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## Chemical composition and lipid profile of mare colostrum and milk of the quarter horse breed

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<b>Abstract:</b>	The objective of this study was to characterize the chemical composition and lipid profile of colostrum and milk of purebred Quarter Horse mares. Thirty-four (34) purebred mares were selected, which were then separated into groups according to age, birth order and lactation stage. Colostrum samples were collected in the first six hours after delivery and milk samples from the 7 <sup>th</sup> postpartum day, with intervals of 14 days until the end of lactation. The samples were refrigerated and sent to the Milk Laboratory of the University ( Laboleite - UFRN ), where they were analyzed for chemical composition. Colostrum was assessed by refractometry. The lipid profile was determined by gas chromatography through a separation of methyl esters. The data were tabulated and submitted to descriptive statistics and analysis of variance by the F-Test, and the groups were compared by the Tukey test using a significance level of 5%. There was a high protein content and reduced lactose content for the colostrum of the Quarter Horse mares. The milk composition was not influenced by the mares' age. However, variations in the lactation stage and in the birth order of the Quarter Horse mares altered the milk's chemical composition. There is variation in the lipid composition of milk according to the lactation stage, without changing the characteristic profile of the mares' milk or diminishing the nutritional quality of the lipid fraction.
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31 **Abstract**

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The objective of this study was to characterize the chemical composition and lipid profile of colostrum and milk of purebred Quarter Horse mares. Thirty-four (34) purebred mares were selected, which were then separated into groups according to age, birth order and lactation stage. Colostrum samples were collected in the first six hours after delivery and milk samples from the 7<sup>th</sup> postpartum day, with intervals of 14 days until the end of lactation. The samples were refrigerated and sent to the Milk Laboratory of the University (*Laboleite - UFRN*), where they were analyzed for chemical composition. Colostrum was assessed by refractometry. The lipid profile was determined by gas chromatography through a separation of methyl esters. ~~The data were tabulated and submitted to descriptive statistics and analysis of variance by the F-Test, and the groups were compared by the Tukey test using a significance level of 5%. There~~ was a high protein content and reduced lactose content for the colostrum of the Quarter Horse mares. The milk composition was not influenced by the mares' age. However, variations in the lactation stage and in the birth order of the Quarter Horse mares altered the milk's chemical composition. There is variation in the lipid composition of milk according to the lactation stage, without changing the characteristic profile of the mares' milk or diminishing the nutritional quality of the lipid fraction.

**Keywords:** Unsaturated fatty acids, lactation stage, Equidae.



## 61 **Introduction**

62

63 A considerable number of horses have been bred in several countries around the world  
64 in order to produce milk [1] because of its nutritional and therapeutic properties. Mares' milk  
65 is consumed by 30 million people worldwide, and has been studied as a milk substitute in  
66 newborns and pre-mature humans [2], in addition to being frequently used as a dietary  
67 supplement for older adults, recovering patients, and mainly children allergic to cow milk [1].

68 On average, equine milk is composed of 6.5% lactose, 1.8% protein, 1.0% fat and 440

69 kcal/kg of energy [3]. It presents a desired protein profile in human food due to the whey:casein  
70 protein ratio and the spongiform structure of the micelles, which make it physiologically more  
71 digestible than cow milk [4]. The nutritional quality of the lipid fraction of equine milk is  
72 composed of small amounts of stearic and palmitic acids and high amounts of linoleic and  
73 linolenic acids [5], which has also supported indications for supplying equine milk to humans.

74 Age, birth order, body weight of the mares, diet, environmental conditions and lactation  
75 stage have an influence on the chemical composition of milk [5, 6]. In addition to these factors,  
76 race and genetics can change the composition of equine milk, especially the protein, fat and  
77 lactose levels [7]. Thus, the objective of this study was to characterize the chemical composition  
78 and lipid profile of colostrum and milk from purebred Quarter Horse mares of different ages,  
79 birth orders and lactation stages.

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81

## 82 **Materials and methods**

### 83 **Animal ethics and experimentation**

84 The trial was submitted to evaluation by the Ethics Committee on the Use of  
85 Animals at *UFRN* (protocol 062/2017), receiving approval registered under opinion number  
86 058.062/2017. All animal management practices followed the recommendations of the National  
87 Council for the Control of Animal Experimentation (*CONCEA*) for the protection of animals  
88 used for animal experimentation and other scientific purposes, in accordance with the  
89 provisions of Law No. 11,794, of 8 October 2008, of Decree No. 6,899, of July 15, 2009.

90

## 91 **Experimental animals, material sampling and laboratory analysis**

92

93 Thirty-four (34) purebred mares were selected from three different stud farms  
94 specialized in breeding and selection of Quarter Horses in Rio Grande do Norte state. The mares  
95 were separated into groups according to age, birth order and lactation stage. The collections  
96 took place between the months of July/2017 and September/2018.

97 Colostrum samples were collected after delivery, not exceeding six hours after the event,  
98 and were obtained by manual milking after cleaning the udder and stored in previously sterilized  
99 plastic bottles. The composition (fat, protein, casein, lactose, total solids and defatted dry  
100 extract), refractometry (Brix percentage determination) and lipid profile of the colostrum  
101 samples were analyzed.

102 Milk samples were collected from the 7<sup>th</sup> postpartum day with 14-day intervals, and thus  
103 continued until the end of lactation (180 days after delivery). The foal remained separated from  
104 the mare during the last two hours preceding the procedure on the day determined for collection  
105 in order to guarantee there being a sufficient volume of milk for sampling accumulated in the  
106 udder. The mares' udders were previously sanitized with a compress soaked with 70% alcohol,  
107 while the milker's hands were washed with clean water and neutral soap, dried with paper

108 towels and also sanitized with 70% alcohol. The first three jets of milk were discarded at the  
109 time of collection, and then the udder was fully milked, with the milk being placed in a glass  
110 container previously sterilized in an autoclave.

111 The samples were identified, placed in an isothermal box containing artificial ice (4 to  
112 8°C) and sent to the *UFRN* Milk Quality Laboratory (*LABOLEITE*). The samples were  
113 subjected to electronic analysis by infrared absorption in DairySpec FT Bentley equipment to  
114 determine the chemical composition of the milk and colostrum. Qualitative analysis of  
115 colostrum was performed using a portable optical refractometer for sugar (Kasvi<sup>®</sup>, model K52-  
116 032, with a measurement range of 0 to 32% Brix and minimum division of 0.2%) after  
117 calibrating it with distilled water, as recommended by the manufacturer. One drop of colostrum  
118 was placed on the refractometer prism with the sample at room temperature and homogenized,  
119 and then reading was conducted through the monocular lens. The result in Brix% was obtained  
120 by the separation between the light area and the dark area formed on the equipment display  
121 after perpendicular disposition of the equipment to light.

122 The milk samples were lyophilized and the fatty acid methyl esters were obtained by  
123 adapting the methodology proposed by Kramer [8] to analyze the lipid profile. Approximately  
124 0.8g of samples were weighed in glass tubes (16 x 150mm) with screw caps and septums in  
125 order to contain 15 to 30 mg of fat. Next, 2mL of hexane and 2mL of sodium methoxide (0.5M  
126 in methanol) were added to the tubes, followed by vortexing (30 seconds) and heating in a water  
127 bath (50°C for 10 minutes). The tubes were subsequently cooled in running water and 3 ml of  
128 acetyl chloride (5% in methanol) were added, which was again heated (80°C for 10 minutes).  
129 Then, 1 ml of hexane and 10 ml of 6% K<sub>2</sub>CO<sub>3</sub> were added, followed by vortexing for 1 minute  
130 and centrifugation (4,000 rpm for 2 minutes). The supernatant was transferred to 15mL Falcon  
131 tubes with approximately 1g of Na<sub>2</sub>SO<sub>4</sub> mixture (previously oven dried) and activated carbon

132 (1:1), followed by stirring (1 min.) and centrifugation (1 min. 4000 rpm). The supernatant was  
133 collected, transferred to an amber vial and then stored in a freezer at -20°C.

134 The separation of the methyl esters from fatty acids was performed in a gas  
135 chromatograph (Focus GC - Thermo Scientific) equipped with flame ionization detector (CG-  
136 DIC) and SPTM-2560 capillary column (100m x 0.25mm x 0.20 μm - Supelco). The analysis  
137 parameters were: injector temperature of 250°C; detector temperature of 280°C; and 30:1 split  
138 ratio. The oven temperature was initially set at 140°C, increasing at a heating rate of 1°C/min  
139 to 220°C; then remaining at that temperature for 25 minutes. Hydrogen gas was used as carrier  
140 gas at a flow rate of 1.5 mL/minute. The injections were performed in duplicates for each  
141 extraction and the injection volume was 1 μL. The identification of the fatty acid methyl esters  
142 was performed by comparing the peak retention times of the samples with the retention time of  
143 the esters of the reference standard (GLC-674, Nu-Chek Prep, Inc.), and the result was obtained  
144 through normalizing the areas with the results expressed as a percentage.

145 The atherogenicity index (AI) and thrombogenicity index (TI) were calculated using the  
146 equation described by Ulbricht and Southgate (1991):

$$147 \quad AI = (C12:0 + 4 \times C14:0 + C16:0) / [\Sigma MUFA + \Sigma(n-6) + \Sigma(n-3)]$$

$$148 \quad TI = (C14:0 + C16:0 + C18:0) / [0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n-6) + 3 \times \Sigma(n-3) + \Sigma(n-3) / \Sigma(n-6)]$$

149

## 150 **Statistical procedures**

151 Data were tabulated in spreadsheets and submitted to descriptive statistics and analysis

152 of variance by the F-test. The groups were divided according to age, birth order and lactation

153 stage, and were then compared using the Tukey test at a significance level of 5% for type I

154 error. Only the different lactation stages were compared for the lipid profile analysis of milk.

155 The statistical analyzes were performed using the SAS (Statistical Analysis System) statistical  
156 package, and the analysis of variance was performed according to the following model:

$$157 \quad Y_{ij} = \mu_i + group_j + residual_{ij}$$

158 In which:

159  $Y_{ij}$  = dependent variables;

160  $\mu_i$  = overall mean;

161  $group_j$  = effect of the  $j^{\text{th}}$  group (age, birth order and lactation stage) on dependent  
162 variables, being group 1 to 3;

163  $residual_{ij}$  = residual effect.

164

## 165 **Results and discussion**

166

167 The values verified for the colostrum composition (Table 1) confirm its nutritional  
168 richness, in which it is important to highlight the high percentage of protein found for the  
169 colostrum of Quarter Horse mares (18.06%) was higher than the average of 15% reported by  
170 Csapó et al. [9] for Hungarian Draught, Haflinger, Breton and Boulonnaise mares; and the 16%  
171 found by Pecka et al. [10] when they evaluated the colostrum of Arabian mares. The lactose  
172 content of the colostrum evaluated in this study also differed from those presented by other  
173 authors, such as: 3.4% cited by Salimei et al. [11]; 2.95% found by Pikul & Wójtowski [12];  
174 and 2.46% presented by Pecka et al. [10]. The results suggest that the colostrum of Quarter  
175 Horse mares may contain more protein and be less dense in energy when compared to other  
176 breeds.

177 The °Brix values obtained were high, following the high protein content of the evaluated  
178 material, since approximately 80% of the colostrum protein corresponds to immunoglobulins

179 [9]. The analyzed colostrum samples fall within the range of 20 to 30% of the refractive index  
 180 established by Nath et al. [13], which classifies them as good, and constitutes an aspect of  
 181 relevant importance for passive transfer of immunity and consequently for establishing the  
 182 newborn's health.

183

184 **Table 1.** Chemical composition of colostrum and milk from purebred Quarter Horse mares.

Variable (%)	Colostrum		Milk	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Fat	1.70 ± 1.05	61.31	0.73 ± 0.45	61.30
Total protein	18.06 ± 2.00	11.09	1.68 ± 0.26	15.44
Casein	13.66 ± 2.00	14.63	1.26 ± 0.20	16.20
Lactose	1.53 ± 0.53	34.81	6.62 ± 0.30	4.45
Total solids	20.49 ± 2.36	11.53	10.00 ± 0.59	5.90
DDE <sup>1</sup>	19.95 ± 1.72	8.66	9.30 ± 0.27	2.89
Brix%	27.40 ± 4.15	15.18	-	-

185 <sup>1</sup>Defatted dry extract; CV = coefficient of variation; SD: standard deviation.

186

187 When analyzing the chemical characterization of milk (Table 1), a reversal between the  
 188 protein and lactose concentrations is noticeable when the two milk secretions (colostrum and  
 189 milk) are compared. However, as lactose does not have as high concentrations in milk as protein  
 190 does in colostrum, the levels of total solids and defatted dry milk extract are considerably lower  
 191 than those observed for colostrum.

192 In studies conducted with Quarter Horse mares, Gibbs et al. [14] and Burns et al. [15]  
 193 reported a variation of 1.8 to 2.9% for total milk protein, constituting values close to those  
 194 found in this study. Lactose is a component which naturally stands out in the composition of  
 195 equine milk [16], and the values verified remained in the average reported by the literature  
 196 (6.1 to 7.3%) for the most diverse breeds studied, as reported by Santos et al. [17], Reis et

197 al.[18], Pecka et al. [10], Markiewicz-Keszycka et al. [19], showing the preponderance of  
198 lactose as a source of carbohydrate in mares' milk [10].

199 The value regarding fat content found in this study is less than the range of 1.0-1.5%  
200 reported by Gibbs et al. [14] for Quarter Horse mares, and also below the average value of  
201 1.25% reported by Salamon et al. [5], but are higher than the 0.62% reported by Reis et al.  
202 [18] for milk from Mangalarga mares. Equine milk certainly has low levels of fat when  
203 compared to milk from other species [2]; however, the measurement of this component in  
204 mares' milk is surrounded by methodological details which are difficult to control and which  
205 translate into the high coefficient of variation (61.30%) presented in Table 1, and therefore  
206 deserve a brief discussion.

207 The small cistern of the mare's udder requires frequent milking and/or breastfeeding  
208 throughout the day, more specifically every 2 or 3 hours [20], and in addition to this  
209 particularity, an accumulation of milk in the breast socket, which requires a reasonable release  
210 of oxytocin for ejection [21]. When extrapolated to the sample collection methodology, these  
211 anatomical and physiological particularities reflect on the difficulty of completely emptying the  
212 udder, which is directly related to the fat content of milk [22], since the residual fraction milk  
213 is rich in fat. Therefore, it is possible that the low fat content of milk found in this study is not  
214 solely and exclusively explained by genetic variations, but also because there is not enough  
215 oxytocin release during the sample collection to remove the residual milk fraction, resulting in  
216 in low-fat samples.

217 There was a significant effect of the lactation stage on the fat, total protein and casein  
218 levels, with the effect of such variations also occurring on the defatted dry extract levels (Table  
219 2).

220

2 **Table 2.** Effect of the **lactation stage** on the milk composition of purebred Quarter Horse  
 222 mares.

Variable (%)	Days in lactation		
	7 - 60	61 - 120	121 - 180
Fat	0.61 ± 0.41 <sup>b</sup>	0.85 ± 0.54 <sup>a</sup>	0.70 ± 0.36 <sup>ab</sup>
Total protein	1.94 ± 0.31 <sup>a</sup>	1.69 ± 0.18 <sup>b</sup>	1.53 ± 0.18 <sup>c</sup>
Casein	1.47 ± 0.24 <sup>a</sup>	1.27 ± 0.14 <sup>b</sup>	1.15 ± 0.15 <sup>c</sup>
Lactose	6.62 ± 0.23	6.60 ± 0.30	6.64 ± 0.32
Total solids	10.11 ± 0.50	10.09 ± 0.73	9.86 ± 0.46
Defatted dry extract	9.49 ± 0.26 <sup>a</sup>	9.29 ± 0.25 <sup>b</sup>	9.20 ± 0.24 <sup>b</sup>

223 <sup>a, b, c</sup> Different letters on the same line indicate statistical difference by the Tukey test (p<0.05).

224

225 According to Markiewicz-Keszycka et al. [19], the evolution of lactation in mares leads  
 226 to producing a milk which is rich in lactose, but low in fat, protein and total solids. The fat  
 227 levels at the end of lactation in this study were higher than those found at the beginning;  
 228 however, they were lower than the 0.9% reported by Burns et al. [15] for milk from Quarter  
 229 Horse mares at 150 days of lactation, thus reaffirming the difficulty of completely emptying the  
 230 udder during collection and the permanence of alveolar milk in the mares evaluated in this  
 231 study. Regarding protein contents and their fractions, there was a gradual reduction during  
 232 lactation exactly as reported by Salimei and Fantuz [22]: a decrease of 20 to 25% of the total  
 233 protein between the 28<sup>th</sup> and 150<sup>th</sup> days of lactation, accompanied by a 20 to 30% decrease of  
 234 casein within the same period.

235 It is noteworthy that variations in the milk composition throughout lactation are  
 236 essential for adjustments in the nutritional management of foals given the early development  
 237 and rapid growth of horses at this stage of life (NRC, 1989). Add to this physiological nature,



238 the expressive and particular muscular development of the Quarter Horse breed, which certainly  
 239 requires an increase in protein intake so that there is no nutritional deficit or consequently losses  
 240 in animal performance.

241 There was a difference ( $p < 0.05$ ) for the lactose levels in the effects of the birth order on  
 242 the milk composition (Table 3), with a notable decrease in this component according to the  
 243 maturity of the glandular breast tissue. Similar behavior was observed for defatted dry extract,  
 244 most likely as a result of lactose variation, since this component is part of the dry extract.

245

246 **Table 3.** Effect of birth order on the milk composition of purebred Quarter Horse mares.

Variable (%)	Birth order					
	1	2	3	4	5	6
Fat	0.75 ± 0.52	0.73 ± 0.40	0.61 ± 0.42	0.84 ± 0.52	0.76 ± 0.39	0.76 ± 0.46
Prot	1.62 ± 0.14	1.62 ± 0.18	1.74 ± 0.33	1.61 ± 0.22	1.67 ± 0.23	1.74 ± 0.30
Cas	1.23 ± 0.12	1.22 ± 0.15	1.31 ± 0.24	1.21 ± 0.18	1.26 ± 0.19	1.30 ± 0.24
Lact	6.82 ± 0.24 <sup>a</sup>	6.70 ± 0.30 <sup>ab</sup>	6.67 ± 0.29 <sup>abc</sup>	6.48 ± 0.27 <sup>c</sup>	6.52 ± 0.26 <sup>bc</sup>	6.59 ± 0.29 <sup>bc</sup>
TS	9.98 ± 0.89	10.09 ± 0.42	9.86 ± 0.68	10.06 ± 0.62	10.01 ± 0.43	10.02 ± 0.45
DDE	9.38 ± 0.16 <sup>a</sup>	9.36 ± 0.20 <sup>ab</sup>	9.34 ± 0.30 <sup>ab</sup>	9.19 ± 0.05 <sup>ab</sup>	9.26 ± 0.22 <sup>ab</sup>	9.27 ± 0.29 <sup>b</sup>

247 <sup>a, b, c</sup> Different letters on the same line indicate statistical difference by the Tukey test ( $p < 0.05$ ).

248 Fat = fat; Prot = total protein; Cas = casein; Lact = lactose; TS = total solids; DDE = defatted dry extract.

249

250 In evaluating the milk production and composition of primiparous and multiparous  
 251 Quarter Horse mares, Pool-Anderson et al. [23] found greater production in multiparous mares,  
 252 but did not report any variation in the milk secretion composition, differently from what  
 253 occurred in this study. The fact that the lactose content is linked to the osmotic function and the  
 254 milk production of the mammary gland [24] generated contrary expectations to the observed  
 255 result, since young mares have lower production, which would lead to smaller lactose content  
 256 in the milk of low birth order mares.

257 Although low birth order mares are generally younger animals, there was no effect  
 258 ( $p>0.05$ ) of age on milk composition (Table 4).

259

260 **Table 4.** Effect of age on the milk composition of purebred Quarter Horse mares.

Variable (%)	Age (years)		
	3 - 5	6 - 10	11 - 19
Fat	0.77 ± 0.43	0.71 ± 0.47	0.76 ± 0.41
Total protein	1.63 ± 0.11	1.67 ± 0.27	1.71 ± 0.31
Casein	1.23 ± 0.08	1.26 ± 0.21	1.29 ± 0.25
Lactose	6.64 ± 0.29	6.59 ± 0.30	6.66 ± 0.29
Total solids	9.99 ± 0.72	9.97 ± 0.61	10.04 ± 0.45
Defatted dry extract	9.32 ± 0.20	9.29 ± 0.29	9.29 ± 0.26

261

262 Milk production in mammals increases with age until physiological maturity is reached  
 263 [25], when there is a tendency to a functional reduction of the mammary gland caused by aging  
 264 of the glandular tissue [26]. In addition to these circumstances is the dilution effect, in which the  
 265 highest production tends to dilute the dry extract components [24], and so variation in the milk  
 266 composition from mares of different ages was expected, especially between the evaluated  
 267 extremes, but this fact did not occur in this study.

268 Tables 5 and 6 show the lipid composition of colostrum and milk from mares and the  
 269 effect of the lactation stage on the lipid profile of milk, while Table 8 shows the relationships  
 270 between fatty acids.

271 Saturated fatty acids prevailed over unsaturated fatty acids in colostrum, with an  
 272 emphasis on palmitic (C16:0), capric (C10:0), lauric (C12:0) and myristic (C14:0) acids, which  
 273 increased the sum of saturated fatty acids (Table 5) and consequently the AGS:AGI ratio (Table  
 274 7). In the case of unsaturated acids, C18:1n9cis, C18:2n6cis and C18:3n3 stood out in

275 comparison to the others. Similar behaviors for such acids have been reported by Pikul et al.  
 276 [12] and Salamon et al. [5] for mares' colostrum; however, the values obtained in this study  
 277 were lower than those reported by these authors.

278

279 **Table 5.** Means and standard deviations of saturated fatty acids (peak area %) in Quarter Horse  
 280 mares' colostrum and milk.

Fatty acid	Colostrum	Milk	Milk - days in milk		
			7 – 60 d	61 – 120 d	121 – 180 d
C4:0	0.035±0.019	0.110±0.020	0.122±0.032 <sup>a</sup>	0.110±0.008 <sup>a</sup>	0.097±0.005 <sup>a</sup>
C6:0	0.142±0.052	0.244±0.055	0.292±0.045 <sup>a</sup>	0.255±0.017 <sup>ab</sup>	0.185±0.288 <sup>bc</sup>
C8:0	2.375±0.033	2.514±0.367	2.715±0.028 <sup>a</sup>	2.747±0.101 <sup>a</sup>	2.080±0.167 <sup>b</sup>
C10:0	10.098±0.066	5.674±1.260	6.192±0.972 <sup>a</sup>	6.685±0.045 <sup>a</sup>	4.145±0.191 <sup>b</sup>
C12:0	8.742±0.083	6.098±0.856	5.882±0.730 <sup>b</sup>	7.075±0.025 <sup>a</sup>	5.335±0.206 <sup>b</sup>
C14:0	6.612±0.243	6.560±0.639	6.095±0.561 <sup>b</sup>	7.312±0.123 <sup>a</sup>	6.272±0.109 <sup>b</sup>
C16:0	19.16±0.472	21.401±1.227	20.295±0.954 <sup>ab</sup>	22.437±0.162 <sup>a</sup>	21.485±1.229 <sup>ab</sup>
C18:0	4.282±0.548	1.626±0.362	1.867±0.286 <sup>a</sup>	1.827±0.080 <sup>a</sup>	1.185±0.051 <sup>b</sup>
∑AGS	53.652±1.364	46.491±3.818	45.322±3.632 <sup>b</sup>	50.795±0.522 <sup>a</sup>	43.357±0.628 <sup>b</sup>

281 <sup>a,b,c</sup> Means followed by different letters on the same line differ statistically from each other using the Tukey test at the  
 282 5% significance level. ∑AGS = sum of saturated fatty acids.

283

284 There was a higher prevalence of unsaturated fatty acids regarding the lipid profile of  
 285 mature milk when compared to colostrum; however, fatty acids (saturated and unsaturated)  
 286 which stood out in the lipid profile of colostrum also did so in milk, with the values obtained  
 287 herein within the threshold presented by Claey's et al. [27]. According to these authors, equine  
 288 milk in fact has higher proportions of unsaturated fatty acids when compared to milk from other  
 289 species (especially cattle), due to the minimal occurrence of biohydrogenation before the  
 290 absorption of unsaturated fatty acids.

291 There was an effect of the lactation stage on the lipid profile of mares' milk, with higher  
 292 values of saturated fatty acids being obtained in the middle third of lactation. This is similar to  
 293 the result presented by Orlandi et al. [28], especially in relation to C12:0, C14:0 and C16:0  
 294 acids. Pikul et al. [12] also found that saturated acids decrease with the lactation progress in  
 295 evaluating the lipid profile of mares' milk throughout lactation. There was a significant  
 296 reduction in linoleic acid in the final third of lactation for the most important unsaturated acids  
 297 in our study, while linolenic acid had its values depressed in the middle of lactation.  
 298 Consequently, the sum of polyunsaturated fatty acids was also lower for the middle third of  
 299 lactation.

300

301 **Tabela 6.** Means and standard deviations of unsaturated fatty acids (peak area %) in Quarter  
 302 Horse mares' colostrum and milk.

Fatty acid	Colostrom	Milk	Milk - days in milk		
			7 – 60 d	61 – 120 d	121 – 180 d
C14:1	0.180±0.020	0.554±0.209	0.352±0.106 <sup>b</sup>	0.497±0.005 <sup>b</sup>	0.812±0.037 <sup>a</sup>
C16:1	1.942±0.140	5.494±1.662	4.200±1.172 <sup>b</sup>	4.882±0.056 <sup>b</sup>	7.400±1.085 <sup>a</sup>
C18:1n9cis	15.700±0.186	16.688±0.971	16.64±1.405 <sup>a</sup>	16.600±0.367 <sup>a</sup>	16.822±1.144 <sup>a</sup>
C18:2n6cis	14.517±0.924	14.011±3.655	16.00±2.547 <sup>a</sup>	16.585±0.416 <sup>a</sup>	9.442±0.600 <sup>b</sup>
C20:1n9	0.470±0.014	0.260±0.029	0.282±0.017 <sup>a</sup>	0.245±0.005 <sup>a</sup>	0.252±0.043 <sup>a</sup>
C18:3n3	4.560±0.155	11.415±5.314	12.212±3.44 <sup>a</sup>	5.387±0.075 <sup>b</sup>	16.645±2.442 <sup>a</sup>
C20:2n6	0.520±0.012	0.278±0.043	0.302±0.005 <sup>a</sup>	0.322±0.009 <sup>a</sup>	0.220±0.008 <sup>b</sup>
C20:3n3	0.147±0.015	0.264±0.097	0.275±0.052 <sup>b</sup>	0.150±0.000 <sup>c</sup>	0.367±0.020 <sup>a</sup>
∑AGM	18.697±0.295	23.099±2.547	21.547±2.636 <sup>a</sup>	22.310±0.390 <sup>a</sup>	25.440±2.312 <sup>a</sup>
∑AGPI	19.980±1.041	26.105±3.236	28.985±0.985 <sup>a</sup>	22.560±0.443 <sup>b</sup>	26.770±2.971 <sup>a</sup>

303 <sup>a,b</sup> Means followed by different letters on the same line differ statistically from each other using the Tukey test at  
 304 the 5% level of significance. ∑AGM = sum of monounsaturated fatty acids; ∑AGP = sum of polyunsaturated fatty  
 305 acids.

306 The relationships between fatty acids presented in Table 7 show that even though some  
 307 values obtained in this study are lower than those contained in the literature, there is a relevant

308 nutritional advantage of the Quarter Horse mares' milk compared to milk of other species. The  
 309 high linoleic and linolenic acid concentrations, which have important biological functions [2]  
 310 bring the relationships between fatty acids close to the ideal value.

311

312 **Table 7.** Means and standard deviations of the relationships between saturated and unsaturated  
 313 fatty acids in colostrum and milk of Quarter Horse mares.

Fatty acid ratios	Colostrom	Milk	Milk - days in milk		
			7 – 60 d	61 – 120 d	121 – 180 d
$\sum n6$	15.177±0.938	14.351±3.697	16.390±2.538 <sup>a</sup>	16.947±0.410 <sup>a</sup>	9.717±0.605 <sup>b</sup>
$\sum n3$	4.707±0.167	11.668±5.382	12.505±3.489 <sup>a</sup>	5.537±0.075 <sup>b</sup>	16.962±2.373 <sup>a</sup>
n6:n3	3.225±0.155	1.692±1.122	1.437±0.603 <sup>b</sup>	3.062±0.065 <sup>a</sup>	0.577±0.044 <sup>b</sup>
AGS:AGI	1.390±0.080	0.954±0.153	0.902±0.136 <sup>b</sup>	1.130±0.0316 <sup>a</sup>	0.830±0.023 <sup>b</sup>
AI	1.415±0.085	1.105±0.173	1.010±0.150 <sup>b</sup>	1.312±0.034 <sup>a</sup>	0.995±0.040 <sup>b</sup>
TI	0.962±0.069	0.597±0.215	0.512±0.129 <sup>b</sup>	0.865±0.019 <sup>a</sup>	0.415±0.058 <sup>b</sup>

314 <sup>a,b</sup> Means followed by different letters on the same line differ statistically from each other using the Tukey test at  
 315 the 5% level of significance.  $\sum n6$  = sum of omega 6 fatty acids;  $\sum n3$  = sum of omega 3 fatty acids; n6:n3 = ratio  
 316 between omega 3 and omega 6 fatty acids; AGS:AGI = ratio between total saturated and unsaturated fatty acids;  
 317 AI = atherogenicity index; TI = thrombogenicity index.

318

319 Lower atherogenicity and thrombogenicity indices indicate the potential for atheroma  
 320 and thrombus prevention [29] and were very close to those presented by Pikul et al. [30] for  
 321 Konik mares. The reduction in indices in the final third of lactation was also observed by  
 322 Markiewicz-Kęszycka et al. [31].

323

## 324 Conclusions

325 Quarter Horse mares produced colostrum with higher protein content and lower lactose  
 326 content when compared to other breeds. The milk does not have a composition influenced by  
 327 the mares' age; however, the lactation stage and the birth order alter the chemical composition  
 328 of the milk of Quarter Horse mares. There is variation in the milk's lipid composition according

329 to the lactation stage without changing the characteristic profile of mares' milk and without  
330 harming the nutritional quality of the lipid fraction.

331

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336

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