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INTERPRETIVE SUMMARY

This study evaluates the impacts of difficult calving on Holstein dairy cow behavior and health. Cows with larger calves had relatively shorter rumination times both during and after calving and presented with comparatively higher inflammation levels, suggesting a more difficult recovery. Determination of calf size before parturition and recognition of small first-calving heifers might help identifying cows at higher risk of health issues at the onset of lactation.

RUNNING HEAD: CALVING AFFECTS RUMINATION AND INFLAMMATION IN COWS

Calving difficulty influences rumination time and inflammatory profile in Holstein dairy cows

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ABSTRACT

Difficult calving may adversely affect dairy cow health and performance. Maternal:fetal disproportion is a major cause of dystocia, therefore the main objective of this study was to assess the effects of dam:calf body weight ratio (**D:C**) on calving difficulty, rumination time, lying time, and inflammatory profile in 25 Holstein dairy cows. Using automatic monitoring systems, we monitored behavior and production in 9 primiparous and 16 pluriparous cows between dry-off and 30 days in milk. During the same period, we collected blood samples to monitor metabolism and inflammatory profile of these cows. Calvings were video recorded to assess calving difficulty and observe the duration of the expulsive stage. After parturition, the cows were separated into 3 classes according to their D:C, namely, easy (**E**, D:C > 17), medium (**M**, 14 < D:C < 17), and difficult (**D**, D:C < 14). The cows in class D showed relatively longer labor durations (108 min. vs. 54 min and 51 min for classes D, M, and E, respectively) and higher calving assistance rates (50% vs. 0% and 11% of calvings for classes D, M, and E, respectively) than those in the other two classes. Compared with the cows in classes M and E, those in class D exhibited shorter rumination times on the day of calving (176 min/d vs. 288 min/d and 354 min/d for classes D, M, and E, respectively) and during the first week of lactation (312 min/d vs. 339 min/d and 434 min/d for classes D, M, and E, respectively) and maintained lower rumination values until 30 DIM (399 min/d vs. 451 min/d and 499 min/d for classes D, M, and E, respectively). Primiparous class D cows had shorter resting times during the first week after calving compared with those in class M (8 h/d vs. 11 h/d for classes D and M, respectively). Interclass differences were found in terms of the levels of inflammation markers such as acute-phase proteins (ceruloplasmin, albumin, retinol, and paraoxonase). Moreover, cows of class D had lower plasma levels of fructosamine and creatinine after calving. Low dam:calf body weight (**BW**) ratio reduced post-calving rumination time and increased inflammation grade, suggesting a lower welfare of these animals at the onset of lactation. D:C

BW ratio might serve as a useful index for the identification of cows at relatively higher risk of metabolic and inflammatory disease, thus helping farmers and veterinarians to improve welfare and health of these cows.

Key words: calving difficulty, dairy cows, inflammation, rumination time.

INTRODUCTION

Parturition is one of the most critical moments in the life of a dairy cow. It constitutes the passage from the dry period to lactation and is characterized by dramatic metabolic and hormonal changes that strongly impact dairy cow welfare (Goff and Horst, 1997; Drackley, 1999). Features of an early lactation period include low dry matter intake (DMI), negative nutrient balance, and immune dysregulation. These manifestations reduce animal welfare and are markedly influenced by peripartum nutrition, environmental conditions, and stressful or painful events (Goff and Horst, 1997; Drackley, 1999; Bradford et al., 2015). Difficult calving may exacerbate this situation by upregulating the inflammatory response and causing reproductive pathology (Bradford et al., 2015). Dystocia lowers both DMI before calving and milk production and increases the risks of disease and perinatal calf mortality (Proudfoot et al., 2009; Mee, 2008). Calf-to-cow disproportion is a major cause of dystocia (Fiems et al., 2001; Noakes et al., 2001; Mee, 2008) and reduces the calf survival rate (Johanson and Berger, 2003). Low cow-to-calf ratio is typical of double-muscled cattle (Fiems et al., 2001), while in dairy cows it is more common in pure Holsteins compared to crossbreeds (Dhakal et al., 2013). Dam:calf mismatch is mainly related to small first-calving heifers, male calves, prolonged gestation and maternal under- and overnutrition during the last month of pregnancy (Mee, 2008). Proudfoot et al. (2009) investigated the impact of a difficult calving on feeding and lying behavior of 22 Holstein cows, from 48h before calving to 48h after. These authors found that cows with dystocia had different behaviors before calving, such as lower dry matter and water intake and higher number of standing bouts. In particular, DMI and standing bouts of cows with dystocia changed significantly 24h before calving, suggesting that these behaviors could be useful to discriminate between cows with or without dystocia. On the contrary, Proudfoot and colleagues didn't find any differences in these behaviors during the 48 hours after calving.

To the best of our knowledge, no studies have attempted to associate calving difficulty and fetal-maternal disproportion with rumination time and inflammation. Rumination time is considered a sensitive indicator of dairy cow health and is used in automated systems for early disease onset detection (Soriani et al., 2012; Calamari et al., 2014). Here, our hypothesis was that low dam:calf body weight ratios could negatively influence behavior and inflammation level in dairy cows during peripartum period, impede cow recovery, and elevate disease risks. Thus, aim of this study was to investigate the relationship between dam:calf body weight ratio and rumination, lying time, and inflammatory profile of cows in the first weeks of lactation.

MATERIALS AND METHODS

The present study was conducted at the teaching dairy farm of the Department of Veterinary Medical Science of the University of Bologna (Ozzano Emilia, Italy). During the experiment, the farm housed 85 milking cows in free stalls with straw-bedded cubicles. Their average daily milk production was 32±1,1 kg. The research was conducted in compliance with Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010 on the Protection of Animals Used for Scientific Purposes. The procedures were approved by the Ethical Committee of the Department of Veterinary Medical Science of University of Bologna, Italy.

Animals, Housing, and Management

Twenty-five Holstein cows (9 primiparous (**PR**) and 16 pluriparous (**PL**)) were selected and enrolled in the trial according to their expected calving dates. Behavior, metabolism, and production traits were monitored from dry-off (60±2 days before calving) to 30 d in milk. Primiparous cows were monitored from 60 days before expected calving date. All calvings occurred between the months of June and August. Temperature and Humidity Index **(THI)** was recorded continuously inside the barn by electronic probes (CMP Impianti s.r.l., Viadana Bresciana (BS) Italy). Daily mean THI during calving period was 75±3.1, average minimum THI was 69.7±3.3, while average maximum THI was 79.7±3.1. During the dry period, the cows were housed in a straw-bedded area, moved to a close-up pen 3 wks before calving, and maintained there until parturition. During the dry period, each animal was allowed 22 m^2 of which 9 m^2 was resting area, 5.5 m^2 was feeding area, and 7.5 m^2 was external paddock. On average 4 (±1) hours after calving, the cows were moved to an early-fresh pen, where they remained for ≥ 10 d, with 10-m^2 of straw bedded area per animal. Depending upon their health condition, they were moved to the same milking cow pen. The lactating pen, equipped with 42 straw bedded cubicles, hosted a total of 40 primiparous and multiparous cows. All dry and milking cow pens were equipped with fans (Vertigo, CMP Impianti s.r.l.). Lactating cows were milked at 0500 and 1800 daily in a double-five herringbone milking parlor equipped with an Afimilk system (Afikim, Kibbutz Afikim, Israel) to measure individual daily milk production (kg) and composition (% fat, protein, and lactose content) by mid-infrared spectroscopy (Afilab, Afikim). After calving, cow body weight was measured twice daily on an automatic weighing scale (Afiweight, Afikim) located at the exit of the milking parlor. The body condition score (BCS) was assessed according to the method of Edmonson et al. (1989) at calving and every 2 wks from dry-off to 30 DIM. During the far-off dry period (from 60 to 21 d before calving), the cows were fed long grass hay ad libitum. In the close-up pen, they received total mixed ration (TMR) twice daily for ad libitum consumption that consisted of chopped grass hay plus 4 kg/h/d prepartum mix. The compositions and analyses of the dry and lactating diets are shown in Table 1.

Calving Data

All calvings were recorded with video cameras in the calving pen for 24 h/d in order to observe calving progress and dam behavior. For each cow, the evolution of the expulsion phase was observed (Noakes et al., 2001). The times of appearance of the amniotic sac and feet, birth, expulsion of the fetal membranes, and any required interventions were recorded. Labor duration was calculated according to the time from the appearance of the amniotic sac or feet to birth. Calving difficulty was assessed according to Schuenemann, who reported timing and evolution of eutocic birth as well as correct calving assistance (Schuenemann et al., 2011). Farm personnel were trained to assist cows during calving and intervened only when recommended or required to do so. Based on Schuenemann et al. (2011), "prolonged labor" was defined as time between appearance of amniotic sac and birth > 60 min. After calving, calf sex and calf and dam body weight within 24 h of delivery were recorded. The ratio between dam and calf body weight, adjusted for BCS = 3.5 (National Research Council, 2001), was used to retrospectively categorize cows by cluster analysis in 3 classes. Cows with dam:calf BW ratios (**D:C**) < 14 were classified as **D**, those with D:C > 17 were classified as **E** and those with 14 < D:C < 17 were classified as **M**.

Behavioral Data

Daily rumination times (**RT**, min) were recorded with a Hi-Tag rumination monitoring system (SCR Engineers Ltd., Netanya, Israel) from dry-off to 30 DIM (Schirmann et al., 2009). Resting behavior of each cow were recorded continuously from calving to 30 DIM using a pedometer fitted with an accelerometer (AfiAct, Afimilk Ltd., Afikim, Kibbutz Afikim, Israel). The minutes and time percentages spent lying per 24 h were segregated by the software into 24-h intervals. Resting behavior was characterized as total daily lying time (**LT**, min), percentage daily time spent lying (**LT %**), and restlessness, calculated as the ratio of daily activity to **LT %**. Characteristics of rumination and resting behaviors were investigated for the various dam:calf BW ratio classes.

Blood Analysis

Blood was sampled from the coccygeal vein between dry-off and 30 DIM at -30 d, -15 d, -5 d, 5 d, 15 d, and 30 d relative to calving (± 1 d). Samples were drawn at 0800 before TMR distribution using 10-mL Vacuette tubes each containing 18 IU Li-heparin/mL (Greiner BioOne GmbH, Kremsmünster, Austria). After sampling, the blood was immediately centrifuged at 3,000 × *g* for 16 min to separate the plasma. The plasma was divided into two 1.5-mL portions and stored at -80 °C until they were analyzed at Istituto di Zootecnica of the University of Piacenza (Italy) according to the procedures described in Calamari et al. (2016). Plasma metabolites were analyzed at 37 °C with an automated clinical analyzer (ILAB 600, Instrumentation Laboratory, Lexington, MA, USA). Commercial kits measured glucose, total cholesterol, urea, calcium, total proteins, albumin, globulins, total bilirubin, creatinine, γ-glutamyltransferase (**GGT**) (Instrumentation Laboratory, Lexington, MA, USA), NEFA, zinc (Wako Chemicals GmbH, Neuss, Germany), and BHBA (Randox, Antrim, UK). Ceruloplasmin and haptoglobin were determined with reagents prepared according to the methods of Bertoni et al. (2008). Fructosamine (Fr) was determined using a commercial fructosamine kit (Randox, Antrim, UK). Total plasma reactive oxygen metabolites (**ROM**), plasma paraoxonase, tocopherol, and retinol were measured as reported by Bionaz et al. (2007).

Statistical Analysis

The cows were retrospectively separated by cluster analysis into three classes representing their dam:calf body weight ratios, namely, D, (D:C < 14), M (14 < D:C < 17), and E (D:C > 17). The data were then compared among these classes. Three cows had incomplete rumination data and so they were excluded from statistical analysis for rumination time data. All data were analyzed with JMP Pro v. 15 (SAS Institute Inc., Cary, NC, USA). Data normality was evaluated with a Shapiro-Wilk test. Variables with non-normal distributions were logarithmically or exponentially transformed. A mixed-model procedure with repeated measures was used to analyze lying and rumination time, plasma variables, and milk yield and composition. Plasma variables were aggregated according to the sampling time points (d): -30, -15, -5, 5, 15, 30. A first-order autoregressive covariance structure was selected according to the Akaike Information Criterion (AIC). Backward elimination was used to choose the best model according to the one with the lowest AIC. The final model included D:C class (D, M, or E), days relative to calving, parity (PR or PL), and their interactions as fixed effects and the cow as random effect. The terms of the repeated measures were days and the cow was the subject. When a significant F-test for class, class × DIM, or class × parity was detected, pairwise means multiple comparisons adjusted by Tukey-Kramer were performed. $P \leq 0.05$ indicated statistically significant differences between treatment means. Health events between classes were compared by Fisher's exact test.

RESULTS

Calving Data

We monitored 25 calvings over 3 mo. Nine of these were first-calving heifers and 16 were pluriparous. Average dam:calf body weight ratio of cows included in the study was 16.5±3.27.

With cluster analysis, cows were segregated into 3 classes based on their D:C ratio: 8 were classified as D (D:C < 14), 8 were classified as medium (M; 14 < D:C < 17), and 9 were classified as E (D:C > 17) (Table 3). The D class was represented mainly by first-calving heifers (6/8), while none of these animals was in the E class (Table 2). Cows body weight was not different among the 3 classes (P = 0.11), they had similar body condition scores (BCS) at calving but delivered relatively heavier calves (P < 0.0001) (Table 3). The calvings were video recorded and the entire duration of the expulsive stage was observed without human interference until birth. The expulsive stage was defined as the time from the appearance of the amniotic sac until birth (Noakes et al., 2001; Schuenemann et al., 2011). The average duration of delivery was 71 min. Animals included in class D had the longest duration of labor (P = 0.002) and relatively higher assistance rate compared with animals of class M and E (P = 0.05) while in contrast, they had similar length of pregnancy (P = 0.93) (Table 3).

Behavioral Data: Rumination and Lying Time

All 22 cows for which rumination data were analyzed presented strong reductions in rumination time on the day of calving. However, the class D cows showed lower RT values than the others, namely, 176.3 min vs. 287.8 min and 353.7 minutes for classes D, M, and E, respectively (P = 0.012). Primiparous class D cows had the lowest absolute daily RT on calving day (154 min) (Table 4). A decline of 68% of the value of RT recorded before calving (from -21d to -15d) was observed in cows of class D on calving day, whereas RT of cows of class E decreased of 35% (P = 0.005). Moreover, class D cows maintained lower RT relative to those of class E and M after parturition (P = 0.031; Figure 1). During the first week of lactation, primiparous class D cows rested less than those of class M (8.6 h/d vs. 11 h/d; P = 0.04). In contrast, pluriparous cows rested 10 h/d on average, without differences between animals of class D and those of class E and M. Taking the primiparous and multiparous cows together, no differences (P= 0.83) were detected between resting time of class D animals and those of the class E and M (Table 5).

Blood Parameters

Complete results of plasma analysis measured before and after calving are reported in Table 6. Figure 2 shows the evolution from 30 days before calving to 30 DIM of those variables that differed (*P*<0.05) between classes. Inflammatory phenomena were evident in the class D cows. These animals showed alterations in certain acute phase proteins compared to the class E and M cows. In these animals, the negative acute-phase proteins (albumin, retinol, and paraoxonase) decreased after parturition and remained low until 15 DIM or 30 DIM (*P*<0.05) Among the positive acute-phase proteins, ceruloplasmin levels at 15 DIM were higher in class M and D cows and differed from those for the class E cows (*P*=0.002). In contrast, haptoglobin peaked 5 d after calving in all animals and decreased thereafter without differences between classes. Markers of energy status (glucose, NEFA and BHBA) did not differ between animals of different classes, except for fructosamine and creatinine that were lower (*P*=0.02) in the class D cows at 5 and 15 DIM. On the contrary, no differences were found in ROM, tocopherol, urea, bilirubin, calcium and zinc, GGT and cholesterol.

Milk Production

Dam:calf BW ratio was not related with milk production nor milk composition (Table 7). Class D cows apparently produced less milk than those of classes M and E. Nevertheless, these data were biased by the comparatively large proportion of primiparous cows in this class. On the other hand, class E was represented exclusively by pluriparous cows. The milk yield was equivalent among the pluriparous cows of all three classes, while the average milk yields of primiparous cows in classes M and D were 26 kg/d and 20 kg/d, respectively (*P*=0.10).

Health Events

Complete health event histories are recorded in Table 8. There were only numerical differences among classes (*P*>0.05). However, 87.5 % of the class D animals were diagnosed with ≥ 1 pathology, and only one cow in this class reached 30 DIM without any health issues. Conversely, the clinical disease rate in class E cows was 55.5%.

DISCUSSION

Cows with lower D:C ratios (class D) had a duration of labor more than doubled compared with animals of other classes (108.0 min vs. 53.7 min and 51.3 min for classes D, M, and E, respectively). Moreover, 50% of D cows required delivery assistance.

In particular, primiparous cows had the longest average calving time (125 min compared to 59 min for multiparous cows). A previous study reported relatively longer durations for the dilation stage of calving in primiparous cows but no influence of parity on the expulsive phase (Schuenemann et al., 2011). Therefore, D:C ratio and parity are strongly correlated. Here, the heifers had lower D:C ratios than the multiparous cows. For this reason, the prolonged labor observed in these animals may be explained by dam:calf size mismatch rather than parity per se. Maternal-fetal disproportion is a main cause of dystocia in first-calving heifers (Mee, 2008). It might account for the higher rates of assistance and greater lengths of labor recorded for the class D animals in the present study (Meijering, 1984; Noakes et al., 2001; Mee et al., 2011). Johanson and Berger (2003) found a strong correlation between dystocia and the ratio of calf to dam body weight. For primiparous and multiparous cows, they calculated an average calf:dam BW ratio of 7% and stated that the target ratio was 7.2% for optimal calf survival (Johanson and Berger, 2003). These values correspond to a ratio of 14 for dam to calf BW. Fiems et al. (2001) reported relatively higher incidences of cesarean sections for cows with an average cow-to-calf ratio of 11.8. In contrast, those presenting with eutocic births had an average cow-to-calf ratio of 14.4 (Fiems et al., 2001). According to these authors and considering the longer labor and the higher rate of assistance of cows with lower dam:calf BW ratio in the present study, we related the D:C ratio to potential calving difficulty. In particular, cows with D:C<14 (D) were considered to have a potential difficult calving, cows with 14 <D:C < 17 (M) as medium and cows with D:C > 17 (E) as easy.

These results confirm the importance of higher body weight at calving particularly for heifers as they require good physical development at first calving (85% of mature body weight) without fattening in order to avoid dystocia and metabolic diseases (Mee, 2009; Gaafar et al., 2011). Nevertheless, a high BCS at calving must be avoided as it might increase the risk of dystocia by narrowing the birth canal caused via fat deposition, as well as the risks of ketosis and other diseases early in lactation (Roche et al., 2009).

Here, class D cows presented with greater relative incidences of retained fetal membranes (RFM), metritis, and delay in uterine involution during the peripartum period. We did not evaluate fertility data here as the follow-up period was too short (30 DIM). However, a previous study reported reductions in the fertility indices for cows with veterinary-assisted calvings compared to unassisted cows including 0.7 more services to conception, +8 d to first service, and calving intervals that were 28 d longer (Eaglen et al., 2011). Other authors reported comparatively lower RFM incidences and improved conception rates following the administration of nonsteroidal anti-inflammatory drugs (NSAID) within 12 h of calving (Giammarco et al., 2016).

In the present study, the dam:calf BW ratio was related with cow behavior such as lying and rumination time during the postpartum period. Unexpectedly, evaluation of the lying behavior around calving showed relatively lower resting times for primiparous class D cows during the first week of lactation. On the contrary, we expected an increase in lying time for animals with relatively longer and more difficult calving. Increased lying time is an illness-related behavior induced by pro-inflammatory cytokines to promote lethargy and anorexia and accelerate disease recovery (Johnson, 2002; Dantzer and Kelley, 2007). Certain studies report comparatively higher lying times for cows affected by clinical or subclinical diseases (Proudfoot et al., 2009; Sepúlveda-Varas et al., 2014). Nevertheless, another study stated that lying time was not associated with health status in primiparous animals, although ketotic and sick multiparous cows had longer lying times after calving than healthy cows (Piñeiro et al., 2019). Moreover, certain authors reported shorter resting times for primiparous than multiparous cows during the transition period (Sepúlveda-Varas et al., 2014; Neave et al., 2017). Thus, the class D primiparous animals in our study had shorter resting times possibly because of their lower hierarchical rank. Lactation stage, age, and body weight have the strongest influences on social hierarchy (Dickson et al., 1970; Grant and Albright, 1995) and fatigued primiparous cows in early postpartum are the most vulnerable to herd competition (Grant and Albright, 1995; Phillips and Rind, 2001; Cook and Nordlund, 2004; Neave et al., 2017). This situation is commonly reported in overcrowded environments where subordinate cows are often displaced by dominant animals from the feed bunk and spend comparatively more time standing without feeding or resting (Fregonesi et al., 2007). Neave et al. (2017) studied the influence of parity on behavior of healthy cows during transition and reported that, with 80% stocking density, primiparous cows were displaced by feeder more frequently than multiparous cows and had more but shorter lying bouts. Excessive standing time could increase the risks of hoof and metabolic disorders around calving. Stressors such as overcrowding and lack of rest upregulate cortisol and DHEA (dehydroepiandrosterone) (Fustini et al., 2017). During early postpartum, adipose tissue is sensitive to stress-related mediators that augment lipolysis and plasma NEFA and increase the risks of metabolic diseases and unsuccessful transition periods (Kushibiki et al., 2002, 2003; Underwood et al., 2003).

Rumination time was markedly reduced in class D animals on the day of calving compared to cows of classes E and M and they required relatively more time compared to the other groups to attain optimal RT values after calving (Figure 1). Reductions in rumination time around calving were reported by several authors who found physiological decreases in rumination time around parturition. Hence, this behavior could be used to detect the approach of calving (Schirmann et al., 2013; Büchel and Sundrum, 2014; Pahl et al., 2014). A novel aspect of our study is that we analyzed this loss of function with respect to potential calving difficulty. Class D, M, and E cows had the same rumination values during the dry period. Therefore, the observed low calving day RT and difficulty in attaining physiological values after calving for class D animals might be associated with their difficulty at delivering larger calves and their comparatively longer labor durations. Another study reported lower DMI at 48 h and 24 h before calving in dystocic cows, showing that cows with difficult parturition exhibit distinct feeding and resting behaviors (Proudfoot et al., 2009). These authors explained this observation by changes in the dam:calf ratios that reduce rumen capacity and increase calving pain (Stanley et al., 1993; Proudfoot et al., 2009) preventing animals from feeding and ruminating. Rumination time is influenced by several factors: adequate physically effective neutral detergent fiber (peNDF) in the diet (Mertens, 1997), forage inclusion and composition (Fustini et al., 2011) and diurnal feed availability (Cavallini et al., 2018). Moreover, health disorders, pain, and distress may inhibit rumination, and indeed, decrease in rumination time is considered a reliable stress and disease indicator (Soriani et al., 2012; Calamari et al., 2014; Schirmann et al., 2016). Calamari et al. (2014) associated slower increases in rumination time after calving with severe inflammation, suggesting the importance of monitoring rumination time after calving in order to identify cows at relatively higher risk of disease (Calamari et al., 2014). The aforementioned study categorized cows as high or low RT on the basis of their average rumination times between days 3 and 6 of lactation and found that low-ruminating cows had greater alterations in their acute-phase response than other cows.

Our findings agree with those of Calamari and colleagues. D animals, that showed lower rumination time during and after calving, presented with markedly alteration in negative acute phase proteins and ceruloplasmin indicating a more severe inflammation process compared to animals of class E and M. These animals experienced relatively longer and more difficult calvings that, together with uterine tissue damage, has been previously associated with increased inflammation (Qu et al., 2014; Bradford et al., 2015; Pohl et al., 2015). Interestingly, some authors, highlighted a more pronounced inflammation after calving in primiparous cows compared with multiparous (Humblet et al., 2006; Schneider et al., 2013; Pohl et al., 2015). These authors speculate that the first calving could drive a more intense acute phase response compared with following calvings and that primiparous cows could be more sensitive to the stress related to parturition. Our results agree with these studies and show that cows with lower dam:calf BW ratio, and therefore particularly primiparous cows, have a more severe inflammation in the post partum period. Thus, we suggest that dam:calf BW ratio could be the leading cause of the high level of inflammation after calving rather than parity per se.

The energy status markers (glucose, NEFA, and BHBA) showed negative energy balance and lipid mobilization characteristic of transition cows without differences among classes, even though fructosamine, that was previously related to the energy balance markers (Caré et al., 2018) was lower in D animals. After calving, cows with lower D:C ratio exhibited also a more pronounced decrease in plasma creatinine compared to other cows, suggesting an higher muscle mobilization in cows of D class compared to cows of class M and E. Plasma creatinine levels, in healthy euhydrated cows, is strictly related to muscle mass and it has been recently suggested as a reliable index to monitor protein mobilization in periparturient cows (Wyss and Kaddurah-Daouk, 2000; Megahed et al., 2019). Therefore, the lower plasma levels of fructosamine and creatinine in D animals suggest a negative relation of dam:calf BW ratio with the energy and protein balance of cows during the post partum period.

According to the blood indices, however, nearly all animals in this study showed alterations in their inflammatory and metabolic profiles. These results confirm the drastic changes typical of transition cows, characterized mainly by metabolic imbalance and inflammatory dysfunction (Sordillo and Raphael, 2013; Bradford et al., 2015).

CONCLUSIONS

Cows with relatively lower dam:calf BW ratios had longer and more difficult calvings. The dam:calf BW ratio was negatively related with the rumination times on the day of calving and during the first month of lactation. Low D:C ratio was also related with lower lying time in primiparous cows and higher inflammation markers during the postpartum period as well as lower fructosamine and creatinine. These findings underscore the importance of avoiding excessively heavy calves especially in first-calving heifers, for which proper physical maturity at breeding is fundamental. The administration of sexed semen could effectively raise the dam:calf BW ratio in smaller heifers. Moreover, fetus sex and size detection during pregnancy and cow's BCS monitoring might help identifying cows at comparatively higher risk of health problems.

Adverse physiological alterations during postpartum can mitigate cow welfare and increase the risk of unsuccessful transitions. In the present study only the first 30 days of lactation were considered and long-term consequences of calving difficulty are far from being clear.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Ingredients and chemical composition of dry and lactating cow diets.

Composition	Close-up dry	Lactating
Ingredient, (% DM)		
Grass hay	70.0	39.8
Corn flakes	5.0	13.1
Sorghum meal	--	26.7
Soy meal	5.0	12.0
Molasses	--	6.0
Minerals and vitamin mix[1]	--	2.4
Close-up cow mix[2]	20.0	--
Nutrients, (% DM)		
Crude protein	11.5	15.03
NDF	55.2	34.21
ADF	36.3	23.87
ADL	6.4	2.98
Starch	9.5	27.31
Ether extract	2.3	3.1

[1]Minerals and vitamin mix: 15,6% Ca, 0,1% P, 14,8% Na, 3,3% Mg, 4000 mg/Kg di Zn, 4000 mg/Kg di Mn, 500 mg/Kg di Cu, 50 mg/Kg I, 30 mg/Kg di Se,700000 IU/Kg di vitamin A, 50000 IU/Kg vitamin D3, e 1500 mg/Kg di vitamin E.

[2]Close-up cow mix: % a.f. moisture 12,50%; crude protein 23,00%; lipids 2,00%; crude fiber18,00%; ash 18,50%; Na 0,80%; Mg 1,60%.

Table 2. Distribution of cows in classes according to their dam:calf BW ratio (D:C).

D:C ratio class	Animals, n	Primiparous	Pluriparous
Total	25	9	16
D[1]	8	6	2
M[2]	8	3	5
E[3]	9	0	9

[1]Dam and calf body weight ratio < 14.

[2]Dam and calf body weight ratio between 14 and 17.

[3]Dam and calf body weight ratio > 17.

Table 3. Dams and calves body weight (BW)[1], BCS[2] and calving characteristics of cows within the 3 classes of dam:calf BW ratio (D:C) (D[3], 8 cows; M[4], 8 cows; E[5], 9 cows). Mean values are reported.

	All cows			Primiparous			Pluriparous				*P*-value		
	E	M	D	E	M	D	E	M	D	SEM	Class	Parity	Parity x Class
Animals, n	9	8	8	0	3	6	9	5	2				
D:C BW[1] ratio	19.4^{a}	16.4^{b}	13.2^{c}	--	18.0^{a}	13.3^{c}	19.4^{a}	15.5^{b}	13.0^{c}	0.5	<0.001	<0.001	0.008
Cows BW[1], kg	718.7	694.4	634.2	--	640.0	620.1	718.7	727.0	676.0	28.7	0.11	<0.001	0.34
Calves BW[1], kg	37.2^{b}	42.7^{b}	49.7^{a}	--	35.7^{c}	48.9^{ab}	37.2^{c}	47.0^{b}	52.5^{a}	1.4	<0.001	<0.001	<0.001
Cows BCS[2], p.ts	3.3	3.5	3.5	--	3.9	3.6	3.3	3.3	3.4	1.8	0.13	<0.001	0.13
Expulsive stage, min.	51.3	53.7	108.0	--	55.7	124.5	51.3	52.6	58.5	19.4	0.002	0.88	0.05
Pregnancy length, days	277.2	278.1	279.0	--	277.7	278.0	277.3	278.4	282.0	3.5	0.93	0.84	0.55
Assisted calving, %	11.1^{b}	0^{b}	50.0^{a}	--	0^{b}	66.7^{a}	11.1^{b}	0^{b}	0^{b}		0.05		

[a,b,c] Values with different superscript letters within a row differ significantly (*P*<0.05)

[1]Body weight recorded within 24 hours after calving.

[2]BCS was scored according to Edmonson et al., 1989, the day of calving.

[3]Dam and calf body weight ratio < 14.

[4]Dam and calf body weight ratio between 14 and 17.

[5]Dam and calf body weight ratio > 17.

Table 4. Rumination time (RT) around calving of cows within the 3 classes of dam:calf BW ratio (D[1], M[2], E[3]). Mean values are reported.

	All cows			Primiparous			Pluriparous				*P*-value		
	E	M	D	E	M	D	E	M	D	SEM	Class	Parity	Parity x Class
Animals, n	7	8	7	0	3	5	7	5	2				
Dry period RT (min./d)	534.3	522.0	563.5	--	515.3	561.6	534.3	542.7	612.0	17.2	0.12	0.55	0.36
Calving day RT (min./d)	353.7^{a}	287.8^{ab}	176.3^{b}	--	286.7	154.0	353.7	288.4	232.0	36.1	0.012	0.98	0.57
RT reduction on calving day (min.)[4]	-191.0^{b}	-261.1^{b}	-376.3^{a}	--	-232.0	-385.6	-191.0	-278.6	-353.0	33.9	0.03	0.50	0.45
RT reduction rate on calving day (%)	-34.7^{a}	-48.3^{ab}	-67.9^{b}	--	-45.7	-71.0	-34.7	-49.9	-60.2	6.0	0.005	0.72	0.42
RT in the 1st week of lactation (min./d)	434.2^{a}	339.6^{b}	312.3^{b}	--	334.3	293.4	434.2	342.7	359.4	13.6	<.0001	0.23	0.19
RT between 25 and 30 DIM (min./d)	496.24^{a}	451.7^{b}	382.3^{c}	--	428.0	403.5	496.2^{a}	467.5^{ab}	350.3^{c}	12.6	< .0001	0.10	0.01

$^{a\text{-}c}$ Values with different superscript letters within a row differ significantly within parity(P<0.05)

[1]Dam and calf body weight ratio < 14.

[2]Dam and calf body weight ratio between 14 and 17.

[3]Dam and calf body weight ratio > 17.

[4] Reduction compared to the average RT recorded between -21 and -15 days before calving.

Table 5. Lying time (means) of cows belonging to the 3 classes of dam:calf BW ratio (D[1], M[2], E[3]) during the first week of lactation (DIM 1-7).

	All cows			Primiparous			Pluriparous				*P*-value		
	E	M	D	E	M	D	E	M	D	SEM	Class	Parity	Parity x Class
Animals, n	9	8	8	0	3	6	9	5	2				
Lying time, h/d	10.2	10.7	9.3	--	11.0^{a}	8.6^{b}	10.2	10.4	10.0	0.3	0.83	0.53	0.04
Lying rate, %/24h	42.6	45.0	39.0	--	46.2^{a}	36.2^{b}	42.6	43.7	41.8	1.4	0.35	0.47	0.03
Restlessness index	1.4	1.2	1.6	--	1.4	1.3	1.4	1.1	1.9	0.1	0.62	0.26	0.12

[a,b] Values with different superscript letters within a row differ significantly within parity (P<0.05).

[1]Dam and calf body weight ratio < 14.

[2]Dam and calf body weight ratio between 14 and 17.

[3]Dam and calf body weight ratio > 17.

Table 6. Plasma variables of cows belonging to the 3 classes of dam:calf BW ratio (D[1], 8 cows; M[2], 8 cows; E[3], 9 cows) before (Pre)[4] and after (Post)[5] calving. Means values are reported.

	E		M		D			*P*-value		
	Pre	Post	Pre	Post	Pre	Post	SEM	Day	Class	Day x Class
Glucose, mmol/l	3.91	3.33	3.85	3.40	3.97	3.24	0.16	<.0001	0.99	0.75
NEFA, mmol/l	0.19	0.56	0.28	0.71	0.26	0.67	0.09	<.0001	0.51	0.85
BHB, mmol/l	0.54	1.02	0.49	1.07	0.56	1.11	0.19	<.0001	0.96	0.73
Fructosamine, µmol/l	192.01	163.84	195.03	157.85	189.99	144.52	4.92	<.0001	0.40	0.02
Bilirubin, µmol/l	1.77	4.37	2.32	5.63	2.28	6.01	0.84	<.0001	0.35	0.93
Haptoglobin, g/l	0.35	0.74	0.40	0.82	0.38	0.78	0.10	<.0001	0.82	0.97
Ceruloplasmin, µmol/l	3.18	3.64	3.19	4.13	2.91	3.79	0.15	<.0001	0.47	0.002
Albumin, g/l	35.34	34.65	35.96	33.81	36.63	32.07	0.62	<.0001	0.84	0.01
Total proteins, g/l	70.97	72.81	72.43	73.66	69.54	72.83	1.06	<.0001	0.55	0.39
Globulins, g/l	35.63	38.05	36.48	39.85	32.91	40.76	1.07	<.0001	0.70	0.04
Paraoxonase, U/ml	88.25	85.86	90.45	74.96	87.81	68.88	4.21	<.0001	0.42	0.002
Retinol, µg/100ml	50.55	45.26	41.18	34.43	43.13	31.93	3.49	<.0001	0.15	0.03
Tocopherol, µg/ml	1.49	1.17	1.46	1.07	1.46	1.05	0.14	<.0001	0.93	0.30
Calcium, mmol/l	2.52	2.46	2.56	2.45	2.56	2.42	0.06	<.0001	0.91	0.61
Zinc, µmol/l	11.87	11.18	12.61	11.58	13.07	10.46	0.67	0.007	0.65	0.20
GGT, U/l	21.44	23.13	22.22	25.19	17.47	27.97	2.33	0.003	0.92	0.55
ROM, mg H_2O_2/100 ml	14.10	16.00	14.80	16.63	13.38	16.76	0.73	<.0001	0.70	0.59
Cholesterol, mmol/l	2.98	3.23	3.21	3.10	2.76	2.96	0.20	<.0001	0.72	0.31
Creatinine, µmol/l	107.16	105.80	121.32	106.48	113.59	93.83	3.56	<.0001	0.04	0.02
Urea, mmol/l	5.30	5.51	6.01	5.34	5.85	4.65	0.53	0.47	0.60	0.78

[1]Dam and calf body weight ratio < 14.

[2]Dam and calf body weight ratio between 14 and 17.

[3]Dam and calf body weight ratio > 17.

[4] mean value of sample collected at -30, -15, -5 days before calving (±1)

[5] mean value of sample collected at 5, 14, 30 days after calving (±1)

Table 7. Milk yield and composition (means) of cows belonging to the 3 classes of dam:calf BW ratio (D[1], M[2], E[3]) during the first month of lactation.

	All cows			Primiparous			Pluriparous				*P*-value		
	E	M	D	E	M	D	E	M	D	SEM	Class	Parity	Class x Parity
Animals, n	9	8	8	0	3	6	9	5	2				
Milk, Kg/d	34.0	27.8	28.3	--	26.0	20.8	34.0	29.6	35.9	0.90	0.10	<0.001	0.25
Fat, %	4.1	4.2	3.8	--	4.1	3.9	4.1	4.3	3.7	0.09	0.23	0.56	0.31
Protein, %	3.4	3.4	3.4	--	3.3	3.4	3.4	3.5	3.4	0.04	0.76	0.87	0.82
Lactose, %	4.9	4.8	4.9	--	4.8	4.8	4.9	4.7	4.9	0.03	0.98	0.84	0.89
Fat:protein	1.2	1.2	1.1	--	1.2	1.1	1.2	1.2	1.1	0.03	0.57	0.64	0.62

[1]Dam and calf body weight ratio < 14.

[2]Dam and calf body weight ratio between 14 and 17.

[3]Dam and calf body weight ratio > 17.

Table 8. Cases of disease within 30 DIM of cows belonging to the 3 classes of dam:calf BW ratio (D[1], 8 cows; M[2], 8 cows; E[3], 9 cows).

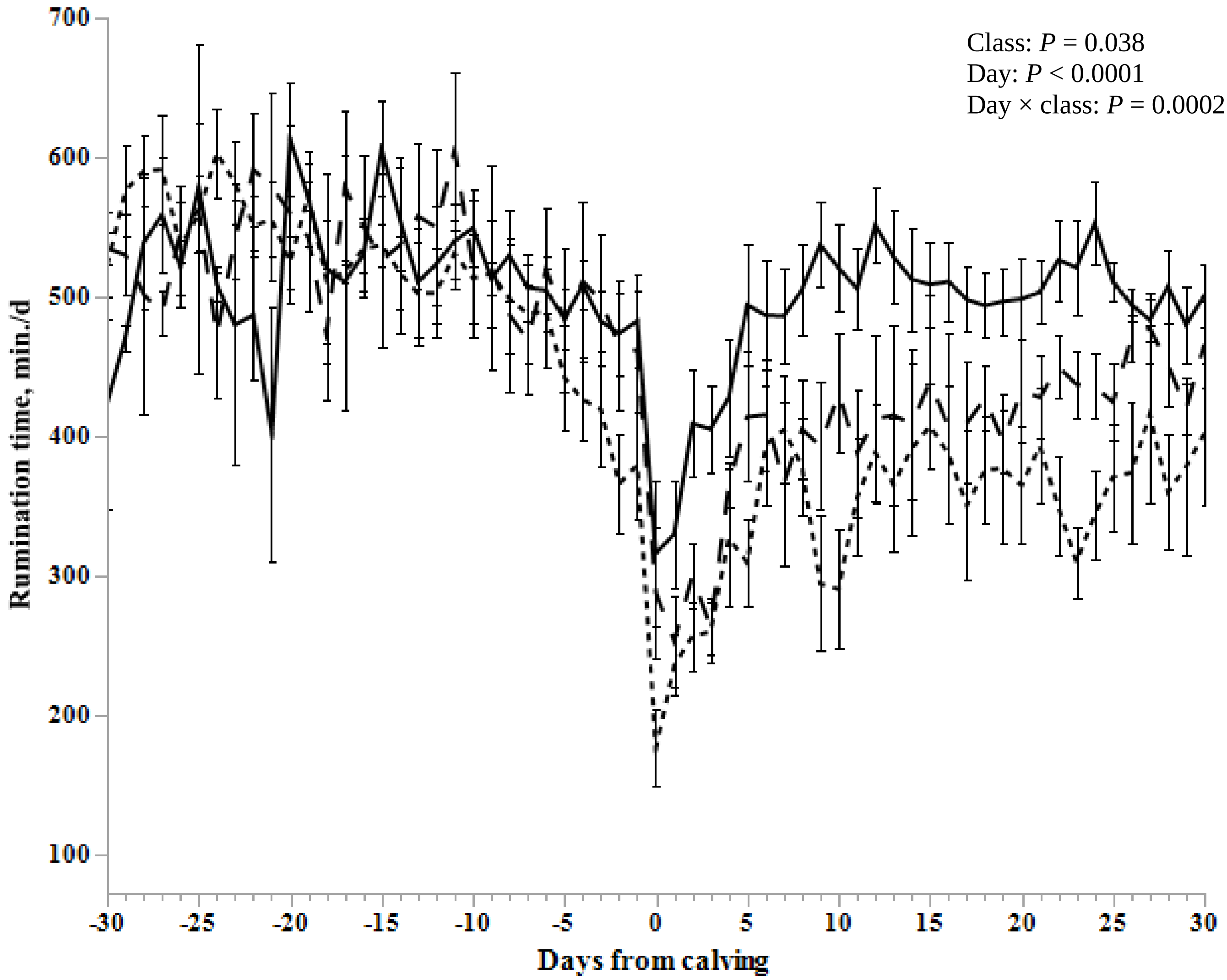
Cases, n	E	M	D	*P*-value
Retained fetal membranes	1	2	2	0.69
Metritis	1	1	2	0.82
Delayed uterine involution	1	1	2	0.82
Ketosis	3	3	4	0.88
Displaced abomasum	0	1	1	0.52
Mastitis	2	2	0	0.50
Absence of pathologies	4	2	1	0.47

[1]Dam and calf body weight ratio < 14.

[2]Dam and calf body weight ratio between 14 and 17.

[3]Dam and calf body weight ratio > 17.

Figure 1. Rumination time (LSM±SEM) of cows belonging to the 3 classes of dam:calf BW ratio (······ D[1], difficult; – – M[2], medium; — E[3],easy) from 30 days before calving to 30 DIM.

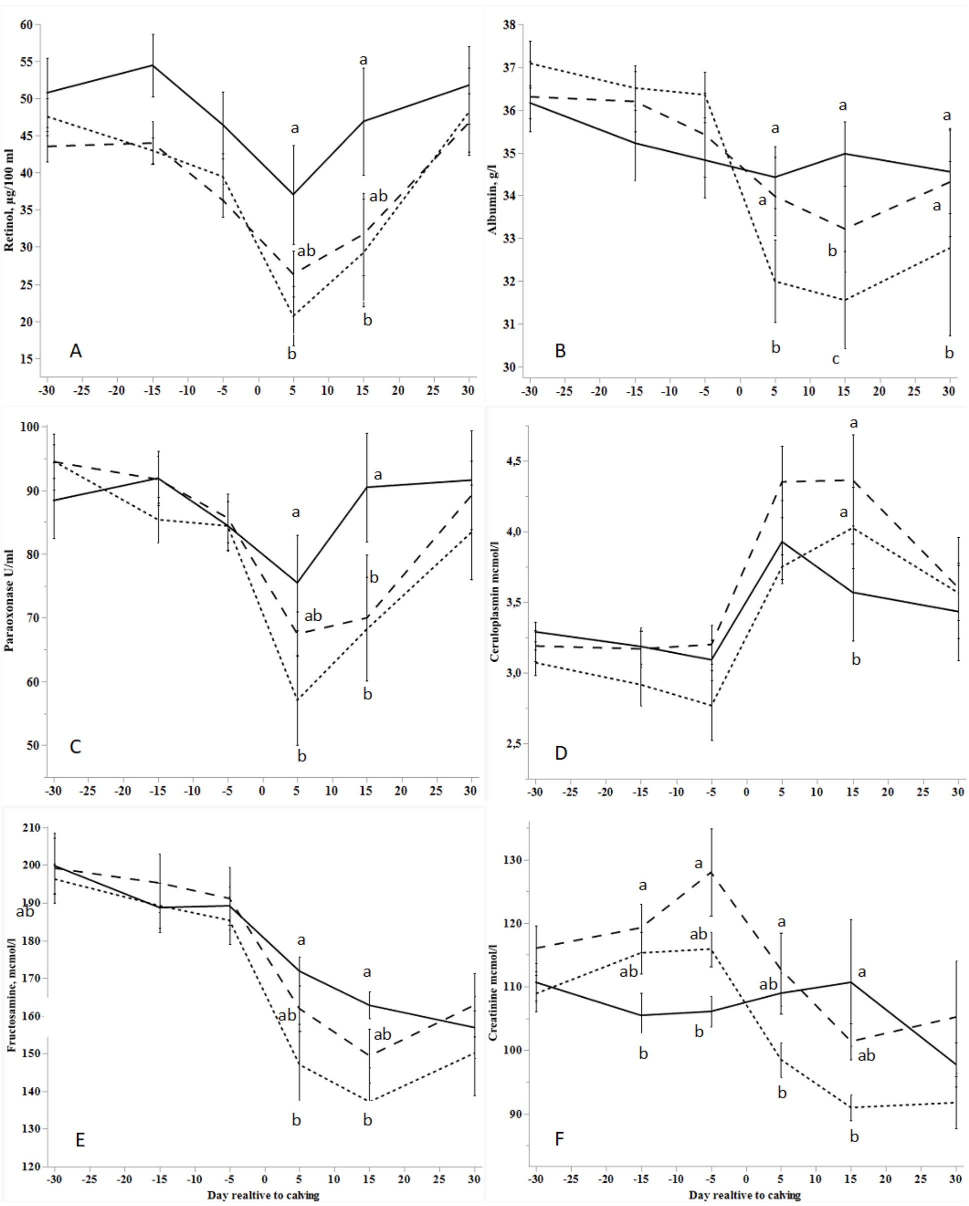


[1]Dam and calf body weight ratio < 14 (7 cows).

[2]Dam and calf body weight ratio between 14 and 17 (8 cows).

[3]Dam and calf body weight ratio > 17 (7 cows).

Figure 2. Pattern of plasma variables (retinol (A), albumin (B), paraoxonase (C), ceruloplasmin (D), fructosamine (E) and creatinine (F)) of cows belonging to the 3 classes of dam:calf BW ratio (···· D[1], difficult; – – M[2], medium; —— E[3], easy) measured from 30 days before calving to 30 DIM. Least square means ±SEM are reported. Different letters at the same day indicate significant differences between classes (P<0.05).



[1]Dam and calf body weight ratio < 14 (8 cows)

[2]Dam and calf body weight ratio between 14 and 17 (8 cows)

[3]Dam and calf body weight ratio > 17 (9 cows)