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Martina Crociati[a], Lakamy Sylla[a], Claudia Floridi[a], Antonella Comin[b], Gabriele Fruganti[a], Maurizio Monaci[a], Giuseppe Stradaioli[b*]

[a]Department of Veterinary Medicine, Via San Costanzo, 4 - 06126 – Perugia, Italy;
[b]Department of Agricultural, Food, Environmental and Animal Sciences (DI4A), Via Delle Scienze, 206 - 33100 - Udine, Italy.

[*]Corresponding author: Giuseppe Stradaioli, Department of Agricultural, Food, Environmental and Animal Sciences (DI4A), Via Delle Scienze, 206 - 33100 Udine, Italy, Tel. +39 0432 558580, Fax. +39 0432 558503.
Email: giuseppe.stradaioli@uniud.it.

Author contributions: M.C., L.S., M.M., and G.S. designed the study. M.C. conducted most of the fields experimental procedures used in the study, i.e., the samples and data collection, the clinical classification of animals, and data analysis, and drafted the manuscript. C.F. performed lipoprotein and blood's biochemical analysis. A.C. performed P4 determinations. M.C. and L.S. performed data analysis and statistics. G.F., M.M., and G.S. supervised experiments and critically revised the manuscript. All authors have read and approved the manuscript.

Abstract

High-yielding dairy cows are metabolically challenged during transition, when intense mobilization and hepatic oxidation of lipids is achieved, thus leading to fatty infiltration, ketosis and generalized inflammation. The condition is associated to periparturient diseases and poor fertility. The aim of this study was to assess whether serum lipoprotein concentrations in the dry period could influence the occurrence of postpartum diseases and reproductive performance in dairy cows. The study was carried out on 30 multiparous Holstein Friesian cows. Blood samples were collected at dry-off (-60 days), 30 days after dry-off and within 12 hours after parturition for biochemical and serum lipoprotein assays. From 10 to 60 days after parturition milk was collected twice weekly after feeding, for milk whey progesterone assay. The Optimal Cutpoint package identified a threshold of 89% for serum High Density Lipoprotein (HDL) concentration at the beginning of the dry period with 95% of confidence interval. Cows with serum HDL greater than 89% (High group, n = 10) showed better reproductive performance when compared to those with low values (Low group, n = 18). The odds ratio for reproductive disorders in High group was 0.6875, however, differences were not significant probably due to both the reduced number of animals per group and overall low incidence of postpartum reproductive disease. First postpartum luteal activity occurred around day 23, while the second one between days 40 and 48. The average calving to first AI interval was 64.00 ± 3.95 days and 94.50 ± 12.32 days in High and Low group, respectively ($P < 0.05$). The calving-conception interval was 129.86 ± 24.42 days and 199.18 ± 24.73 days in High and Low groups, respectively ($P < 0.05$). Low group displayed an increase in liver markers, that is total bilirubin, with 0.46 ± 0.09 mg/dL and 0.23 ± 0.08 mg/dL, in Low and High group respectively ($P < 0.05$), and NEFA/cholesterol ratio, with 0.30 ± 0.06 and 0.14 ± 0.03, in Low and High groups, respectively ($P < 0.05$), at parturition. Concentrations of HDL > 89% at dry-off could be suggestive of improved liver adaptation to the transition, and probably of enhanced fertility in High group.

Key words: dairy cow; dry period; lipoprotein; fertility; reproductive disorders

1 Introduction

In the last weeks of gestation, cow's metabolism has to sustain fetal growth, mammary gland development and after calving the energy demand further increases due to lactation. Usually, during this period the dry matter intake is not sufficient to meet the requirements. In order to cope with this imbalance, dairy cows mobilize great amounts of body reserves, especially lipids [1]. Lipids are released from periphery into the circulation in the form of non-esterified fatty acids (NEFA) and then they are used in liver for gluconeogenesis and ketogenesis. If negative energy balance is excessive, the intake of NEFA overcomes the possibility of complete oxidation in the liver. In this case, NEFA are re-esterified, turned into triglycerides and stored into the cytoplasm of hepatocytes as lipid droplets. This condition, known as fatty liver, is usually associated to impairment of liver function, subclinical or clinical ketosis, periparturient metabolic diseases and poor fertility, which deeply influence the herd profitability [2-5]. Human and bovine hepatocytes are able to synthesize very low-density lipoprotein (VLDL), in order to export triglycerides towards peripheral tissues [6]. However, bovine liver is not able to adjust VLDL synthesis based on NEFA absorption and re-esterification into cytoplasmic droplets [2]; therefore, the prevalence of lipidosis among transition dairy cows can reach 50% [7-8]. Very-low density lipoprotein represents only 3 to 5% of all circulating lipoproteins in cattle, while other classes, such as high-density (HDL) and low-density lipoprotein (LDL), account for 80-85% and 10-15%, respectively. As liver can export triglycerides only through VLDL, it is generally assumed that low levels of circulating VLDL are representative of increased fatty acids infiltration [6, 9].

It has been reported a decrease of pregnancy at first artificial insemination when more than 50% of cows in a herd have serum NEFA $\geq$ 0.5 mEq/L one week before calving, as indicative of intense lipids mobilization [3]. An increased odds ratio for retention of fetal membranes and metritis in cows with prepartum NEFA $\geq$ 0.3 mEq/L was reported [4]. It has also been highlighted that the peculiar metabolic status of the dairy cows during periods of lipids mobilization could cause lipid accumulation in oocytes and the regenerating endometrium, which impairs fertility via reduction in

79 embryo survival and increased inflammatory changes, respectively [10]. The majority of studies
80 concerning the association between biochemistry profiles of dry cows in late gestation and
81 postpartum performance are focused on the last two or three weeks before calving [3-4, 9].
82 However, Dervishi et al. [11] reported alterations in inflammatory and metabolic profiles as early as
83 eight weeks before calving, in dairy cows that will develop metritis. Brscic et al. [12], recently
84 reported some reference limits for metabolic profiles in Holstein late-pregnant heifers and dry cows,
85 but postpartum performance was not assessed.
86 Moved by the need of finding new predictive indexes of reduced reproductive efficiency, we
87 hypothesized that the level of circulating lipoproteins during the last period of pregnancy could be
88 indicative of the degree of adaptation to transition of dairy cows and of their susceptibility to both
89 postpartum reproductive diseases and reduced fertility. Moreover, we evaluated biochemistry
90 profiles from dry-off until calving as a tool to verify the overall health status in our experimental
91 animals and to exclude alterations in lipoprotein metabolism due to subclinical pathological
92 conditions.
93
94 **2 Materials and Methods**
95 *2.1 Animals and husbandry*
96 In the present study, 30 healthy multiparous Holstein Friesian cows were selected with parity
97 ranging from 2 to 5; they belonged to a commercial dairy farm located in Central Italy (42°95′ N,
98 12°39′ E), with a herd size ranging between 100 and 120 lactating cows. Each cow was randomly
99 selected through the herd-management software before entering into the dry period. Mean heard
100 intercalving period was 410.27 ± 15.87 days, the mean dry period length was 60.25 ±10.14 days
101 and the voluntary waiting period averaged 50.16 ± 7.08 days. Average milk production was above
102 10,900 kg/lactation/cow. All lactating cows were housed in free stalls with cubicles and milked with
103 two Automatic milking systems (DeLaval S.p.A., VMS, Milano, Italy); dry cows were kept in a free
104 stall barn with straw. The dry cows had free access to a total mixed ration (TMR) offered *ad*

105 *libitum*, composed of 4.5 kg wheat straw (4.60% CP, 78.90% NDF, 48.40% ADF), 4.5 kg oat hay
106 (8.70% CP, 61.30% NDF, 38.20% ADF), 3.0 kg concentrate (28.50% CP, 20.20% NDF, 9.70%
107 ADF) per head. During the close-up period this ration was supplemented with 10 kg of fresh cow
108 TMR/head. The overall TMR composition was: 4 kg alfalfa and lolium mixed hay (7.67% CP,
109 37.86% NDF, 20.84% ADF), 3.5 kg alfalfa hay (14.91% CP, 42.70% NDF, 33.10% ADF), 11.5 kg
110 concentrate (28.50% CP, 20.20% NDF, 9.70% ADF), 22 kg corn silage (9.20% CP, 45.90% NDF,
111 27.00% ADF) and 0.8 kg molasses (4.30 % CP, 0% NDF, 0% ADF). Mycotoxins content in feed
112 was within the legislative established limits (Italian Law 149/2004). Contents of organic acid in
113 silage and total mixed ration from dry cow feed-bunk were evaluated by HPLC analysis. Only
114 butyric acid was fairly present in both silage and total mixed ration, with mean values of 0.02 ±
115 0.01 and 0.03 ± 0.001 g/100g, respectively.
116

117 *2.2 Experimental procedures and samples collection*
118 The study was conducted from January to December 2015; all experimental cows calved before the
119 end of April 2015. Two cows were excluded from the experiment due to abortion and premature
120 calving. Body condition score (BCS), assessed through a five-point scale [13], and blood sampling
121 were performed at 60 (T1) and 30 days (T2) before the expected calving and within 12 hours
122 postpartum (T3). Samples were obtained by coccygeal vein puncture into plain and EDTA vacuum
123 tubes (BD Vacutainer Systems, Plymouth, UK) between 5.30 and 6.00 AM, half an hour before
124 feeding. Samples were stored at 4°C and delivered within 1 hour to the Laboratory; serum was
125 obtained through centrifugation at 1,300 g for 10 minutes and immediately processed. An aliquot of
126 serum from each sample was stored at 4°C until electrophoresis analysis. From 10 to 60 days after
127 parturition milk was collected twice weekly after feeding, between 6.00 and 7.00 AM, in empty
128 eppendorf tubes and immediately frozen at -20°C, until analysis.
129 To evaluate healthy condition of the newborn calf, the APGAR score described by Vannucchi et al.

130 [14] was employed. Briefly, the following symptoms were considered and scored: mucous color (0
131 = cyanotic; 1 = pale; 2 = normal), heart rate (0 = absent; 1 = bradycardia, < 80 beats per minute or
132 irregular; 2 = regular, > 100 beats per minute), muscle tone (0 = flaccid; 1 = slight flexion; 2 =
133 flexion), activity (0 = absent; 1 = some movement; 2 = active calf), and respiration (0 = absent; 1 =
134 irregular < 24 respiration per minute; 2 = regular > 36 respiration per minute).
135 Postpartum uterine diseases were diagnosed according to Sheldon et al. [15]. Briefly, animals were
136 monitored once weekly after calving until complete uterine involution was achieved. The fetal
137 membranes were considered retained when not released within 12 hours after parturition. Cows that
138 presented an enlarged uterus with watery red-brown to viscous off-white purulent uterine discharge,
139 from 0 to 21 days after calving were considered suffering from metritis. Clinical endometritis was
140 defined by the presence of pathological uterine discharge 21 days or more postpartum.
141 Reproductive parameters such as calving to first insemination interval, calving to conception
142 interval and number of inseminations per pregnancy were retrieved from the herd management
143 software, at least until 200 days after parturition.
144 The experimental activity was carried out in accordance to the guidelines of animals experiments as
145 set by the Italian Law 26/2014 (national application of EU Directive 2010/63/EU) and has been
146 approved by the Ethical Committee of the University of Perugia.
147
148 *2.3 Serum lipoprotein's electrophoresis, biochemical profiles and CBC*
149 Electrophoresis of serum lipoproteins was obtained with the Hydrasys – LC Sebia automatic system
150 and Hydragel 7 LIPO + Lp(a) Kit (Sebia® Electrophoresis, Sebia Inc., Norcross, GA USA). Briefly,
151 lipoprotein classes were separated by electrophoresis on agarose gel buffered plates (pH 8.5);
152 different bands were then read with densitometry scanner at 570 nm (Epson Perfection V700
153 PHOTO, Seiko Epson Corporation, Japan) and lipoproteins were expressed in term of relative
154 percentage. Three main bands were identified, namely HDL (or α-lipoproteins), VLDL (or pre-β-
155 lipoproteins) and LDL (or β-lipoproteins).

156 The serum concentrations of albumin (ALB), β -hydroxybutyrate (BHB), total proteins (TP),
157 triglycerides (TG), total bilirubin, direct bilirubin, indirect bilirubin, glucose, urea, creatinine, lactic
158 dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), non-esterified
159 fatty acids (NEFA), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), creatine
160 kinase (CK), cholesterol (CHOL), magnesium (Mg), calcium (Ca) and phosphorus (P) were
161 determined with an Hitachi 904 chemistry analyzer (Boehringer Mannheim, Germany).
162 All diagnostic kits were purchased from DiaSys – Diagnostic Systems (GmbH, Alte Strasse 9, D –
163 65558 Holzheim, Germany). Count of blood cells (CBC) was obtained in EDTA samples with a
164 Heco Vet S (SEAC, Florence, Italy) electric impedance system.
165
166 *2.4 Milk whey progesterone assay*
167 Milk progesterone was quantified into the whey fraction through direct enzyme immunoassay as
168 described by Comin et al. [16]. Briefly, whey aliquots were added to micro plates, with 25 µL/well
169 of anti-progesterone antibody with 50 µL of progesterone-peroxidase conjugate in ELISA buffer.
170 Plates were incubated one night at 4°C and then washed five times with buffer solution.
171 Chromogenic substrate was added and plates were incubated for 30 minutes at room temperature.
172 Finally, absorbance was read at 450 nm using a plate reader. Resumption of ovarian activity was
173 considered when progesterone concentrations was above 300 pg/mL [16], and presence of a corpus
174 luteum was confirmed by trans rectal ultrasound examination.
175
176 *2.5 Statistical analysis*
177 Data were analyzed through SPSS software [17]. Normal distribution was assessed for each
178 biochemical and reproductive outcome through Kolmogorov-Smirnov test with 95% of confidence
179 interval. However, due to the limited number of animals involved and in order to be conservative,
180 non-parametric statistics were used. All tests were set with $\alpha < 0.05$. Data were expressed through
181 mean and standard error of the mean (SEM). Differences between groups were analyzed with

Kruskal-Wallis test. R software v3.2.4 [18] was used to run Optimal Cutpoints package [19] in order to estimate threshold in lipoproteins concentration at T1, T2 and T3 in relation to postpartum diseases. The optimal cutpoint for the diagnostic test (HDL, VLDL and LDL in each of the three periods) was computed by means of a Receiver Operating Characteristic (ROC), using the disease status (e.g. sick or healthy) as dependent categorical variable and optimizing the Younden's Index, so that the sum of Specificity and Sensitivity was maximized. Cows were then assigned to Low and High HDL groups; differences in reproductive outcomes and mean progesterone concentrations were investigated with Kruskal Wallis test in SPSS. If any significant difference was found between High and Low HDL cows, data were discussed as belonging to a single group based on sampling interval.

3 Results

3.1 Clinical outcomes and resumption of ovarian activity

Cows entered the dry period with a mean BCS of 3.23 ± 0.07 that decreased to 3.04 ± 0.05 at calving ($P < 0.05$). Nineteen out of 28 animals had normal postpartum while 3 cows showed retained fetal membranes and 6 metritis. All calving were eutocic and all calves were born healthy and with a mean APGAR score of 8.21± 1.15.

Left abomasal displacement was observed in 3 animals without any reproductive disorder.

Milk whey progesterone in High (n = 10) and Low (n = 18) group of cows ranged between 150 and 500 pg/mL, but no significant difference was identified. First postpartum luteal activity occurred around day 23, while the second one from day 40 to 48. One animal in High and one in the Low group revealed an increase in P4 concentrations only after 50 days post calving. One cow per group also showed a prolonged period of elevated milk P4.

3.2 *Biochemistry profiles and CBC*

208 *3.2.1 Serum lipoprotein electrophoresis*

209 Relative proportion of lipoproteins in experimental dairy cows is reported in Table 1. VLDL
210 lipoproteins ranged between 3.27 ± 0.29 % and 2.69 ± 1.43 %, without any difference between
211 groups; HDL decreased ($P < 0.001$) from dry-off (87.52 ± 0.87 %) towards T2 (82.41 ± 1.21 %),
212 then increased at calving (87.39 ± 1.07 %). LDL lipoproteins presented a reverse trend; their
213 concentration was greater at T2, with mean value of 14.32 ± 1.18 % ($P < 0.01$).
214 The Optimal Cutpoint package identified a threshold of 89% for serum HDL concentration at the
215 beginning of the dry period with 95% of confidence interval. As shown in Table 2, cows with serum
216 HDL greater than 89% (n = 10) tended to show better reproductive performances when compared to
217 others (n = 18). More deeply, the average calving to first AI interval was 64.00 ± 3.95 days and
218 94.50 ± 12.32 days in High and Low group, respectively ($P < 0.05$). The calving - conception
219 intervals were 129.86 ± 24.42 days and 199.18 ± 24.73 days in High and Low groups, respectively
220 ($P < 0.05$). The odds ratio for reproductive disorders in High HDL cows was 0.6875, even if the P
221 was not significant.
222
223 *3.2.2 Energy metabolism analytes*
224 Beta-hydroxybutyrate, glucose, NEFA and triglyceride concentration are reported in Table 3. Beta-
225 hydroxybutyrate was constantly below reference limit of 1.40 mmol/L and did not show any
226 significant variation throughout the period of study. Mean serum glucose level decreased from
227 66.11 ± 1.85 mg/dL at T1 to 55.75 ± 2.24 mg/dL at parturition ($P < 0.001$) while NEFA increased
228 from 0.18 ± 0.02 mmol/L at dry-off to 0.58 ± 0.08 mmol/L at calving ($P < 0.001$). Triglycerides
229 increased both at T1 and T2, while they declined at calving time ($P < 0.001$).
230
231 *3.2.3 Markers of liver function*
232 Low group displayed significantly higher concentration of both total and indirect bilirubin, and
233 NEFA/CHOL ratio than High group, only at calving (Table 4).

In all cows, urea and LDH did not show any significant variation during the dry period. Aspartate transaminase activity was higher at calving, with a mean value of 106.57 ± 6.98 UI/L, compared to what found during the dry period ($P < 0.001$). Concerning GGT, it decreased at parturition with a mean value of 15.22 ± 1.31 UI/L ($P < 0.05$) and ALT showed a similar trend, averaging 20.21 ± 0.96 UI/L at calving ($P < 0.001$). Cholesterol declined progressively from 192.79 ± 5.30 mg/dL to 112.57 ± 6.96 mg/dL ($P < 0.001$; Table 5).

3.2.4 Protein metabolism analytes

Protein metabolism analyses are reported in Table 6. Albumin and CK did not show any significant variation throughout the period of study. Conversely, total serum proteins decreased from T1 (7.56 ± 0.16 g/dL) until parturition, when they reached an average value of 6.43 ± 0.11 g/dL ($P < 0.001$); CK remained constant and averaged 265.69 ± 52.59 UI/L throughout the study.

3.2.5 Mineral metabolism and other analytes

Mineral and other analytes are reported in Table 7. At dry-off, calcium and phosphorus averaged 9.65 ± 0.12 mg/dL and 6.55 ± 0.28 mg/dL, respectively; thereafter they decreased at calving, with mean values of 8.80 ± 0.29 mg/dL ($P < 0.05$) and 4.83 ± 0.28 mg/dL ($P < 0.001$), respectively. Alkaline phosphatase activity was higher at dry-off, with a mean value of 84.57 ± 4.68 UI/L and then decreased to 64.46 ± 5.04 UI/L at calving ($P < 0.001$). Creatinine constantly averaged 1.25 ± 0.06mg/dL. Magnesium and Creatinine did not show any significant variation throughout the period of study.

3.2.6 Count blood cells

The CBC features were within the referenced limits [20] and there were any significant difference among groups (data not shown).

4 Discussion

The transition dairy cow has to cope with negative energy balance, lipomobilization and fatty liver, all of them influencing the susceptibility to postpartum diseases and the reproductive outcomes. However, there is lack of data concerning lipoprotein metabolism during the full dry period and their interaction with postpartum health and fertility.

High-density lipoproteins, herein reported, were greater at dry-off and at calving, compared to T2, while LDL reached a peak one month before expected delivery. This opposite trend could be due to LDL peripheral catabolism, as mammary gland absorbs triglycerides from LDL for milk fatty acids synthesis; in the middle of dry period, this metabolism is not active and LDL is accumulated, thus determining a relative decline of HDL concentration. We also observed a progressive decline of cholesterol until reaching the lower values at calving, as also previously reported [7, 12, 21-24]. Generally, it is accepted that cholesterol is representative of liver synthesis of VLDL and that a decline in VLDL concentration is indicative of poor adaptation of the liver to fat mobilization, even if currently, there is no reference limit for VLDL in dry high-producing dairy cows [9, 21, 25-26]. Through Optimal Cutpoint package, we defined a cut-off value of 89% of serum HDL at the beginning of the dry period as a possible threshold to identify cows, which are more prone to experience postpartum reproductive diseases. Cows belonging to High group presented better calving to first AI and calving to conception intervals when compared to Low group ($P<0.05$). Cows with a greater concentrations of HDL had also lower incidence of retention of fetal membranes and/or metritis, with an odds ratio of 0.6875; however, differences were not significant probably due to both the reduced number of animal per group and the overall low incidence of postpartum reproductive diseases. In the present study, in fact, the prevalence of fetal membrane retention and/or metritis was 32%, which was lower than the rate of 36-50% reported by Sheldon et al. [15]. At parturition, bilirubin and NEFA/CHOL ratio were higher in Low group, as indicative of increased liver metabolic load, even if values remained within reference limits [9, 22].

All cows resumed ovarian activity within the first 3 week s postpartum. Only one cow per group showed a prolonged period of milk P4 due to persistent ovarian luteal cyst. Emergence and growth of first follicular wave after calving is regulated by nutritional status and energy availability which also influence hypothalamic release of gonadotropin releasing hormone [27]. Since resumption of ovarian activity was similar between groups, we hypothesized that serum lipoproteins did not influence the hypothalamic-pituitary-gonadal axis.

The reduction in reproductive efficiency observed in the Low group could be related to oocyte-embryo quality. Considering that dairy cows have to cope with generalized inflammatory condition 4-5 weeks before calving [23] and that HDL have anti-inflammatory activity [29], we hypothesized that their high concentration in dry cows could protect primordial ovarian follicles against metabolic stress, enhancing oocyte quality, and increasing fertility rate, as observed in High group. In relation to postpartum follicular development and their steroidogenic capacity, Golini et al. [28] showed that there is a down-regulation of genes involved in cell proliferation and steroidogenesis of granulosa cells harvested from preovulatory follicles collected from early lactating cows, until 90 days after calving. Moreover, results reported by Wu et al. [30] show that ovarian cells preferentially use HDL-bound cholesterol for steroidogenesis, suggesting their fundamental role in follicle development and maturation. Even if those results are referred to active and growing follicles, it could be speculated that decreased HDL availability in conjunction with lower follicle's steroidogenic capacity and pro-oxidant environment could negatively affect cholesterol absorption storage and metabolism in ovarian follicles during the dry period.

To our knowledge, only other two studies reported data about concentration of lipoproteins in dairy cows during dry period [7, 29]. The lack of uniformity in separation and detection methods (electrophoresis *versus* centrifugation) or the investigation of lipoprotein concentration during lactation makes comparison with some studies difficult [25, 31]. Bernabucci et al. [7] used the same analytical procedure as in the present study, but they considered a different period of samples collection (35 days before calving and 3 and 30 days after calving). They reported a lower

312 proportion of serum VLDL and higher HDL after calving, while LDL did not significantly change
313 during the transition period. This difference could be due to the matrix used for the test, since
314 Bernabucci et al. [7] worked on plasma while Sebia Lipo Lp (a) electrophoresis method is
315 standardized for serum. Newman et al. [29] investigated the effect of several diets with different
316 energy density on serum lipoprotein composition, from week 6 before expected calving until week
317 6 of lactation. The study [29] was aimed to verify if a provision of adequate total metabolizable
318 energy would better allow the cow to maintain total cholesterol and an HDL-rich profile throughout
319 the transition period while concurrently limiting the rise in NEFA. However, they did not find
320 influence of energy density in the diet on lipoprotein. This suggests that lipoprotein metabolism is
321 dependent on liver and peripheral secretion and absorption rather than on feeding management.
322 They reported a decline in HDL from week 6 until week 1 before calving and a subsequent increase.
323 In their study, mean concentrations of HDL at the beginning and at the end of the dry period were
324 similar to those found in the present work, for instance 80-85%. Since in their study VLDL was not
325 detectable, LDL relative concentrations were complementary to that of HDL, thus ranging from
326 15% to 25%. This difference could be due to the methods used, such as the duration of
327 electrophoresis and migration, the voltage applied or the staining technique, which may affect the
328 degree of lipoprotein separation and identification.
329 All other analysis carried out to verify animal's overall health status and to exclude alterations in
330 lipoprotein metabolism due to subclinical pathological conditions confirmed the good health
331 condition of the cows. Only during the last month before calving some modifications, indicated a
332 certain degree of lipid mobilization and reactivation of liver function, typical of the dairy cows
333 during the transition period. In particular, BCS was evaluated to assess the overall energy balance
334 and it was fairly constant, while mean NEFA were within reference limit of 0.4 mmol/L, as reported
335 in pending calving cows [4, 9, 32]. Only at calving BCS significantly decreased, as generally
336 described in transition dairy cows [4, 24, 33-34]. Correspondingly, NEFA increased, but remained
337 lower than the threshold value of 0.7 mmol/L established for the postpartum cows [32]. Triglyceride

338 decreased significantly from dry-off to calving, as previously observed by Weber et al. [24]. Herein,
339 cows showed normal glucose concentrations throughout the dry period, similarly to what reported
340 by Weber et al. [24]; however, a significant decrease was found at parturition. We observed BHB
341 was constantly greater than 1.00 mmol/L but below the threshold value of 1.20 - 1.40 mmol/L. Feed
342 analysis excluded the presence of ketogenetic compounds, as only butyric acid was fairly present in
343 both silage and total mixed ration. Subclinical ketosis diagnosis has been defined by BHB
344 concentrations of 1200 or 1400 μmol/L [35-37]. Although BHB above 0.96 mmol/L is associated
345 with increased risk of postpartum disease [38-39], prepartum BHB concentrations are not predictive
346 of disease [4].
347 Concerning the other analytes, mean concentrations of GGT, ALT and TP were in agreement with
348 results reported by Brscic et al. [12], without significant difference between groups. The increase of
349 mean AST levels toward calving in all our experimental groups could be representative of increased
350 muscle labour. As ALP, Ca and P concentrations decreased at parturition but remained within
351 reference limits; we excluded subclinical hypocalcemia or other mineral imbalance.
352

353 **5 Conclusions**

354 The growing knowledge on the role of lipid metabolism in the adaptation of dairy cows to the
355 transition lead to an increased interest in lipoproteins. In our study, animal with HDL levels greater
356 than 89% at dry-off showed better reproductive performance when compared to the other
357 experimental group. We suggest that HDL due to their role of main carrier of cholesterol in ovarian
358 steroidogenetic cells and to anti-inflammatory and antioxidant activities can exert some influences
359 on liver adaptation to the transition and on the fertility in cows. However, future studies are needed
360 to improve our knowledge on lipoprotein metabolism and to validate the quantification of HDL in
361 dry and transition phases using larger groups of animals and under different management or
362 nutritional conditions.
363

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460 Veterinaria Scandinavica 2003;44(Suppl 1):28.
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462 Table 1. Relative proportion of lipoproteins in the 28 experimental dairy cows.

%	Time	Mean ± SEM	P
High-density lipoproteins	T1	87.52 ± 0.88[a]	P < 0.001
	T2	82.41 ± 1.21[b]	
	T3	87.39 ± 1.07[a]	
Low-density lipoproteins	T1	9.65 ± 0.71[a]	P < 0.01
	T2	14.32 ± 1.18[b]	
	T3	9.92 ± 1.10[a]	
Very low-density lipoproteins	T1	2.83 ± 0.29	NS
	T2	3.27 ± 0.29	
	T3	2.69 ± 1.43	

463 T1 = 60 days before expected calving; T2 = 30 days before expected calving; T3 = calving; P =
464 Kruskal-Wallis test significance; NS = not significant. [a,b] = Values with different superscripts are
465 significantly different.

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Table 2. Mean (± SEM) reproductive outcomes in dairy cows of the Low and High HDL groups.

	Cut-off HDL > 89.0 %					
	n	HDL (%)	C - 1AI (days)	C-C (days)	AI (n°)	OR
Low	18	85.07 ± 0.88	94.50 ± 12.32	199.18 ± 24.73	3.36 ± 0.58	
High	10	91.94 ± 0.67	64.00 ± 3.95	129.86 ± 24.42	3.67 ± 0.78	0.6875
P		P < 0.001	P < 0.05	P < 0.05	NS	NS

n = number of animals; C – 1AI = calving to first artificial insemination interval; C – C = calving to conception interval; n° AI = number of artificial insemination per pregnancy; OR = odds ratio for postpartum uterine diseases; P = Kruskal-Wallis test significance; NS = not significant.

478 Table 3. Mean (± SEM) values of energy metabolism analytes in the 28 experimental dairy cows 60
479 (T1) and 30 (T2) days before expected calving, and at calving (T3).

	Time	Values	P
BHB (mmol/L)	T1	1.16 ± 0.08	NS
	T2	1.29 ± 0.85	
	T3	1.03 ± 0.53	
NEFA (mmol/L)	T1	0.18 ± 0.02[a]	P < 0.001
	T2	0.24 ± 0.04[a]	
	T3	0.58 ± 0.08[b]	
Glucose (mg/dL)	T1	66.10 ± 1.85[a]	P < 0.001
	T2	62.21 ± 1.73[a]	
	T3	55.75 ± 2.24[b]	
Triglycerides (mg/dL)	T1	16.32 ± 1.51[a]	P < 0.001
	T2	18.39 ± 1.13[a]	
	T3	11.41 ± 1.20[b]	

480 P = Kruskal-Wallis test significance; NS = not significant. [a,b] = Values with different superscripts are
481 significantly different.
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Table 4. Mean (±SEM) bilirubin and NEFA/CHOL ratio in the experimental dairy cows of Low and High HDL groups at calving.

Cut-Off HDL > 89%				
	n	T-Bil	Ind-Bil	NEFA/CHOL
Low	18	0.46 ± 0.09	0.27 ± 0.07	0.30 ± 0.06
High	10	0.23 ± 0.08	0.12 ± 0.05	0.14 ± 0.03
P		P < 0.05	P < 0.05	P < 0.05

n = number of animals; T-Bil = total bilirubin; Ind-Bil = indirect bilirubin; NEFA/CHOL = NEFA: cholesterol ratio; P = Kruskal-Wallis test significance; NS = not significant.

493 Table 5. Mean (± SEM) hepatic function analytes values in the 28 experimental dairy cows 60 (T1)
494 and 30 (T2) days before expected calving, and at calving (T3).

	Time	Values	P
Urea (mg/dL)	T1	29.39 ± 1.54	NS
	T2	30.54 ± 1.89	
	T3	30.89 ± 4.03	
GGT (U/L)	T1	21.00 ± 2.48[a]	P < 0.05
	T2	16.71 ± 1.68[b]	
	T3	15.22 ± 1.31[b]	
LDH (U/L)	T1	883.21 ± 87.59	NS
	T2	1113.04 ± 36.80	
	T3	1174.71 ± 60.14	
AST (U/L)	T1	82.36 ± 3.95[a]	P < 0.001
	T2	77.00 ± 3.93[a]	
	T3	106.57 ± 6.98[b]	
ALT (U/L)	T1	29.21 ± 0.97[a]	P < 0.001
	T2	24.54 ± 1.11[b]	
	T3	20.21 ± 0.96[b]	
Cholesterol (mg/dL)	T1	192.79 ± 5.30[a]	P < 0.001
	T2	148.54 ± 5.72[b]	
	T3	112.57 ± 6.97[c]	

495 P = Kruskal-Wallis test significance; NS = not significant. [a,b,c] = Values with different superscripts
496 are significantly different.

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498 Table 6. Mean (± SEM) protein metabolism analytes values in the 28 experimental dairy cows 60

499 (T1) and 30 (T2) days before expected calving, and at calving (T3).

	Time	Values	P
Albumin (g/L)	T1	4.10 ± 0.08	NS
	T2	4.04 ± 0.08	
	T3	4.05 ± 0.08	
Total Proteins (g/L)	T1	7.56 ± 0.16[a]	P < 0.001
	T2	7.11 ± 0.13[a]	
	T3	6.43 ± 0.11[b]	
CK (U/L)	T1	246.96 ± 59.40	NS
	T2	340.34 ± 144.31	
	T3	209.76 ± 28.52	

500 P = Kruskal-Wallis test significance; NS = not significant. [a,b] = Values with different superscripts are
501 significantly different.

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505 Table 7. Mean (± SEM) mineral metabolism and non-specific analytes values in the 28 experimental
506 dairy cows 60 (T1) and 30 (T2) days before expected calving, and at calving (T3).

	Time	Values	P
Creatinine (mg/dL)	T1	1.16 ± 0.03	NS
	T2	1.22 ± 0.05	
	T3	1.38 ± 0.18	
ALP (U/L)	T1	84.57 ± 4.68[a]	P < 0.001
	T2	76.07 ± 5.72[a]	
	T3	64.46 ± 5.05[b]	
Calcium (mg/dL)	T1	9.65 ± 0.12[a]	P < 0.05
	T2	9.34 ± 0.21[a]	
	T3	8.80 ± 0.29[b]	
Magnesium (mg/dL)	T1	2.51 ± 0.11	NS
	T2	2.31 ± 0.08	
	T3	2.39 ± 0.62	
Phosphorous (mg/dL)	T1	6.55 ± 0.28[a]	P < 0.001
	T2	6.40 ± 0.17[a]	
	T3	4.83 ± 0.28[b]	

507 P = Kruskal-Wallis test significance; NS = not significant. [a,b] = Values with different superscripts are
508 significantly different.

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