# Differences in Blood and Milk Fatty Acid Profile of Primiparous and Multiparous Mediterranean Buffaloes Cows During Transition Period and Early Lactation

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**Abstract:** The objective of this study was to determine the differences in blood and fatty acid (FA) profile between primiparous and multiparous Mediterranean buffaloes cows from 28 days of expected calving date until 56 days in milk. Nine multiparous (MUL) and 7 primiparous (PRI) cows were used in the present study, animals grazed in *Brachiaria decumbens* and supplemented with 2.0 kg of concentrate/day. Blood and milk samples were collected once a week and data were submitted to repeated measures analysis using PROC MIXED of SAS. Multiparous cows presented higher concentrations of glucose and non-esterified FA during pre- and post-partum period, higher cholesterol during post-partum when compared to PRI. Milk yield and fat content were higher for MUL (6.44 kg/d and 7.89%) when compared to PRI (5.66 kg/d and 6.75%). Palmitic and palmitoleic FA were lower and C18:0, C18:1 *trans*-11 and C18:1 *cis*-9 FAwere higher in MUL cows. Multiparous buffalo cows demonstrated higher milk yield and fat content than PRI, and milk FA profile of MUL had higher amounts of C18:0 FA. Multiparous buffalos presented higher non-esterified fatty acid, which altered milk fat content, and higher milk yield than primiparous cows. Furthermore, multiparous cows presented a higher content of FA from incomplete biohydrogenation process.

**Keywords:** Beta-hydroxybutyrate, heifer, milk fat, milk yield, non-esterified fatty acid, saturated fatty acid.

## INTRODUCTION

Transition period represents the last 3 weeks before parturition to 3 weeks after parturition [1] in dairy cows. Despite buffalos seems to not have a tumultuous period as Holstein cows, great physiologic changes occur due to transition from the pregnant, non-lactating state to the non-pregnant, lactating state. Furthermore, heifers are less susceptible than multiparous cows to negative energy balance due to lower milk yield, body fat content and lower body reserves mobilization. Some blood metabolites may be used as an indirect measure of lipid mobilization from adipose tissue, as the non-esterified FA, which is greater in early lactation [2].

Studies related to the major milk FA are of interest because some specifically FA may be biological modifiers in humans [3] and may affect nutritional value and technological properties of milk [4]. The milk of buffalos cows differ from cows due to higher content of protein, fat and minerals as calcium and phosphorus [5]. Despite the increase of researches related to buffalo performance, no studies have been carried out to evaluate differences in metabolism and milk FA profile of multiparous and primiparous buffalo cows. The objective of this study was to determine the differences in blood metabolites and milk FA profile of primiparous and multiparous cows. Our hypothesis was that primiparous cows presented lower blood non-esterified FA concentration and lower content of saturated FA in milk than multiparous cows.

## MATERIAL AND METHODS

The experiment was conducted in "Setor de Bubalinocultura da Coordenadoria do Campus de Pirassununga" of University of São Paulo, from March until November. Seventeen Mediterranean buffalo cows, nine multiparous and seven primiparous, grazing in *Brachiaria brizantha* pasture were used in the experiment. The pasture presented 35.63% DM, 70.15% NDF, 39.12% ADF and 6.52% CP of average chemical composition. Animals were milked once a day at 7h00, with portable milk machine. During the milking animals received 2.0 kg of grain mixture, and the ration were formulated to achieve nutrient requirement of

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ISSN: 1927-5196 / E-ISSN: 1927-520X/15 

lactating cows with 690 kg of live weigh, milking 8 kg/d and 5.5% of fat content according to NRC [6].

**Table 1: Concentrate Composition During Experimental Period**

| Ingredient (%DM) | |
|---|---|
| Ground corn | 60.00 |
| Extrused soybean | 10.00 |
| Soybean meal | 24.45 |
| Urea | 1.00 |
| Dicalcium phosphate | 1.63 |
| Limestone | 0.63 |
| Salt | 0.70 |
| Mineral mix | 1.60 |
| Composition (%DM) | |
| Dry matter (%as fed) | 89.88 |
| Organic matter | 88.80 |
| Crude protein | 25.53 |
| Ether extract | 4.58 |
| Neutral detergent fiber | 15.55 |
| Acid detergent fiber | 7.4 |
| Lignin | 1.22 |

Containing per kilogram of mineral and vitamin mix: calcium - 120 g; phosphorus - 73 g; sulfur - 30 g; magnesium - 44 g; copper - 340 mg; zinc - 1.350 mg; manganese - 940 mg; cobalt - 3 mg; iodine - 16 mg; selenium - 10 mg; iron - 1.064 mg; vitamin A - 100.000 IU; vitamin D - 40.000 IU; vitamin E – 600 IU.

Blood samples were collected weekly from 28 days of expected calving date, at day of partum until 56 days of lactation. Sampling was performed after milking from all cows by puncture of coccigean vein, in sterile vacutainers, without clot activator (BD Vacutainer®, USA). The samples were centrifuged at 2000 x g for 15 minutes. The supernatant serum was transferred to plastic tubes, and stored at -20ºC until laboratory analysis.Blood serum was analyzed for glucose, total cholesterol, HDL-cholesterol, urea, ureic nitrogen, total protein, albumin, non-esterified FA andβ-hydroxy-butyrate (BHB). These analysis were performed using commercial kits (CELM®, São Caetano do Sul - Brazil) using enzymatic colorimetric endpoint method or kinetic. The reading was performed in automatic biochemistry analyzer (SBA-200 automatic biochemis-try – CELM®, São Caetano do Sul - Brazil).Analysis of non-esterified FA and β-hydroxybutyrate were made using commercial kits (RB 1007, Randox®) and reading performed by a micro-plate reader (ASYS Brand, Model Expert Plus UV-340- Analytic, São Paulo, Brazil).

Milk yield was recorded daily through weighing, and milk samples were collected once a week from partum until 56 days of lactation andanalyzed for fat (Funke-Gerber, Labortechnik GmbH, Berlin, Germany). The fat corrected milk (3.5% FCM) was calculated according to Sklan *et al*. [7], as follows: FCM = (0.0432 + 0.1625*milk fat content)*milk yield. Milk samples for analysis were obtained proportionally during milking. Fat extraction procedure of milk samples was performed according to Feng *et al*. [8] and separated fat was methylated and the methyl esters were formed according to Kramer *et al*. [9]. Fatty acids were quantified by gas chromatography (GC Shimatzu 2010 with automatic injection, Shimadzu Corporation, Kyoto - Japan) using an SP-2560 capillary column (100 m × 0.25 mm i.d. with 0.02-µm film thickness; Supelco, Bellefonte, PA). Similarly to Huang *et al*. [10], oven temperature was 70°C for 4 minutes, then increased 13°C minute$^{-1}$ to 175 ºC, and held for 27 minutes. A further increase of 4$^{o}$C minute$^{-1}$ was camed out until 215$^{o}$C, and maintained during 31 minutes. Hydrogen was used as the carrier gas and flowed 40 cm$^{3}$ s$^{-1}$. Four standards were used for FA identification: standard C4-C24 FA (TM 37; Supelco Sigma-Aldrich Group, Bellefonte, Pennsylvania, USA), vaccenic acid C18: 1 trans-11 FA (V038- 1G,; Supelco Sigma-Aldrich Group, Bellefonte, Pennsylvania, USA), conjugated linoleic acid (CLA) C18: 2 trans-10, cis-12 (UC-61M 100 mg;NU-CHEKPREP, Inc. Elysian, Minnesota, USA) and C18: 2 cis-9, trans-11 (UC-60M 100 mg; NU-CHEKPREP, Inc. Elysian, Minnesota, USA).

**Statistical Analyses**

The data were subjected to SAS (Version 9.1.3, SAS Institute, Cary, NC 2004), verifying the normality of residuals and homogeneity of variances by PROC UNIVARIATE. Data were analyzed by PROC MIXED according to the following model of time repeated measures [11]:

$$Y_{ijk} = \mu + Ci + t_j + C_i*T_j + a_k + e_{ijk}$$

Where: $Y_{ijk}$ = dependent variable; µ = overall mean; $C_i$ = fixed effect of animal class; $T_j$ = random effect of time; $C_i*T_j$) = interaction between class and time; $a_k$ =random effect of animal; $e_{ijk}$ = residual error. Autoregressive method was used to calculate covariance structure. The degrees of freedom were calculated according to the Satterthwaite's method (ddfm = satterth). Autoregressive 1 was the best covariance structure based upon the smallest Akaike's information criterion values. Other covariance structure

tested includes compound symmetry, heterogeneous compound symmetry, unstructured and heterogeneous autoregressive 1.

## RESULTS

### Blood Profile

Blood glucose and non-esterified FA concentrations were higher in MUL cows during pre- and post-partum (Table **2**). Total and HDL cholesterol were higher in MUL cows only during post-partum period. Total protein was lower in MUL cows. Time effect was observed for total cholesterol, ureic nitrogen and albumin during pre-partum period. Time effect was observed for total, HDL cholesterol, urea, ureic nitrogen, albumin, non-esterified FA during post-partum period. No interaction effect was observed in blood parameters.

**Table 2: Differences in Blood Profile between Primiparous and Multiparous Mediterranean Buffaloes Cows During Transition Period and Early Lactation**

| Item | Class | | SEM | *p-Value* | | |
|---|---|---|---|---|---|---|
| | Primiparous | Multiparous | | Class | Time | Interaction |
| *mg/dL* | | | | | | |
| Glucose | | | | | | |
| Pre-partum | 58.54 | 73.46 | 1.79 | 0.009 | 0.431 | 0.966 |
| Post-partum | 66.91 | 62.62 | 1.39 | 0.013 | 0.365 | 0.886 |
| Total cholesterol | | | | | | |
| Pre-partum | 70.82 | 71.65 | 2.04 | 0.677 | 0.031 | 0.876 |
| Post-partum | 78.91 | 83.52 | 1.26 | 0.004 | 0.001 | 0.752 |
| HDL-cholesterol | | | | | | |
| Pre-partum | 58.50 | 49.58 | 2.53 | 0.859 | 0.564 | 0.212 |
| Post-partum | 62.41 | 61.19 | 1.68 | 0.859 | 0.030 | 0.212 |
| Urea | | | | | | |
| Pre-partum | 19.95 | 18.53 | 1.01 | 0.651 | 0.537 | 0.977 |
| Post-partum | 28.50 | 31.78 | 0.91 | 0.375 | 0.001 | 0.593 |
| Ureic nitrogen | | | | | | |
| Pre-partum | 9.32 | 8.65 | 0.47 | 0.521 | 0.017 | 0.733 |
| Post-partum | 12.97 | 14.84 | 0.43 | 0.230 | 0.001 | 0.321 |
| *g/L* | | | | | | |
| Total protein | | | | | | |
| Pre-partum | 5.56 | 5.10 | 0.14 | 0.002 | 0.156 | 0.778 |
| Post-partum | 4.81 | 4.91 | 0.03 | 0.680 | 0.271 | 0.141 |
| Albumin | | | | | | |
| Pre-partum | 2.16 | 2.26 | 0.03 | 0.207 | 0.031 | 0.957 |
| Post-partum | 2.09 | 2.22 | 0.09 | 0.144 | 0.039 | 0.111 |
| *mmol/L* | | | | | | |
| Non-esterified fatty acid | | | | | | |
| Pre-partum | 0.563 | 0.710 | 0.03 | 0.032 | 0.156 | 0.778 |
| Post-partum | 0.692 | 0.823 | 0.02 | 0.029 | 0.012 | 0.937 |
| β -hydroxybutyrate | | | | | | |
| Pre-partum | 0.452 | 0.433 | 0.01 | 0.207 | 0.531 | 0.957 |
| Post-partum | 0.429 | 0.455 | 0.01 | 0.368 | 0.173 | 0.592 |

### Milk Yield and Fatty Acid Profile

Milk yield and fat content were higher for MUL than PRI. Furthermore, MUL cows presented lower C16:0, C16:1 and total C16:0 FA and higher C18:0, C18:1 *trans*-11 and C18:1 *cis*-9 FA than PRI (Table **3**). Time effect was observed for milk yield and for almost all FA, excepted for C4:0, C8:0, C10:0, C12:0, C17:1, C18:0 and C18:2 FA.

**Table 3: Differences in Milk Yield and Fatty Acid Profile between Primiparous and Multiparous Mediterranean Buffaloes Cows During Early Lactation**

| Item | Class | | SEM | *p-Value* | | |
|---|---|---|---|---|---|---|
| | Primiparous | Multiparous | | Class | Time | Interaction |
| Kg/day | | | | | | |
| Milk yield | 5.66 | 6.44 | 0.12 | 0.004 | 0.003 | 0.937 |
| % | | | | | | |
| Fat | 6.75 | 7.89 | 0.21 | 0.001 | 0.455 | 0.943 |
| g/100g of FA | | | | | | |
| Fatty acid | | | | | | |
| C4:0 | 1.75 | 1.68 | 0.01 | 0.155 | 0.746 | 0.688 |
| C6:0 | 1.25 | 1.21 | 0.01 | 0.470 | 0.090 | 0.640 |
| C8:0 | 0.68 | 0.667 | 0.01 | 0.770 | 0.148 | 0.809 |
| C10:0 | 1.39 | 1.38 | 0.02 | 0.945 | 0.228 | 0.711 |
| C11:0 | 0.058 | 0.053 | 0.02 | 0.333 | 0.001 | 0.331 |
| C12:0 | 1.93 | 1.95 | 0.02 | 0.842 | 0.099 | 0.788 |
| C13:0 | 0.242 | 0.227 | 0.05 | 0.443 | 0.001 | 0.477 |
| C14:0 | 10.38 | 10.40 | 0.07 | 0.964 | 0.036 | 0.397 |
| C14:1 | 1.36 | 1.27 | 0.02 | 0.350 | 0.001 | 0.264 |
| C15:0 | 1.11 | 1.03 | 0.01 | 0.169 | 0.001 | 0.912 |
| C15:1 | 0.238 | 0.222 | 0.01 | 0.192 | 0.003 | 0.880 |
| C16:0 | 33.22 | 32.89 | 0.21 | 0.041 | 0.050 | 0.401 |
| C16:1 | 2.24 | 1.97 | 0.03 | 0.050 | 0.003 | 0.535 |
| C17:0 | 0.686 | 0.691 | 0.02 | 0.861 | 0.037 | 0.955 |
| C17:1 | 0.217 | 0.199 | 0.02 | 0.289 | 0.125 | 0.312 |
| C18:0 | 9.08 | 11.35 | 0.10 | 0.002 | 0.576 | 0.837 |
| C18:1 n9t | 0.041 | 0.039 | 0.01 | 0.906 | 0.001 | 0.858 |
| C18:1 *trans*-11 | 1.02 | 2.23 | 0.04 | 0.009 | 0.001 | 0.621 |
| C18:1 *cis*-9 | 22.00 | 24.48 | 0.22 | 0.006 | 0.006 | 0.696 |
| C18:2 | 0.542 | 0.612 | 0.01 | 0.247 | 0.443 | 0.918 |
| C20:0 | 0.118 | 0.114 | 0.01 | 0.694 | 0.001 | 0.833 |
| C18:3 | 0.201 | 0.196 | 0.01 | 0.676 | 0.001 | 0.577 |
| CLA *cis*-9, *trans*-11 | 0.783 | 0.759 | 0.01 | 0.556 | 0.008 | 0.526 |
| Total | 93.28 | 93.04 | 0.11 | 0.627 | 0.010 | 0.808 |
| <C16 | 20.43 | 20.14 | 0.16 | 0.702 | 0.071 | 0.576 |
| C16 | 35.46 | 34.81 | 0.26 | 0.031 | 0.050 | 0.401 |
| >C16 | 37.39 | 38.11 | 0.25 | 0.462 | 0.257 | 0.682 |
| Total C18 | 35.37 | 38.11 | 0.02 | 0.433 | 0.271 | 0.671 |
| C18 unsat/sat | 2.61 | 2.60 | 0.17 | 0.917 | 0.066 | 0.983 |
| Total | | | | | | |
| Saturated | 62.80 | 62.54 | 0.20 | 0.767 | 0.022 | 0.660 |
| Unsaturated | 30.47 | 30.52 | 0.23 | 0.944 | 0.468 | 0.777 |
| Unsaturated / saturated | 0.48 | 0.49 | 0.01 | 0.829 | 0.173 | 0.709 |

## DISCUSSION

Glucose is a primary nutrient for milk synthesis, being the substrate of lactose synthesis, which is osmotic determinant of milk volume [12]. Multiparous cows presented lower glucose concentration than PRI, because the glucose requirement for milk synthesis is higher for MUL than PRI cows, since the milk yield is higher. This result agrees with Santos *et al*. [13] which reported higher glucose concentrations in PRI than MUL Holstein cows during post-partum period, but the authors also observed this effect during pre-partum period.

Non-esterified concentrations may be considered as an index of lipid mobilization [14], since they are released from body reserves and absorbed by the mammary gland to provide milk triglycerides or are metabolized into hepatocytes to form alternative energetic compounds. Due to higher milk yield of multiparous cows, energy requirements are consequently greater than primiparous cows, thus multiparous cows are more susceptible to mobilize body lipids and increase NEFA blood concentrations. Despite the differences in NEFA blood concentrations, BHB concentrations were not affected by number of lactations. β-Hydroxybutyrate is the major ketone body in blood and is related with FA oxidation in liver [15]. Therefore the increased NEFA concentration of MUL was routed to udder for fat synthesis and MUL cows presented higher milk fat content. The higher total cholesterol and total protein presented by MUL cows than PRI are related with lipid transport made by lipoproteins in secretors organs (as intestine and liver). Lipoproteins are macromolecular complexes of protein, phospholipid, cholesterol and triglycerides [16]. Urea blood concentration presented time effect only during post-partum period. This fact is related to the amino acids body reserves mobilization, presented in skeletal muscle [17]. Santos *et al*. [13] also observed similar effects of higher milk yield and milk fat content in MUL than PRI Holstein cows during the first 120 days in milk and entire lactation.

The higher content of C18:1 trans-11 and C18:1 cis-11 FA for MUL may indicate an incomplete biohydrogenation process, probably because the higher passage rate of multiparous cows when compared to PRI. The C18:1 trans-11 FA is a substrate to formation of C18:2 cis-9 trans-11 FA in the animal organism [18], which is a potential health-promoting, contributing with immune response, cancer prevention and decreased arthrosclerosis [19-21]. No differences were found in total saturated and unsaturated FA in milk. We expected that saturated would be higher in MUL because a considered part of triglycerides to milk fat synthesis is from NEFA on blood, which is formed mainly by C16:0 and C18:0 FA. Indeed, C18:0 FA was higher in MUL than PRI, but C16:0 FA was lower and the reason why it occurs is unknown. Data of milk FA profile of buffalo cows are scarce. However, Varrichio *et al*. [22] described similar concentrations of saturated FA in milk (65.48%) of Mediterranean buffalos of different herds that reported in the present experiment.

## CONCLUSION

Multiparous buffalos presented higher non-esterified fatty acid, which altered milk fat content, and higher milk yield than primiparous cows. Furthermore, multiparous cows presented a higher content of FA from incomplete biohydrogenation process.

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Received on 14-07-2015 Accepted on 22-07-2015 Published on 07-08-2015

DOI: http://dx.doi.org/10.6000/1927-520X.2015.04.02.2