, P = 0.014) and behenic acids (C22:0, P = 0.023) decreased linearly with the inclusion of cow milk. In turn, the caprylic acid (C8:0, P = 0.006) and capric acid (C10:0, P = 0.002) contents increased.

Buffaloes present a more significant degradation of the dietary fibre in the rumen compared with cows and, consequently, more of the volatile fatty acids (acetate, butyrate and propionate); however, dietary fiber also generate a higher molar ratio of acetate and butyrate, precursors of the short-chain SFA observed in milk (Terramocchia et al., 2005; Shen et al., 2019), especially C4:0 (starter). These facts may explain the decrease in C4:0 content with the inclusion of bovine milk since studies have indicated a higher content of C4 in buffalo milk than cow milk (Zotos & Bampidis, 2014; Correddu et al., 2017; Pegolo et al., 2017; Teng et al., 2017). Accordingly, the pure mozzarella had a higher C4 content than the others prepared from the blended milk. It is interesting to note that higher levels of C4 in foods are sought because of beneficial effects on the human body, such as antiproliferative, anti-inflammatory and apoptotic properties (Mills et al., 2011; Teng et al., 2017). The MUFA were not affected by the inclusion of bovine milk in buffalo milk (P > 0.05), except for palmitoleic acid (C16:1, P = 0.040), which decreased linearly. Some of the C16:0 originates via *de novo* synthesis in the mammary gland, with  $\beta$ -hydroxybutyrate as the precursor (Bauman & McGuire, 2011). The activity of  $\Delta 9$ -desaturase enzyme is higher in the mammary gland of buffaloes when compared with the mammary gland of cows (Fernandes et al., 2007). Thus, the higher C16:0 concentration in buffalo milk, associated with the higher activity of  $\Delta 9$ -desaturase in the buffalo mammary gland, may explain these results.

The addition of cow milk did not affect (P > 0.05) the PUFA composition of buffalo mozzarella cheese (Table 3). It is possible to find some reports about the effects of ingestion of fatty acids by humans. There is convincing scientific evidence that some PUFA positively affects health (Glick & Fischer, 2013), among them, the rumenic acid (C18:2 *cis-9, trans-11*), the main

**Table 2.** Chemical composition of buffalo mozzarella frauded with cow milk.

Variables	Cow milk level (%)						P-value <sup>1</sup>		
	0	10	20	30	40	50	L	Q	C
FDM (%) <sup>2</sup>	61.0	54.0	52.2	54.0	<b>51.3</b>	<b>51.4</b>	0.122	<b>0.003</b>	0.097
<b>Protein (%)<sup>3</sup></b>	<b>23.8</b>	<b>22.3</b>	<b>22.3</b>	<b>20.8</b>	<b>20.8</b>	<b>20.2</b>	<b>0.000</b>	0.383	0.809
Moisture (%)	46.3	45.6	46.4	48.1	46.6	47.5	0.206	0.885	0.527
Ash (%)	2.89	2.90	3.04	2.98	3.12	2.85	0.375	0.425	0.212
TS (%)	53.7	54.4	53.6	51.9	53.4	52.5	0.206	0.885	0.527

<sup>1</sup>P-value (p>0.05); L, Q, and C: linear, quadratic, and cubic effects, respectively; <sup>2</sup> $\hat{y} = 0.0067x^2 - 0.4705x + 59.578$  (R<sup>2</sup> = 0.86); <sup>3</sup> $\hat{y} = -0.0688x + 23.424$  (R<sup>2</sup> = 0.92); FDM = Fat in dry matter; TS = total solids.

**Table 3.** Fatty acid composition of buffalo mozzarella frauded with cow milk (mg.g<sup>-1</sup>).

Fatty acid (mg.g <sup>-1</sup> )	Cow milk level (%)						P-Value <sup>1</sup>		
	0	10	20	30	40	50	L	Q	C
Saturated									
<b>C4:0<sup>2</sup></b>	<b>60.57</b>	<b>63.48</b>	<b>55.92</b>	<b>42.67</b>	<b>54.51</b>	<b>45.77</b>	<b>0.012</b>	0.647	0.501
C6:0	27.00	26.95	26.48	21.97	28.24	26.35	0.767	0.236	0.786
<b>C8:0<sup>3</sup></b>	<b>11.15</b>	<b>11.77</b>	<b>11.56</b>	<b>10.78</b>	<b>13.44</b>	<b>13.22</b>	<b>0.006</b>	0.144	0.793
<b>C10:0<sup>4</sup></b>	<b>19.17</b>	<b>19.19</b>	<b>21.21</b>	<b>20.00</b>	<b>24.06</b>	<b>25.28</b>	<b>0.002</b>	0.240	0.935
C12:0	26.27	25.67	27.65	26.18	25.95	30.09	0.699	0.646	0.551
C14:0	138.79	140.39	136.35	127.31	134.13	129.16	0.080	0.989	0.832
C15:0	17.35	16.88	17.03	15.67	17.41	16.41	0.202	0.636	0.461
<b>C16:0<sup>5</sup></b>	<b>405.08</b>	<b>413.63</b>	<b>386.61</b>	<b>337.10</b>	<b>372.16</b>	<b>341.28</b>	<b>0.014</b>	0.783	0.538
C17:0	10.54	11.42	10.68	9.85	10.91	7.44	0.060	0.144	0.532
C18:0	105.83	114.19	106.34	109.54	134.86	106.36	0.911	0.684	0.845
C20:0	2.00	1.63	1.79	1.90	2.15	1.67	0.805	0.957	0.063
<b>C22:0<sup>6</sup></b>	<b>0.84</b>	<b>0.82</b>	<b>0.80</b>	<b>0.76</b>	<b>0.69</b>	<b>0.67</b>	<b>0.023</b>	0.660	0.598
Monounsaturated									
C14:1	6.67	6.41	6.18	5.62	5.93	4.82	0.107	0.640	0.569
C15:1	3.68	3.74	3.60	3.56	3.96	3.07	0.328	0.343	0.274
<b>C16:1<sup>7</sup></b>	<b>18.08</b>	<b>17.25</b>	<b>17.98</b>	<b>13.56</b>	<b>14.51</b>	<b>14.73</b>	<b>0.040</b>	0.665	0.345
C17:1	4.93	5.41	5.40	5.04	5.57	4.45	0.514	0.152	0.712
C18:1t11	21.22	19.85	18.75	16.29	19.89	17.61	0.108	0.287	0.656
C18:1c9	175.99	199.65	187.03	185.72	223.45	171.90	0.703	0.239	0.391
Polyunsaturated									
C18:2n-6	4.83	6.25	5.59	4.70	6.05	5.43	0.685	0.594	0.194
C18:2c9.t11	6.84	6.63	6.25	6.07	7.10	6.14	0.746	0.798	0.583
C18:2t10.c12	3.22	2.97	2.95	2.84	2.87	2.74	0.140	0.672	0.671
C18:3n-6	0.14	0.22	0.15	0.15	0.20	0.17	0.502	0.594	0.315
C18:3n-3	3.31	3.80	2.85	2.86	3.11	2.79	0.079	0.782	0.582

<sup>1</sup> P-value = p > 0.05; L, Q, and C: linear, quadratic, and cubic effects, respectively. <sup>2</sup> $\hat{y} = -0.3262x + 61.974$  (R<sup>2</sup> = 0.56); <sup>3</sup> $\hat{y} = 0.0417x + 10.945$  (R<sup>2</sup> = 0.51); <sup>4</sup> $\hat{y} = 0.1256x + 18.346$  (R<sup>2</sup> = 0.81); <sup>5</sup> $\hat{y} = -1.4083x + 411.19$  (R<sup>2</sup> = 0.68); <sup>6</sup> $\hat{y} = -0.0037x + 0.8548$  (R<sup>2</sup> = 0.95); <sup>7</sup> $\hat{y} = -0.084x + 18.118$  (R<sup>2</sup> = 0.63).

conjugated linoleic acid isomer (CLA), playing a significant role (Rodríguez-Alcalá et al., 2014). In this study, rumenic acid varied between 68-71% of the total CLA. Previous work indicated that the C18:2 *cis-9 trans-11* content in mozzarella cheese can account for more than 80% of the CLA in milk (Romano et al., 2011), but factors, such as milk origin and processing, may determine variations (Martini et al., 2016; Ruiz et al., 2016).

However, the controversy regarding the effects of SFA is evident due to the complexity of the interaction of fatty acids and other biomolecules present in milk (Gómez-Cortés et al., 2018). In this group, myristic (C14:0), palmitic (C16:0) and lauric (C12:0) acids, which have a hypercholesterolemic action, but C14:0 is the most active (Ulbricht & Southgate, 1991). Among these fatty acids, only C16:0 was affected (P = 00.014), decreasing with the inclusion of cow milk. In this sense, the origin of the milk (species) can explain the results observed in this work since buffaloes and cows were fed the same diet (*Brachiaria decumbens* and *B. ruziziensis* pasture).

### 3.3 Nutritional indices of cheeses

The inclusion of cow milk for the production of buffalo mozzarella cheese did not alter the sum of fatty acids and nutritional quality indices (P > 0.05), except for the cholesterol content

(Table 4). Linoleic acid (C18:2 n-6) and α-linolenic (C18:3 n-3) are precursors of several metabolites, such as eicosanoids, thromboxanes, prostacyclins, prostaglandins and leukotrienes, which are associated with immune and inflammatory responses (Ricciotti & FitzGerald, 2012; Samuelsson, 2012; Glick & Fischer, 2013). These fatty acids are known as precursors of important metabolites (Martini et al., 2016). Studies indicate that diets rich in linoleic acid can favour the formation of eicosanoids from arachidonic acid, which, in turn, should favour the synthesis of inflammatory eicosanoids (Glick & Fischer, 2013; Harnack et al., 2009). Contrariwise, the ingestion of foods rich in n-3 fatty acids causes an increase in the formation of docosahexaenoic acid (DHA) and eicosapentaenoic acid, forming anti-inflammatory eicosanoids (Ricciotti & FitzGerald, 2012; Samuelsson, 2012). The scientific community has been studying the relationship between the intake of essential fatty acids and cardiac diseases (Simopoulos, 2008; Harnack et al., 2009; Russo, 2009; Simopoulos, 2016; Sheppard & Cheatham, 2018). In general, the n-6/n-3 ratio intake ranging from 1:1 to 10:1 (Chardigny et al., 2001; Harnack et al., 2009; Russo, 2009). In an influential article, (Masters (1996) indicated the ideal ratio as 2-3:1, because this ratio preferentially favours the conversion of α-linolenic acid to DHA, which leads to the balanced intake of these fatty acids. Thus, the data observed in this study put the cheeses studied among the foods with adequate n-6/n-3 ratios (Marshall & van der Meij, 2018).

**Table 4.** Effect of the inclusion of cow milk to buffalo milk on the nutritional quality of buffalo mozzarella.

Fatty acid	Cow milk level (%)						P-Value <sup>1</sup>		
	0	10	20	30	40	50	L	Q	C
	Sum (mg.g <sup>-1</sup> )								
Σ SFA <sup>2</sup>	824.92	846.27	802.65	718.06	799.66	735.12	0.058	0.832	0.664
Σ MUFA <sup>3</sup>	230.58	252.31	238.95	229.79	273.31	216.59	0.907	0.299	0.429
Σ PUFA <sup>4</sup>	18.78	20.39	17.79	17.15	19.94	17.28	0.475	0.879	0.814
Σ CLA <sup>5</sup>	10.32	9.59	9.19	8.90	9.97	8.88	0.492	0.730	0.534
Σ <i>Trans</i> <sup>6</sup>	31.54	29.45	27.95	25.19	29.86	26.49	0.207	0.178	0.245
Σ n-6 <sup>7</sup>	4.97	6.47	5.75	4.85	6.25	5.60	0.564	0.566	0.169
Σ n-3 <sup>8</sup>	3.75	4.32	2.85	3.40	3.72	2.79	0.197	0.689	0.526
	Nutritional indexes (ratio)								
PUFA/SFA <sup>9</sup>	0.02	0.02	0.02	0.02	0.02	0.02	0.232	0.998	0.989
n-6/n-3 <sup>10</sup>	1.33	1.49	2.03	1.44	1.72	2.01	0.081	0.306	0.514
DFA (mg.g <sup>-1</sup> ) <sup>11</sup>	355.18	386.89	363.08	356.49	403.28	340.22	0.198	0.527	0.059
Rumenic/ΣCLA <sup>12</sup>	2.10	2.08	2.06	1.82	1.99	1.97	0.346	0.450	0.089
Vaccenic/ΣTrans <sup>13</sup>	0.67	0.67	0.67	0.65	0.67	0.66	0.531	0.274	0.280
Cholesterol (mg.100 g <sup>-1</sup> ) <sup>14</sup>	<b>67.0</b>	<b>74.6</b>	<b>77.1</b>	<b>82.1</b>	<b>82.6</b>	<b>88.4</b>	<b>0.014</b>	0.154	0.216

<sup>1</sup>P-Value = p > 0.05; L, Q, and C: linear, quadratic, and cubic effects, respectively; <sup>2</sup>Sum of the saturated fatty acid; <sup>3</sup>Sum of the monounsaturated fatty acid; <sup>4</sup>Sum of the polyunsaturated fatty acid; <sup>5</sup>sum of the conjugated linoleic acid; <sup>6</sup>Sum of the *trans* fatty acid; <sup>7</sup>Sum of the omega-6; <sup>8</sup>Sum of the omega-3; <sup>9</sup>Polyunsaturated fatty acid/Saturated fatty acid ratio; <sup>10</sup>Omega-6, and omega-3 ratio; <sup>11</sup>Desirable fatty acid; <sup>12</sup>Rumenic acid and total conjugated fatty acid ratio; <sup>13</sup>Vaccenic fatty acid and total *trans* ratio; <sup>14</sup> $\hat{y} = 0.3871x + 68.95$  (R<sup>2</sup> = 0.95).

The rumenic acid/ΣCLA ratio did not change with the increase of cow milk in the buffalo milk for the production of mozzarella, remaining around 2 because the respective fatty acids did not change. These are the same for the total *trans*/total *trans* fatty acid ratio (Table 3). However, there was an increasing effect (P < 0.05) in the cheese mozzarella with the inclusion of cow milk (Table 3). With the inclusion of cow milk into mozzarella processing, the cholesterol content increased to 32%. Buffalo milk contains less cholesterol compared with cow milk (Pignata et al., 2014; Manuelian et al., 2017), and the fraud of mozzarella with the inclusion of cow milk to the mixture and consequent increase in the content of cholesterol, altered an important nutritional parameter for consumers of animal products. Buffalo milk fraud is being fought on a legal basis in many countries, especially in Europe (European Union, 1996; Russo et al., 2012) and frequently occurs despite advanced food fraud technologies (Zarei et al., 2016). Many techniques exist to detect fraud in milk and buffalo derivatives (Cozzolino et al., 2002; Enne et al., 2005; Feligini et al., 2005; Czerwenka et al., 2010; Pesic et al., 2011; Poonia et al., 2017), but they are expensive, time-consuming and require skilled technicians, and safety when working with reagents, but these techniques are accurate (Roncada et al., 2012). Thus, the use of analytical techniques that are cheaper and precede proper quantitative techniques may help to combat fraud of buffalo milk derivatives, due to the possibility of increasing the number of samples evaluated. The results of the current study showed an increase in the amount of cholesterol in buffalo mozzarella, due to the inclusion of cow milk. Since the cholesterol content is lower in buffalo milk than cow milk (Zotos & Bampidis, 2014; Manuelian et al., 2017), being a species-specific parameter, samples that present a non-standard cholesterol content of the species should be sent for qualitative and confirmatory tests (Roncada et al., 2012; Zarei et al., 2016).

## 4 Conclusion

The addition of cow milk to buffalo milk for mozzarella production altered the content of short-chain SFA and the cholesterol content, thereby modifying the nutritional indices.

## References

- Bannon, Cecil D., Breen, Geoffrey J., Craske, John D., Hai, Ngo Trong, Harper, N. L., & O'Rourke, K. L. (1982). Analysis of fatty acid methyl esters with high and reliability Accuracy. III. Literature review of and investigations into the development of rapid procedures for the methoxide-catalysed methanolysis of fats and oils. *Journal of Chromatography A*, 247, 71-89. [http://dx.doi.org/10.1016/S0021-9673\(00\)84857-8](http://dx.doi.org/10.1016/S0021-9673(00)84857-8).
- Bauer, L. C., Santana, D. A., Macedo, M. S., Torres, A. G., Souza, N. E., & Simionato, J. I. (2014). Method validation for simultaneous determination of cholesterol and cholesterol oxides in milk by RP-HPLC-DAD. *Journal of the Brazilian Chemical Society*, 25, 161-168. <http://dx.doi.org/10.5935/0103-5053.20130283>.
- Bauman, D. E. M. A., & McGuire, K. J. H. (2011). Mammary gland, milk biosynthesis and secretion. In J. W. Fuquay (Ed.), *Encyclopedia of dairy sciences* (2nd ed., pp. 352-358). USA: Elsevier Inc.
- Bergamo, P., Fedele, E., Iannibelli, L., & Marzillo, G. (2003). Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. *Food Chemistry*, 82(4), 625-631. [http://dx.doi.org/10.1016/S0308-8146\(03\)00036-0](http://dx.doi.org/10.1016/S0308-8146(03)00036-0).
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917. <http://dx.doi.org/10.1139/o59-099>. PMID:13671378.
- Bondan, C., Folchini, J. A., Noro, M., Quadros, D. L., Machado, K. M., & González, F. H. D. (2018). Milk composition of Holstein cows: a retrospective study. *Ciência Rural*, 48(12), 1-8. <http://dx.doi.org/10.1590/0103-8478cr20180123>.
- Bontempo, L., Barbero, A., Bertoldi, D., Camin, F., Larcher, R., Perini, M., Sepulcri, A., Zicarelli, L., & Piasentier, E. (2019). Isotopic and elemental profiles of Mediterranean buffalo milk and cheese and

- authentication of Mozzarella di Bufala Campana PDO: an initial exploratory study. *Food Chemistry*, 285:316-323. <http://dx.doi.org/10.1016/j.foodchem.2019.01.160>.
- Boro, P., Debnath, J., Kumar Das, T., Naha, B. C., Debarma, N., Deabbarma, P., Debbarma, C., Devi, L. S. B. & Devi, T. G. (2018). Milk composition and factors affecting it in dairy buffaloes: a review. *Journal of Entomology and Zoology Studies JEZS*, 340, 340-343.
- Brasil. Ministério da Agricultura (2018). *Manual de Métodos Oficiais para Análise de Alimentos de Origem Animal* (1ª ed.) Brasília: MAPA.
- Calandrelli, M. (2007). *Manual on the production of traditional buffalo mozzarella cheese*. Rome: FAO.
- Chardigny, J. M., Bretillon, L., & Sébédo, J.-L. (2001). New insights in health effects of trans  $\alpha$ -linolenic acid isomers in humans. *European Journal of Lipid Science and Technology*, 103(7), 478-482. [http://dx.doi.org/10.1002/1438-9312\(200107\)103:7<478::AID-EJLT478>3.0.CO;2-A](http://dx.doi.org/10.1002/1438-9312(200107)103:7<478::AID-EJLT478>3.0.CO;2-A).
- Correddu, F., Serdino, J., Manca, M. G., Cosenza, G., Pauciullo, A., Ramunno, L., & Macciotta, N. P. P. (2017). Use of multivariate factor analysis to characterize the fatty acid profile of buffalo milk. *Journal of Food Composition and Analysis*, 60, 25-31. <http://dx.doi.org/10.1016/j.jfca.2017.03.008>.
- Cozzolino, R., Passalacqua, S., Salemi, S., & Garozzo, D. (2002). Identification of adulteration in water buffalo mozzarella and in ewe cheese by using whey proteins as biomarkers and matrix-assisted laser desorption / ionization mass spectrometry. *Journal of Mass Spectrometry*, 37(9), 985-991. <http://dx.doi.org/10.1002/jms.358>.
- Czerwenka, C., Lukáš, M., & Lindner, W. (2010). Detection of the adulteration of water buffalo milk and mozzarella with cow's milk by liquid chromatography – mass spectrometry analysis of b-lactoglobulin variants. *Food Chemistry*, 122(3), 901-908. <http://dx.doi.org/10.1016/j.foodchem.2010.03.034>.
- Dal Bosco, C., Panero, S., Navarra, M. A., Tomai, P., Curini, R., & Gentili, A. (2018). Screening and assessment of low-molecular-weight biomarkers of milk from cow and water buffalo: an alternative approach for the rapid identification of adulterated water buffalo mozzarellas. *Journal of Agricultural and Food Chemistry*, 66(21), 5410-5417. <http://dx.doi.org/10.1021/acs.jafc.8b01270>. PMID:29746108.
- Dalmaso, A., Civera, T., La Neve, F., & Bottero, M. T. (2011). Simultaneous detection of cow and buffalo milk in mozzarella cheese by Real-Time PCR assay. *Food Chemistry*, 124(1), 362-366. <http://dx.doi.org/10.1016/j.foodchem.2010.06.017>.
- Enne, G., Elez, D., Fondrini, F., Bonizzi, I., Feligini, M., & Aleandri, R. (2005). High-performance liquid chromatography of governing liquid to detect illegal bovine milk's addition in water buffalo Mozzarella: comparison with results from raw milk and cheese matrix. *Journal of Chromatography. A*, 1094(1-2), 169-174. <http://dx.doi.org/10.1016/j.chroma.2005.09.004>. PMID:16257304.
- European Union. (1996). *Indication whose name has already been registered as a designation of origin or the protected geographical indication may not be registered where, in the light of a trade*. Bruxelles: European Commission.
- European Union. (2017). *Monthly summary of articles on food fraud and adulteration*. Bruxelles: European Commission.
- Farag, R. S., Hewedi, M. M., Abo-Raya, S. H., & Khalifa, H. H. (1984). Detection of cow's milk admixture to buffalo's milk. *Journal of the American Oil Chemists' Society*, 61(5), 913-916. <http://dx.doi.org/10.1007/BF02542165>.
- Feligini, M., Bonizzi, I., Curik, V. C., Parma, P., Greppi, G. F., & Enne, G. (2005). Detection of adulteration in Italian mozzarella cheese using mitochondrial DNA templates as biomarkers. *Food Technology and Biotechnology*, 43, 91-95.
- Feltes, G. L., Michelotti, V. T., Prestes, A. M., et al. (2016). *Milk production and percentages of fat and protein in Holstein breed cows raised in Rio Grande do Sul, Brazil*. 700-706
- Fernandes, S. A. A., Mattos, W. R. S., Matarazzo, S. V., Tonhati, H., Gama, M. A. S., & Lanna, D. P. D. (2007). Total fatty acids in murrha buffaloes milk on commercial farms in Brazil. *Italian Journal of Animal Science*, 6(Suppl 2), 1063-1066. <http://dx.doi.org/10.4081/ijas.2007.s2.1063>.
- Food and Agriculture Organization – FAO. (2019). *Dairy market review - overview of global dairy market developments in 2018*. Rome: FAO.
- Gagliostro, G. A., Negrette, M. S., Sager, G., Castelli, L., Antonacci, L. E., Raco, F., Gallelo, L., Rodríguez, M. A., Cañameras, C., Zampatti, M. L., & Bernal, C. (2015). Milk fatty acid profile from grazing buffaloes fed a blend of soybean and linseed oil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 67(3), 927-934. <https://doi.org/10.1590/1678-4162-7811>.
- Glick, N. R., & Fischer, M. H. (2013). The role of essential fatty acids in human health. *Journal of Evidence-Based Complementary & Alternative Medicine*, 18(4), 268-289. <http://dx.doi.org/10.1177/2156587213488788>.
- Golay, P. A., Moulin, J., Alewijn, M., Braun, U., Choo, L. F., Crujisen, H., Delmonte, P., Fontecha, J., Holroyd, S., Hostetler, G., Lacoste, E., Lehmann, C., Nagelholt, L., Phillips, S., Ritvanen, T., Rizzo, A., Shimelis, O., Srigley, C., Sullivan, D., & Trossat, P. (2016). Determination of labeled fatty acids content in milk products, infant formula, and adult/pediatric nutritional formula by capillary gas chromatography: Collaborative study, final action 2012.13. *Journal of AOAC International*, 99(1), 210-222. <http://dx.doi.org/10.5740/jaoacint.15-0140>. PMID:26864245.
- Gómez-Cortés, P., Juárez, M., & de la Fuente, M. A. (2018). Milk fatty acids and potential health benefits: An updated vision. *Trends in Food Science & Technology*, 81, 1-9. <http://dx.doi.org/10.1016/j.tifs.2018.08.014>.
- Gonçalves, B.-H. R. F., Silva, G. D. J., Conceição, D. G., Egitto, A. S., & Ferrão, S. P. B. (2017). Buffalo mozzarella chemical composition and authenticity assessment by electrophoretic profiling. *Semina: Ciências Agrárias*, 38(4), 1841-1851. <http://dx.doi.org/10.5433/1679-0359.2017v38n4p1841>.
- Gunning, Y., Fong, L. K. W., Watson, A. D., Philo, M., & Kemsley, E. K. (2019). Quantitative authenticity testing of buffalo mozzarella via  $\alpha$ 1-Casein using multiple reaction monitoring mass spectrometry. *Food Control*, 101, 189-197. <http://dx.doi.org/10.1016/j.foodcont.2019.02.029>.
- Hansen, P. W., & Holroyd, S. E. (2019). Development and application of Fourier transform infrared spectroscopy for detection of milk adulteration in practice. *International Journal of Dairy Technology*, 72:321-331. <http://dx.doi.org/10.1111/1471-0307.12592>.
- Harnack, K., Andersen, G., & Somoza, V. (2009). Quantitation of alpha-linolenic acid elongation to eicosapentaenoic and docosahexaenoic acid as affected by the ratio of n6/n3 fatty acids. *Nutrition & Metabolism*, 6(1), 1-11. <http://dx.doi.org/10.1186/1743-7075-6-8>. PMID:19228394.
- Ilić, K., Jakovljević, E., & Skodrić-Trifunović, V. (2011). Social-economic factors and irrational antibiotic use as reasons for antibiotic resistance of bacteria causing common childhood infections in primary healthcare. *European Journal of Pediatrics*, 171(5), 767-777. <http://dx.doi.org/10.1007/s00431-011-1592-5>.
- Italy. (2003, November 6). *Disciplinare di produzione della Denominazione di Origine Protetta "Mozzarella di Bufala Campana"*. Allegato al Decreto del Ministero delle Politiche Agricole e Forestali del 18 settembre 2003 (G.U. n. 258 del 6.11.2003). *Gazzetta Ufficiale*.
- Locci, F., Ghiglietti, R., Francolino, S., Iezzi, R., Oliviero, V., Garofalo, A., Mucchetti, G. (2008). Detection of cow milk in cooked buffalo

- Mozzarella used as Pizza topping. *Food Chemistry*, 107(3), 1337-1341. <http://dx.doi.org/10.1016/j.foodchem.2007.09.040>.
- Lopez, C., Briard-Bion, V., Ménard, O., Beaucher, E., Rousseau, F., Fauquant, J., Leconte, N., & Robert, B. (2011). Fat globules selected from whole milk according to their size: different compositions and structure of the biomembrane, revealing sphingomyelin-rich domains. *Food Chemistry*, 125(2), 355-368. <http://dx.doi.org/10.1016/j.foodchem.2010.09.005>.
- Lucey, J. A., Otter, D., & Horne, D. S. (2017). A 100-year review : progress on the chemistry of milk and its components 1. *Journal of Dairy Science*, 100(12), 9916-9932. <http://dx.doi.org/10.3168/jds.2017-13250>. PMID:29153180.
- Manuelian, C. L., Currò, S., Penasa, M., Cassandro, M., & De Marchi, M. (2017). Characterization of major and trace minerals, fatty acid composition, and cholesterol content of Protected Designation of Origin cheeses. *Journal of Dairy Science*, 100(5), 3384-3395. <http://dx.doi.org/10.3168/jds.2016-12059>. PMID:28237598.
- Marshall, S., & van der Meij, B. (2018). Fish and omega-3 intake and health in older people. *Maturitas*, 115, 117-118. <http://dx.doi.org/10.1016/j.maturitas.2018.04.002>. PMID:29681428.
- Martini, M., Altomonte, I., Santana, A. M. S., & Salari, F. (2016). Nutritional composition of four commercial cheeses made with buffalo milk. *Journal of Food and Nutrition Research*, 55, 256-262.
- Masters, C. (1996). Omega-3 fatty acids and the peroxisome. *Molecular and Cellular Biochemistry*, 165(2), 83-93. <http://dx.doi.org/10.1007/BF00229469>. PMID:8979256.
- Mills, S., Ross, R. P., Hill, C., Fitzgerald, G. F., & Stanton, C. (2011). Milk intelligence: mining milk for bioactive substances associated with human health. *International Dairy Journal*, 21(6), 377-401. <http://dx.doi.org/10.1016/j.idairyj.2010.12.011>.
- Nawaz, H., Yaqoob, M., Sarwar, M., Abdulla, M., Sultan, J. I., & Kan, B. B. (2009). Effect of feeding different sources of supplemental fat on the performance of lactating Nili-Ravi buffaloes. *The Indian Journal of Animal Sciences*, 79(2), 188-192.
- Ottavian, M., Facco, P., Barolo, M., Berzaghi, P., Segato, S., Novelli, E., & Balzan, S. (2012). Near-infrared spectroscopy to assist authentication and labeling of Asiago d'allevato cheese. *Journal of Food Engineering*, 113(2), 289-298. <http://dx.doi.org/10.1016/j.jfoodeng.2012.05.037>.
- Pegolo, S., Stocco, G., Mele, M., Schiavon, S., Bittante, G., & Cecchinato, A. (2017). Factors affecting variations in the detailed fatty acid profile of Mediterranean buffalo milk determined by 2-dimensional gas chromatography. *Journal of Dairy Science*, 100(4), 2564-2576. <http://dx.doi.org/10.3168/jds.2016-11696>. PMID:28189314.
- Penchev, P., Ilieva, Y., Ivanova, T., & Kalev, R. (2016). Fatty acid composition of buffalo and bovine milk as affected by roughage source - silage versus hay. *Emirates Journal of Food and Agriculture*, 28(4), 264. <http://dx.doi.org/10.9755/ejfa.2015-11-974>.
- Pesic, M., Barac, M., Vrvic, M., Ristic, N., Macej, O., & Stanojevic, S. (2011). Qualitative and quantitative analysis of bovine milk adulteration in caprine and ovine milks using native-PAGE. *Food Chemistry*, 125(4), 1443-1449. <http://dx.doi.org/10.1016/j.foodchem.2010.10.045>.
- Phogat, J. B., Pandey, A. K., & Singh, I. (2016). Seasonality in buffaloes reproduction. *International Journal of Plant, Animal, Environmental Sciences*, 6, 46-54.
- Pignata, M. C., Fernandes, S. A. A., Ferrão, S. P. B., Faleiro, A. S., & Conceição, D. G. (2014). Estudo comparativo da composição química, ácidos graxos e colesterol de leites de búfala e vaca. *Revista Caatinga*, 2125, 226-233.
- Pignata, M. C., Ferrão, S. P. B., Oliveira, C. P., Faleiro, A. S., Bonomo, R. C., Silva, W. S., Rodrigues, L. B., & Fernandes, S. A. A. (2015). Mechanical parameters of the mozzarella from buffalo with inclusion levels of the cow's milk: preliminary study at the lab scale. *Journal of Bioanalysis & Biomedicine*, 7(6), 191-196. <http://dx.doi.org/10.4172/1948-593X.1000143>.
- Poonia, A., Jha, A., Sharma, R., Singh, H. B., Rai, A. K., & Sharma, N. (2017). Detection of adulteration in milk: a review. *International Journal of Dairy Technology*, 70(1), 23-42. <http://dx.doi.org/10.1111/1471-0307.12274>.
- R Core Team. (2015). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ramesha, K. P., Rao, A., Basavaraju, M., Alex, R., Katakataware, M. A., Jayakumar, S., & Varalakshmi, S. (2016). Genetic variants of  $\beta$ -casein in cattle and buffalo breeding bulls in Karnataka state of India. *Indian Journal of Biotechnology*, 15, 178-181.
- Ricciotti, E., & FitzGerald, G. A. (2012). Prostaglandins and Inflammation. *Art Thromb Vas Biol*, 31(5), 986-1000. <http://dx.doi.org/10.1161/ATVBAHA.110.207449>.
- Rodríguez-Alcalá, L. M., Alonso, L., & Fontecha, J. (2014). Stability of fatty acid composition after thermal, high pressure, and microwave processing of cow milk as affected by polyunsaturated fatty acid concentration. *Journal of Dairy Science*, 97(12), 7307-7315. <http://dx.doi.org/10.3168/jds.2013-7849>. PMID:25459902.
- Romano, R., Giordano, A., Chianese, L., Addeo, F., & Musso, S. S. (2011). Journal of Food Composition and Analysis Triacylglycerols, fatty acids and conjugated linoleic acids in Italian Mozzarella di Bufala Campana cheese. *Journal of Food Composition and Analysis*, 24(2), 244-249. <http://dx.doi.org/10.1016/j.jfca.2010.10.004>.
- Roncada, P., Piras, C., Soggiu, A., Turk, R., Urbani, A., & Bonizzi, L. (2012). Farm animal milk proteomics. *Journal of Proteomics*, 75(14), 4259-4274. <http://dx.doi.org/10.1016/j.jprot.2012.05.028>. PMID:22641156.
- Ruiz, J. P. A., Alonzo, M. W., & Pertíñez, M. D. (2016). Conjugated linoleic acid of dairy foods is affected by cows' feeding system and processing of milk. *Scientia Agrícola*, 73(2), 103-108. <http://dx.doi.org/10.1590/0103-9016-2015-0051>.
- Russo, G. L. (2009). Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochemical Pharmacology*, 7(6), 937-946. <http://dx.doi.org/10.1016/j.bcp.2008.10.020>.
- Russo, R., Severino, V., Mendez, A., Lliberia, J., Parente, A., & Chambery, A. (2012). Detection of buffalo mozzarella adulteration by an ultra-high performance liquid chromatography tandem mass spectrometry methodology. *Journal of Mass Spectrometry*, 47(11), 1407-1414. <http://dx.doi.org/10.1002/jms.3064>. PMID:23147815.
- Sakaridis, I., Ganopoulos, I., Argiriou, A., & Tsafaris, A. (2013). High resolution melting analysis for quantitative detection of bovine milk in pure water buffalo mozzarella and other buffalo dairy products. *International Dairy Journal*, 28(1), 32-35. <http://dx.doi.org/10.1016/j.idairyj.2012.08.006>.
- Salman M, Khaskheli M, Israr-Ul-Haq, Talpur, A. R., Khuhro, A. P., Rauf, M., Hamid, H., & Aziz, A. (2014). Comparative studies on nutritive quality of buffalo and cow milk. *International Journal of Research in Applied Natural and Social Sciences*, 2, 2321-8851.
- Samuelsson, B. (2012). Role of basic science in the development of new medicines: Examples from the eicosanoid field. *The Journal of Biological Chemistry*, 287(13), 10070-10080. <http://dx.doi.org/10.1074/jbc.X112.351437>. PMID:22318727.
- Santiago-López, L., Aguilar-toalá, J. E., Hernández-mendoza, A., Vallejo-Cordoba, B., Liceaga, A. M., & González-Córdova, A. F. (2018). Invited review: bioactive compounds produced during

- cheese ripening and health effects associated with aged cheese consumption. *Journal of Dairy Science*, 101(5), 3742-3757. <http://dx.doi.org/10.3168/jds.2017-13465>. PMID:29477517.
- Shen, H., Xu, Z., Shen, Z., & Lu, Z. (2019). The Regulation of Ruminant Short-Chain Fatty Acids on the Functions of Rumen Barriers. *Frontiers in Physiology*, 10, 1-13. <http://dx.doi.org/10.3389/fphys.2019.01305>.
- Sheppard, K. W., & Cheatham, C. L. (2018). Omega-6/omega-3 fatty acid intake of children and older adults in the U.S.: dietary intake in comparison to current dietary recommendations and the Healthy Eating Index. *Lipids in Health and Disease*, 17(1), 1-12. <http://dx.doi.org/10.1186/s12944-018-0693-9>. PMID:29523147.
- Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine (Maywood, N.J.)*, 233(6), 674-688. <http://dx.doi.org/10.3181/0711-MR-311>. PMID:18408140.
- Simopoulos, A. P. (2016). An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*, 8(3), 1-17. <http://dx.doi.org/10.3390/nu8030128>. PMID:26950145.
- Talpur, F. N., Bhanger, M. I., Khooharo, A. A., & Memon, G. Z. (2008). Seasonal variation in fatty acid composition of milk from ruminants reared under the traditional feeding system of Sindh, Pakistan. *Livestock Science*, 118(1-2), 166-172. <http://dx.doi.org/10.1016/j.livsci.2008.04.008>.
- Teng, F., Wang, P., Yang, L., Ma, Y., & Day, L. (2017). Quantification of fatty acids in human, cow, buffalo, goat, yak, and camel milk using an improved one-step GC-FID method. *Food Analytical Methods*, 10(8), 2881-2891. <http://dx.doi.org/10.1007/s12161-017-0852-z>.
- Terramocchia, S., Bartocci, S., & Borghese, A. (2005). New acquisitions on the digestive physiology of the mediterranean Buffalo. In: A. Borghese (Ed.), *REU Technical Series* (pp. 161-172). Rome: FAO.
- Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *Lancet*, 338(8773), 985-992. [http://dx.doi.org/10.1016/0140-6736\(91\)91846-M](http://dx.doi.org/10.1016/0140-6736(91)91846-M). PMID:1681350.
- Zarei, M., Maktabi, S., Yousefvand, A., & Tajbakhsh, S. (2016). Fraud identification of undeclared milk species in composition of sheep yogurt and cheese using multiplex PCR assay. *Journal of Food Quality and Hazards Control*, 3, 15-19.
- Zotos, A., & Bampidis, V. A. (2014). Journal of food composition and analysis milk fat quality of greek buffalo (*Bubalus bubalis*). *Journal of Food Composition and Analysis*, 33(2), 181-186. <http://dx.doi.org/10.1016/j.jfca.2013.12.004>.