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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

45
46 Difficult calving may adversely affect dairy cow health and performance. Maternal:fetal
47 disproportion is a major cause of dystocia, therefore the main objective of this study was to
48 assess the effects of dam:calf body weight ratio (**D:C**) on calving difficulty, rumination time,
49 lying time, and inflammatory profile in 25 Holstein dairy cows. Using automatic monitoring
50 systems, we monitored behavior and production in 9 primiparous and 16 pluriparous cows
51 between dry-off and 30 days in milk. During the same period, we collected blood samples to
52 monitor metabolism and inflammatory profile of these cows. Calvings were video recorded to
53 assess calving difficulty and observe the duration of the expulsive stage. After parturition, the
54 cows were separated into 3 classes according to their D:C, namely, easy (**E**, $D:C > 17$), medium
55 (**M**, $14 < D:C < 17$), and difficult (**D**, $D:C < 14$). The cows in class D showed relatively longer
56 labor durations (108 min. vs. 54 min and 51 min for classes D, M, and E, respectively) and
57 higher calving assistance rates (50% vs. 0% and 11% of calvings for classes D, M, and E,
58 respectively) than those in the other two classes. Compared with the cows in classes M and E,
59 those in class D exhibited shorter rumination times on the day of calving (176 min/d vs. 288
60 min/d and 354 min/d for classes D, M, and E, respectively) and during the first week of lactation
61 (312 min/d vs. 339 min/d and 434 min/d for classes D, M, and E, respectively) and maintained
62 lower rumination values until 30 DIM (399 min/d vs. 451 min/d and 499 min/d for classes D,
63 M, and E, respectively). Primiparous class D cows had shorter resting times during the first
64 week after calving compared with those in class M (8 h/d vs. 11 h/d for classes D and M,
65 respectively). Interclass differences were found in terms of the levels of inflammation markers
66 such as acute-phase proteins (ceruloplasmin, albumin, retinol, and paraoxonase). Moreover,
67 cows of class D had lower plasma levels of fructosamine and creatinine after calving. Low
68 dam:calf body weight (**BW**) ratio reduced post-calving rumination time and increased
69 inflammation grade, suggesting a lower welfare of these animals at the onset of lactation. D:C

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

73

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

75

76

INTRODUCTION

77 Parturition is one of the most critical moments in the life of a dairy cow. It constitutes the
78 passage from the dry period to lactation and is characterized by dramatic metabolic and
79 hormonal changes that strongly impact dairy cow welfare (Goff and Horst, 1997; Drackley,
80 1999). Features of an early lactation period include low dry matter intake (DMI), negative
81 nutrient balance, and immune dysregulation. These manifestations reduce animal welfare and
82 are markedly influenced by peripartum nutrition, environmental conditions, and stressful or
83 painful events (Goff and Horst, 1997; Drackley, 1999; Bradford et al., 2015). Difficult calving
84 may exacerbate this situation by upregulating the inflammatory response and causing
85 reproductive pathology (Bradford et al., 2015). Dystocia lowers both DMI before calving and
86 milk production and increases the risks of disease and perinatal calf mortality (Proudfoot et al.,
87 2009; Mee, 2008). Calf-to-cow disproportion is a major cause of dystocia (Fiems et al., 2001;
88 Noakes et al., 2001; Mee, 2008) and reduces the calf survival rate (Johanson and Berger, 2003).
89 Low cow-to-calf ratio is typical of double-muscled cattle (Fiems et al., 2001), while in dairy
90 cows it is more common in pure Holsteins compared to crossbreeds (Dhakal et al., 2013).
91 Dam:calf mismatch is mainly related to small first-calving heifers, male calves, prolonged
92 gestation and maternal under- and overnutrition during the last month of pregnancy (Mee,
93 2008). Proudfoot et al. (2009) investigated the impact of a difficult calving on feeding and lying
94 behavior of 22 Holstein cows, from 48h before calving to 48h after. These authors found that

95 cows with dystocia had different behaviors before calving, such as lower dry matter and water
96 intake and higher number of standing bouts. In particular, DMI and standing bouts of cows
97 with dystocia changed significantly 24h before calving, suggesting that these behaviors could
98 be useful to discriminate between cows with or without dystocia. On the contrary, Proudfoot
99 and colleagues didn't find any differences in these behaviors during the 48 hours after calving.
100 To the best of our knowledge, no studies have attempted to associate calving difficulty and
101 fetal-maternal disproportion with rumination time and inflammation. Rumination time is
102 considered a sensitive indicator of dairy cow health and is used in automated systems for early
103 disease onset detection (Soriani et al., 2012; Calamari et al., 2014). Here, our hypothesis was
104 that low dam:calf body weight ratios could negatively influence behavior and inflammation
105 level in dairy cows during peripartum period, impede cow recovery, and elevate disease risks.
106 Thus, aim of this study was to investigate the relationship between dam:calf body weight ratio
107 and rumination, lying time, and inflammatory profile of cows in the first weeks of lactation.

108

109

MATERIALS AND METHODS

110 The present study was conducted at the teaching dairy farm of the Department of Veterinary
111 Medical Science of the University of Bologna (Ozzano Emilia, Italy). During the experiment,
112 the farm housed 85 milking cows in free stalls with straw-bedded cubicles. Their average daily
113 milk production was $32 \pm 1,1$ kg. The research was conducted in compliance with Directive
114 2010/63/EU of the European Parliament and the Council of September 22, 2010 on the
115 Protection of Animals Used for Scientific Purposes. The procedures were approved by the
116 Ethical Committee of the Department of Veterinary Medical Science of University of Bologna,
117 Italy.

118

119 *Animals, Housing, and Management*

120 Twenty-five Holstein cows (9 primiparous (**PR**) and 16 pluriparous (**PL**)) were selected and
121 enrolled in the trial according to their expected calving dates. Behavior, metabolism, and
122 production traits were monitored from dry-off (60 ± 2 days before calving) to 30 d in milk.
123 Primiparous cows were monitored from 60 days before expected calving date. All calvings
124 occurred between the months of June and August. Temperature and Humidity Index (**THI**) was
125 recorded continuously inside the barn by electronic probes (CMP Impianti s.r.l., Viadana
126 Bresciana (BS) Italy). Daily mean THI during calving period was 75 ± 3.1 , average minimum
127 THI was 69.7 ± 3.3 , while average maximum THI was 79.7 ± 3.1 . During the dry period, the cows
128 were housed in a straw-bedded area, moved to a close-up pen 3 wks before calving, and
129 maintained there until parturition. During the dry period, each animal was allowed 22 m² of
130 which 9 m² was resting area, 5.5 m² was feeding area, and 7.5 m² was external paddock. On
131 average 4 (± 1) hours after calving, the cows were moved to an early-fresh pen, where they
132 remained for ≥ 10 d, with 10-m² of straw bedded area per animal. Depending upon their health
133 condition, they were moved to the same milking cow pen. The lactating pen, equipped with 42
134 straw bedded cubicles, hosted a total of 40 primiparous and multiparous cows. All dry and
135 milking cow pens were equipped with fans (Vertigo, CMP Impianti s.r.l.). Lactating cows were
136 milked at 0500 and 1800 daily in a double-five herringbone milking parlor equipped with an
137 Afimilk system (Afikim, Kibbutz Afikim, Israel) to measure individual daily milk production
138 (kg) and composition (% fat, protein, and lactose content) by mid-infrared spectroscopy
139 (Afilab, Afikim). After calving, cow body weight was measured twice daily on an automatic
140 weighing scale (Afiweight, Afikim) located at the exit of the milking parlor. The body
141 condition score (BCS) was assessed according to the method of Edmonson et al. (1989) at
142 calving and every 2 wks from dry-off to 30 DIM. During the far-off dry period (from 60 to 21
143 d before calving), the cows were fed long grass hay ad libitum. In the close-up pen, they

144 received total mixed ration (TMR) twice daily for ad libitum consumption that consisted of
145 chopped grass hay plus 4 kg/h/d prepartum mix. The compositions and analyses of the dry and
146 lactating diets are shown in Table 1.

147

148 *Calving Data*

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

164

165 *Behavioral Data*

166 Daily rumination times (**RT**, min) were recorded with a Hi-Tag rumination monitoring system
167 (SCR Engineers Ltd., Netanya, Israel) from dry-off to 30 DIM (Schirmann et al., 2009).
168 Resting behavior of each cow were recorded continuously from calving to 30 DIM using a

169 pedometer fitted with an accelerometer (AfiAct, Afimilk Ltd., Afikim, Kibbutz Afikim, Israel).
170 The minutes and time percentages spent lying per 24 h were segregated by the software into
171 24-h intervals. Resting behavior was characterized as total daily lying time (**LT**, min),
172 percentage daily time spent lying (**LT %**), and restlessness, calculated as the ratio of daily
173 activity to **LT %**. Characteristics of rumination and resting behaviors were investigated for the
174 various dam:calf BW ratio classes.

175

176 ***Blood Analysis***

177 Blood was sampled from the coccygeal vein between dry-off and 30 DIM at -30 d, -15 d, -5 d,
178 5 d, 15 d, and 30 d relative to calving (± 1 d). Samples were drawn at 0800 before TMR
179 distribution using 10-mL Vacuette tubes each containing 18 IU Li-heparin/mL (Greiner
180 BioOne GmbH, Kremsmünster, Austria). After sampling, the blood was immediately
181 centrifuged at $3,000 \times g$ for 16 min to separate the plasma. The plasma was divided into two
182 1.5-mL portions and stored at -80°C until they were analyzed at Istituto di Zootechnica of the
183 University of Piacenza (Italy) according to the procedures described in Calamari et al. (2016).
184 Plasma metabolites were analyzed at 37°C with an automated clinical analyzer (ILAB 600,
185 Instrumentation Laboratory, Lexington, MA, USA). Commercial kits measured glucose, total
186 cholesterol, urea, calcium, total proteins, albumin, globulins, total bilirubin, creatinine, γ -
187 glutamyltransferase (**GGT**) (Instrumentation Laboratory, Lexington, MA, USA), NEFA, zinc
188 (Wako Chemicals GmbH, Neuss, Germany), and BHBA (Randox, Antrim, UK).
189 Ceruloplasmin and haptoglobin were determined with reagents prepared according to the
190 methods of Bertoni et al. (2008). Fructosamine (Fr) was determined using a commercial
191 fructosamine kit (Randox, Antrim, UK). Total plasma reactive oxygen metabolites (**ROM**),
192 plasma paraoxonase, tocopherol, and retinol were measured as reported by Bionaz et al. (2007).

193

194 *Statistical Analysis*

195 The cows were retrospectively separated by cluster analysis into three classes representing their
196 dam:calf body weight ratios, namely, D, (D:C < 14), M (14 < D:C < 17), and E (D:C > 17).
197 The data were then compared among these classes. Three cows had incomplete rumination data
198 and so they were excluded from statistical analysis for rumination time data. All data were
199 analyzed with JMP Pro v. 15 (SAS Institute Inc., Cary, NC, USA). Data normality was
200 evaluated with a Shapiro-Wilk test. Variables with non-normal distributions were
201 logarithmically or exponentially transformed. A mixed-model procedure with repeated
202 measures was used to analyze lying and rumination time, plasma variables, and milk yield and
203 composition. Plasma variables were aggregated according to the sampling time points (d): -30,
204 -15, -5, 5, 15, 30. A first-order autoregressive covariance structure was selected according to
205 the Akaike Information Criterion (AIC). Backward elimination was used to choose the best
206 model according to the one with the lowest AIC. The final model included D:C class (D, M, or
207 E), days relative to calving, parity (PR or PL), and their interactions as fixed effects and the
208 cow as random effect. The terms of the repeated measures were days and the cow was the
209 subject. When a significant F-test for class, class × DIM, or class × parity was detected,
210 pairwise means multiple comparisons adjusted by Tukey-Kramer were performed. $P \leq 0.05$
211 indicated statistically significant differences between treatment means. Health events between
212 classes were compared by Fisher's exact test.

213

214 **RESULTS**

215

216 *Calving Data*

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

219 With cluster analysis, cows were segregated into 3 classes based on their D:C ratio: 8 were
220 classified as D ($D:C < 14$), 8 were classified as medium (M; $14 < D:C < 17$), and 9 were
221 classified as E ($D:C > 17$) (Table 3). The D class was represented mainly by first-calving heifers
222 (6/8), while none of these animals was in the E class (Table 2). Cows body weight was not
223 different among the 3 classes ($P = 0.11$), they had similar body condition scores (BCS) at
224 calving but delivered relatively heavier calves ($P < 0.0001$) (Table 3). The calvings were video
225 recorded and the entire duration of the expulsive stage was observed without human
226 interference until birth. The expulsive stage was defined as the time from the appearance of the
227 amniotic sac until birth (Noakes et al., 2001; Schuenemann et al., 2011). The average duration
228 of delivery was 71 min. Animals included in class D had the longest duration of labor ($P =$
229 0.002) and relatively higher assistance rate compared with animals of class M and E ($P = 0.05$)
230 while in contrast, they had similar length of pregnancy ($P = 0.93$) (Table 3).

231

232 ***Behavioral Data: Rumination and Lying Time***

233 All 22 cows for which rumination data were analyzed presented strong reductions in rumination
234 time on the day of calving. However, the class D cows showed lower RT values than the others,
235 namely, 176.3 min vs. 287.8 min and 353.7 minutes for classes D, M, and E, respectively ($P =$
236 0.012). Primiparous class D cows had the lowest absolute daily RT on calving day (154 min)
237 (Table 4). A decline of 68% of the value of RT recorded before calving (from -21d to -15d)
238 was observed in cows of class D on calving day, whereas RT of cows of class E decreased of
239 35% ($P = 0.005$). Moreover, class D cows maintained lower RT relative to those of class E and
240 M after parturition ($P = 0.031$; Figure 1). During the first week of lactation, primiparous class
241 D cows rested less than those of class M (8.6 h/d vs. 11 h/d; $P = 0.04$). In contrast, pluriparous
242 cows rested 10 h/d on average, without differences between animals of class D and those of
243 class E and M. Taking the primiparous and multiparous cows together, no differences ($P = 0.83$)

244 were detected between resting time of class D animals and those of the class E and M (Table
245 5).

246

247 ***Blood Parameters***

248 Complete results of plasma analysis measured before and after calving are reported in Table 6.

249 Figure 2 shows the evolution from 30 days before calving to 30 DIM of those variables that

250 differed ($P<0.05$) between classes. Inflammatory phenomena were evident in the class D cows.

251 These animals showed alterations in certain acute phase proteins compared to the class E and

252 M cows. In these animals, the negative acute-phase proteins (albumin, retinol, and

253 paraoxonase) decreased after parturition and remained low until 15 DIM or 30 DIM ($P<0.05$)

254 Among the positive acute-phase proteins, ceruloplasmin levels at 15 DIM were higher in class

255 M and D cows and differed from those for the class E cows ($P=0.002$). In contrast, haptoglobin

256 peaked 5 d after calving in all animals and decreased thereafter without differences between

257 classes. Markers of energy status (glucose, NEFA and BHBA) did not differ between animals

258 of different classes, except for fructosamine and creatinine that were lower ($P=0.02$) in the

259 class D cows at 5 and 15 DIM. On the contrary, no differences were found in ROM, tocopherol,

260 urea, bilirubin, calcium and zinc, GGT and cholesterol.

261

262 ***Milk Production***

263 Dam:calf BW ratio was not related with milk production nor milk composition (Table 7). Class

264 D cows apparently produced less milk than those of classes M and E. Nevertheless, these data

265 were biased by the comparatively large proportion of primiparous cows in this class. On the

266 other hand, class E was represented exclusively by pluriparous cows. The milk yield was

267 equivalent among the pluriparous cows of all three classes, while the average milk yields of

268 primiparous cows in classes M and D were 26 kg/d and 20 kg/d, respectively ($P=0.10$).

269 ***Health Events***

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

274

275

DISCUSSION

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

279 In particular, primiparous cows had the longest average calving time (125 min compared to 59
280 min for multiparous cows). A previous study reported relatively longer durations for the
281 dilation stage of calving in primiparous cows but no influence of parity on the expulsive phase
282 (Schuenemann et al., 2011). Therefore, D:C ratio and parity are strongly correlated. Here, the
283 heifers had lower D:C ratios than the multiparous cows. For this reason, the prolonged labor
284 observed in these animals may be explained by dam:calf size mismatch rather than parity per
285 se. Maternal-fetal disproportion is a main cause of dystocia in first-calving heifers (Mee, 2008).

286 It might account for the higher rates of assistance and greater lengths of labor recorded for the
287 class D animals in the present study (Meijering, 1984; Noakes et al., 2001; Mee et al., 2011).

288 Johanson and Berger (2003) found a strong correlation between dystocia and the ratio of calf
289 to dam body weight. For primiparous and multiparous cows, they calculated an average
290 calf:dam BW ratio of 7% and stated that the target ratio was 7.2% for optimal calf survival
291 (Johanson and Berger, 2003). These values correspond to a ratio of 14 for dam to calf BW.
292 Fiems et al. (2001) reported relatively higher incidences of cesarean sections for cows with an
293 average cow-to-calf ratio of 11.8. In contrast, those presenting with eutocic births had an

294 average cow-to-calf ratio of 14.4 (Fiems et al., 2001). According to these authors and
295 considering the longer labor and the higher rate of assistance of cows with lower dam:calf BW
296 ratio in the present study, we related the D:C ratio to potential calving difficulty. In particular,
297 cows with $D:C < 14$ (D) were considered to have a potential difficult calving, cows with 14
298 $< D:C < 17$ (M) as medium and cows with $D:C > 17$ (E) as easy.

299 These results confirm the importance of higher body weight at calving particularly for heifers
300 as they require good physical development at first calving (85% of mature body weight)
301 without fattening in order to avoid dystocia and metabolic diseases (Mee, 2009; Gaafar et al.,
302 2011). Nevertheless, a high BCS at calving must be avoided as it might increase the risk of
303 dystocia by narrowing the birth canal caused via fat deposition, as well as the risks of ketosis
304 and other diseases early in lactation (Roche et al., 2009).

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

314 In the present study, the dam:calf BW ratio was related with cow behavior such as lying and
315 rumination time during the postpartum period. Unexpectedly, evaluation of the lying behavior
316 around calving showed relatively lower resting times for primiparous class D cows during the
317 first week of lactation. On the contrary, we expected an increase in lying time for animals with
318 relatively longer and more difficult calving. Increased lying time is an illness-related behavior

319 induced by pro-inflammatory cytokines to promote lethargy and anorexia and accelerate
320 disease recovery (Johnson, 2002; Dantzer and Kelley, 2007). Certain studies report
321 comparatively higher lying times for cows affected by clinical or subclinical diseases
322 (Proudfoot et al., 2009; Sepúlveda-Varas et al., 2014). Nevertheless, another study stated that
323 lying time was not associated with health status in primiparous animals, although ketotic and
324 sick multiparous cows had longer lying times after calving than healthy cows (Piñeiro et al.,
325 2019). Moreover, certain authors reported shorter resting times for primiparous than
326 multiparous cows during the transition period (Sepúlveda-Varas et al., 2014; Neave et al.,
327 2017). Thus, the class D primiparous animals in our study had shorter resting times possibly
328 because of their lower hierarchical rank. Lactation stage, age, and body weight have the
329 strongest influences on social hierarchy (Dickson et al., 1970; Grant and Albright, 1995) and
330 fatigued primiparous cows in early postpartum are the most vulnerable to herd competition
331 (Grant and Albright, 1995; Phillips and Rind, 2001; Cook and Nordlund, 2004; Neave et al.,
332 2017). This situation is commonly reported in overcrowded environments where subordinate
333 cows are often displaced by dominant animals from the feed bunk and spend comparatively
334 more time standing without feeding or resting (Fregonesi et al., 2007). Neave et al. (2017)
335 studied the influence of parity on behavior of healthy cows during transition and reported that,
336 with 80% stocking density, primiparous cows were displaced by feeder more frequently than
337 multiparous cows and had more but shorter lying bouts. Excessive standing time could increase
338 the risks of hoof and metabolic disorders around calving. Stressors such as overcrowding and
339 lack of rest upregulate cortisol and DHEA (dehydroepiandrosterone) (Fustini et al., 2017).
340 During early postpartum, adipose tissue is sensitive to stress-related mediators that augment
341 lipolysis and plasma NEFA and increase the risks of metabolic diseases and unsuccessful
342 transition periods (Kushibiki et al., 2002, 2003; Underwood et al., 2003).

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

367 rumination times between days 3 and 6 of lactation and found that low-ruminating cows had
368 greater alterations in their acute-phase response than other cows.

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

383 The energy status markers (glucose, NEFA, and BHBA) showed negative energy balance and
384 lipid mobilization characteristic of transition cows without differences among classes, even
385 though fructosamine, that was previously related to the energy balance markers (Caré et al.,
386 2018) was lower in D animals. After calving, cows with lower D:C ratio exhibited also a more
387 pronounced decrease in plasma creatinine compared to other cows, suggesting an higher
388 muscle mobilization in cows of D class compared to cows of class M and E. Plasma creatinine
389 levels, in healthy euhydrated cows, is strictly related to muscle mass and it has been recently
390 suggested as a reliable index to monitor protein mobilization in periparturient cows (Wyss and
391 Kaddurah-Daouk, 2000; Megahed et al., 2019). Therefore, the lower plasma levels of

392 fructosamine and creatinine in D animals suggest a negative relation of dam:calf BW ratio with
393 the energy and protein balance of cows during the post partum period.
394 According to the blood indices, however, nearly all animals in this study showed alterations in
395 their inflammatory and metabolic profiles. These results confirm the drastic changes typical of
396 transition cows, characterized mainly by metabolic imbalance and inflammatory dysfunction
397 (Sordillo and Raphael, 2013; Bradford et al., 2015).

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CONCLUSIONS

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

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

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420

Conflict of Interest

422 The authors declare no conflict of interest.

423

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593 **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

594 ¹Minerals 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,
595 500 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
596 mg/Kg di vitamin E.

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

599

600 **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

601 ¹Dam and calf body weight ratio < 14.

602 ²Dam and calf body weight ratio between 14 and 17.

603 ³Dam and calf body weight ratio > 17.

604 **Table 3.** Dams and calves body weight (BW)¹, BCS² and calving characteristics of cows within
605 the 3 classes of dam:calf BW ratio (D:C) (D³, 8 cows; M⁴, 8 cows; E⁵, 9 cows). Mean values
606 are reported.

607

	All cows			Primiparous			Pluriparous			SEM	P-value		
	E	M	D	E	M	D	E	M	D		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		

608 ^{a,b,c} Values with different superscript letters within a row differ significantly ($P < 0.05$)

609 ¹Body weight recorded within 24 hours after calving.

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

611 ³Dam and calf body weight ratio < 14.

612 ⁴Dam and calf body weight ratio between 14 and 17.

613 ⁵Dam and calf body weight ratio > 17.

614

615 **Table 4.** Rumination time (RT) around calving of cows within the 3 classes of dam:calf BW ratio (D¹, M², E³). Mean values are reported.

	All cows			Primiparous			Pluriparous			SEM	P-value		Parity x Class
	E	M	D	E	M	D	E	M	D		Class	Parity	
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 1 st 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

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

617 ¹Dam and calf body weight ratio < 14.

618 ²Dam and calf body weight ratio between 14 and 17.

619 ³Dam and calf body weight ratio > 17.

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

621 **Table 5.** Lying time (means) of cows belonging to the 3 classes of dam:calf BW ratio (D¹, M²,
622 E³) during the first week of lactation (DIM 1-7).

	All cows			Primiparous			Pluriparous			SEM	P-value		
	E	M	D	E	M	D	E	M	D		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

623 ^{a,b} Values with different superscript letters within a row differ significantly within parity ($P < 0.05$).

624 ¹Dam and calf body weight ratio < 14.

625 ²Dam and calf body weight ratio between 14 and 17.

626 ³Dam and calf body weight ratio > 17.

627

628 **Table 6.** Plasma variables of cows belonging to the 3 classes of dam:calf BW ratio (D¹, 8 cows;
629 M², 8 cows; E³, 9 cows) before (Pre)⁴ and after (Post)⁵ calving. Means values are reported.

	E		M		D		SEM	P-value		
	Pre	Post	Pre	Post	Pre	Post		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 ₂ O 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

630 ¹Dam and calf body weight ratio < 14.

631 ²Dam and calf body weight ratio between 14 and 17.

632 ³Dam and calf body weight ratio > 17.

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

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

635 **Table 7.** Milk yield and composition (means) of cows belonging to the 3 classes of dam:calf
 636 BW ratio (D¹, M², E³) during the first month of lactation.

	All cows			Primiparous			Pluriparous			SEM	<i>P</i> -value		Class x Parity
	E	M	D	E	M	D	E	M	D		Class	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

637 ¹Dam and calf body weight ratio < 14.

638 ²Dam and calf body weight ratio between 14 and 17.

639 ³Dam and calf body weight ratio > 17.

640

641 **Table 8.** Cases of disease within 30 DIM of cows belonging to the 3 classes of dam:calf BW
 642 ratio (D¹, 8 cows; M², 8 cows; E³, 9 cows).

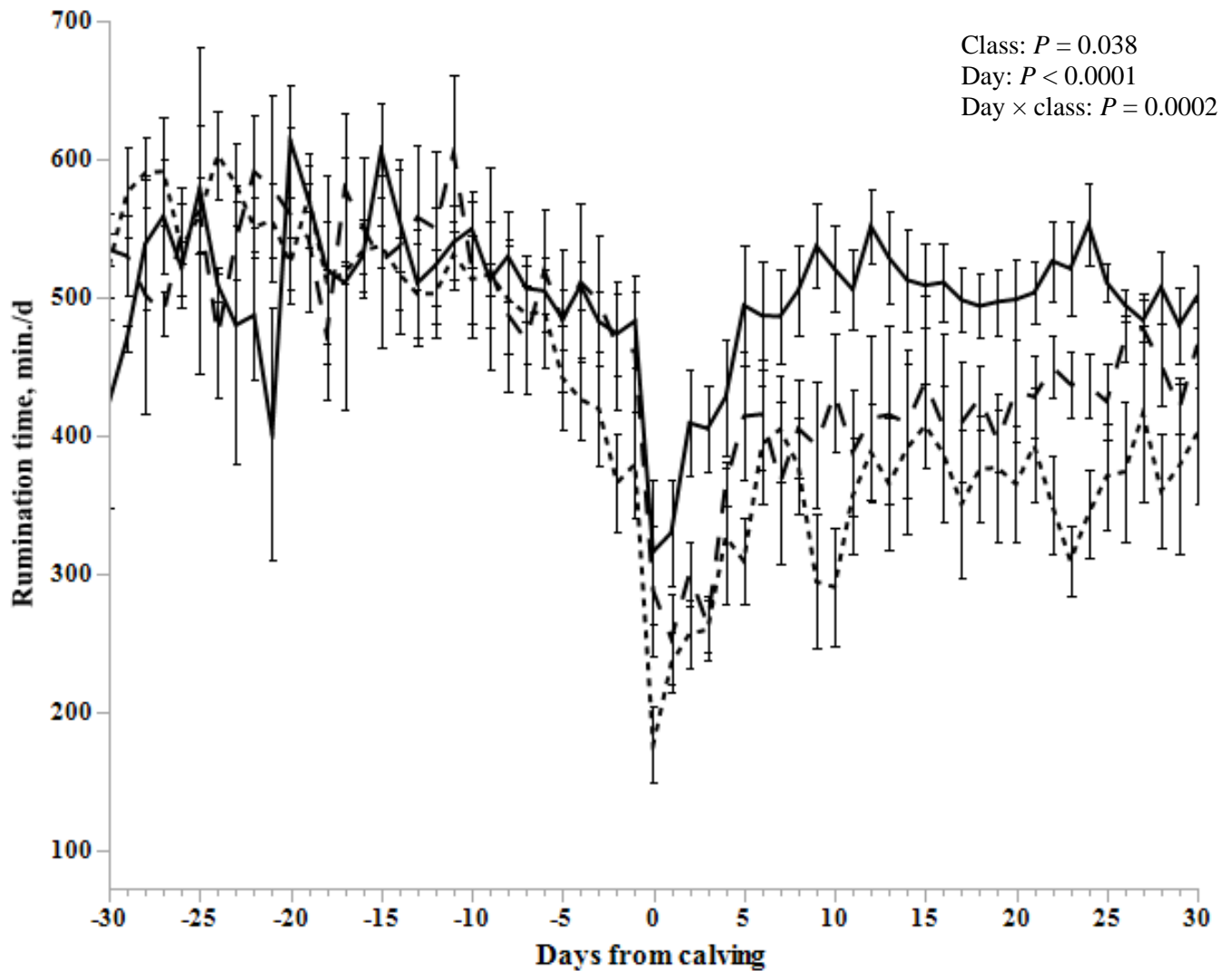
Cases, n	E	M	D	<i>P</i> -value
Retained fetal membranes	1	2	2	0.69
Metritis	1	1	2	0.82
Delayed uterine involution	1	1	2	0.82
Ketosis	3	3	4	0.88
Displaced abomasum	0	1	1	0.52
Mastitis	2	2	0	0.50
Absence of pathologies	4	2	1	0.47

643 ¹Dam and calf body weight ratio < 14.

644 ²Dam and calf body weight ratio between 14 and 17.

645 ³Dam and calf body weight ratio > 17.

646 **Figure 1.** Rumination time (LSM±SEM) of cows belonging to the 3 classes of dam:calf BW
647 ratio (..... D¹, difficult; - - M², medium; —E³,easy) from 30 days before calving to 30 DIM.



648

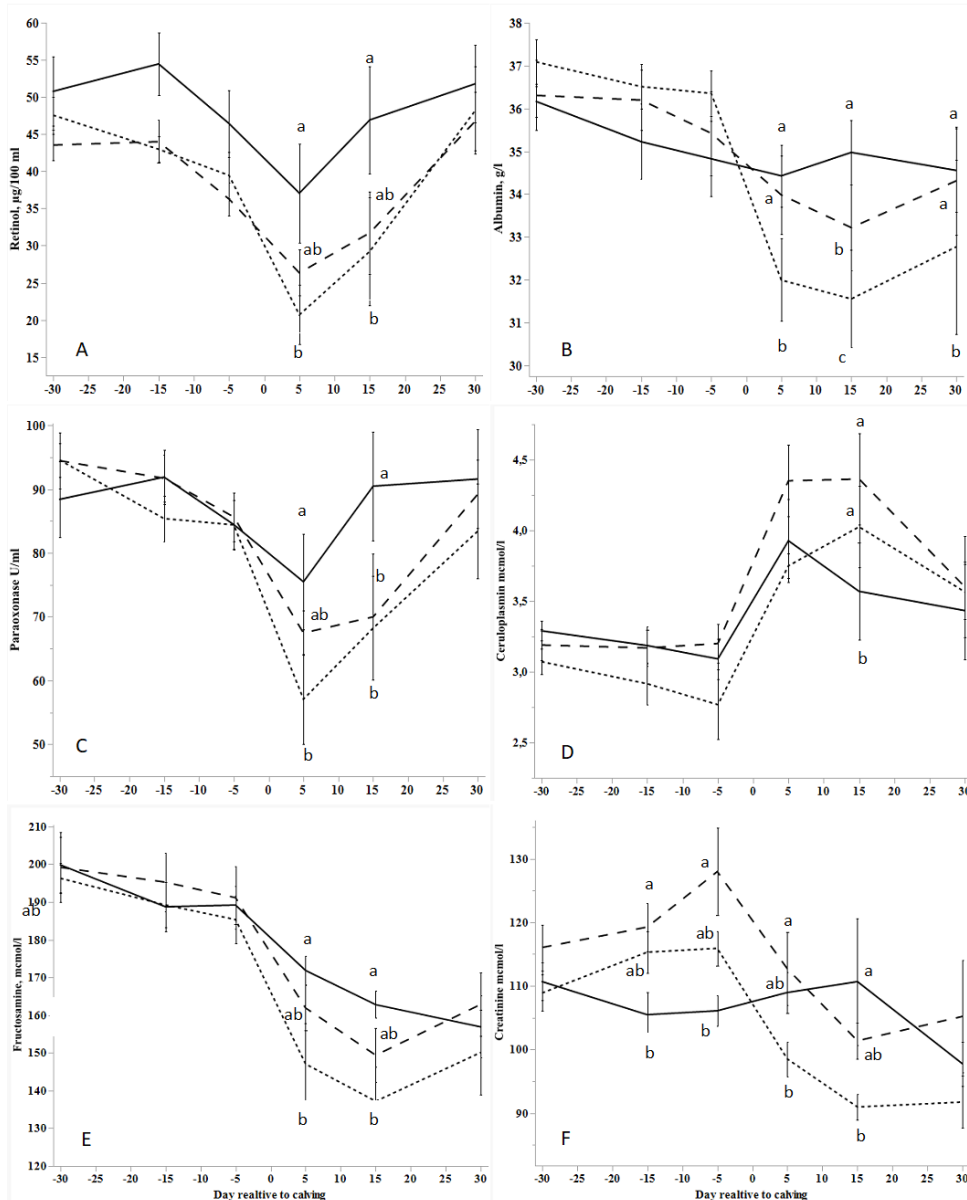
649 ¹Dam and calf body weight ratio < 14 (7 cows).

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

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

652

653 **Figure 2.** Pattern of plasma variables (retinol (A), albumin (B), paraoxonase (C),
 654 ceruloplasmin (D), fructosamine (E) and creatinine (F)) of cows belonging to the 3 classes of
 655 dam:calf BW ratio (....D¹, difficult; -- M², medium; — E³, easy) measured from 30 days
 656 before calving to 30 DIM. Least square means ±SEM are reported. Different letters at the same
 657 day indicate significant differences between classes (*P*<0.05).



658

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

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

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