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Metabolic predictors of peri-partum diseases and their association with parity in dairy cows

Gretel Ruprechter[a,*], Maria de Lourdes Adrien[b], Alejandro Larriestra[c], Otavio Meotti[d], Chester Batista[e], Ana Meikle[a], Mirela Noro[d]

[a] *Animal Endocrine and Metabolism Laboratory, Veterinary Faculty, Montevideo, Uruguay*
[b] *Health in Livestock Systems Department, Veterinary Faculty, Paysandú, Uruguay*
[c] *Veterinary Faculty, National University of Rio Cuarto, Argentina*
[d] *Veterinary Faculty, Federal University of Pampa, Uruguaiana, Brazil*
[e] *Faculty of Veterinary Sciences, The Federal University of Rio Grande do Sul, Brazil*

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ABSTRACT

The predictive values of plasma non-esterified fatty acid (NEFA), beta-hydroxybutyrate (BHB), cholesterol, albumin and calcium to predict risk of peripartum diseases in primiparous (PP) and multiparous (MP) Holstein cows was investigated. Besides it was assessed if the health status interacted with parity on body condition score and metabolic profiles during the transition period. Dairy cows (126 PP and 182 MP) from a commercial dairy *free stall* herd (loose-housing system) were weekly body condition scored and tail bled for metabolites determination from −3 to +4 weeks relative to calving. Peripartum diseases were diagnosed by a single trained veterinarian, while subclinical diseases (ketosis and hypocalcemia) were diagnosed at the laboratory. Cows were classified as healthy cows, cows with one event, or cows with two clinical events following a prospective observational cohort study, with only healthy cows enrolled at the beginning of the study. The largest incidence was for metritis (26.6%) followed by retained placenta (17.2%) and mastitis (15.2%) with no effect of parity, while subclinical hypocalcemia incidence was greater in MP than PP cows (43% vs 9.5%) respectively. In MP cows albumin concentrations were predictive for metritis at week −2 and for retained placenta at weeks −2 and −1, while cholesterol was predictive for mastitis at week −2, −1 and at calving. The interaction between health status and parity affected all metabolites during the transition period. This study showed a different evolution of metabolic profiles in healthy and sick cows during the transition according to parity, pointing out albumin and cholesterol as diseases predictors before calving.

1. Introduction

Over the past decades dairy cows have undergone intensive genetic selection, which has increased milk yield to a level where the demands for nutrients from the diet and body tissue reserves often results in ill-health and infertility (Mulligan and Doherty, 2008). The relationship of 'the transition cow' metabolism and the pathogenesis of peri-partum diseases has increased, and maintaining health and productivity is one of the most difficult tasks for dairy herds. Approximately 75% of diseases in dairy cows typically happen in the first month after calving (LeBlanc et al., 2006). These problems are increasingly known to be rooted in immune function and feed intake 2 to 3 wks before calving, arguing for the importance of nutritional management of the transition dairy cow (LeBlanc et al., 2006).

The negative energy balance (NEB) that takes place during this period is associated with the development of many of these diseases (Herdt, 2000). Body condition score (BCS) during the transition period and the risk of peri-partum diseases has been established; e.g., cows with BCS ≥ 3.75 have a higher risk to develop clinical ketosis or displacement of the abomasum (DA) (Seifi et al., 2011). Moreover, a direct effect of BCS on feed intake was suggested (Garnsworthy, 2006); e.g., cows with BCS ≤ 3.25 at calving lost less BCS after calving and reached their maximum dry matter intake (DMI) earlier than cows with higher BCS. Parity is also a well-known risk factor for some diseases; e.g., multiparous cows are more likely to develop ketosis and hypocalcemia (Seifi et al., 2011; Reinhardt et al., 2011). As NEB is reflected in some metabolites, many metabolic markers can be used for evaluating the cow adaptation to the NEB and the risk of peri-partum diseases (Van Saun, 2009). Elevated serum non-esterified fatty acid (NEFA) concentrations 7 to 10 days pre-partum is a risk factor for DA (LeBlanc

* Corresponding author.
E-mail address: gcruprechter@gmail.com (G. Ruprechter).

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et al., 2005). Also elevated serum post-partum β-hydroxybutyrate (BHB) concentrations were reported as a risk factor for ketosis (Duffield et al., 2005; LeBlanc et al., 2005), metritis and mastitis (Duffield et al., 2009). Moreover, subclinical hypocalcemia may make cows more susceptible to secondary diseases (Reinhardt et al., 2011), as it reduces the ability of immune cells to respond to stimuli (Kimura et al., 2006). This could contribute to infections, such as mastitis or metritis (Martinez et al., 2012). In the first week post-partum, confined cows with an altered hepatic function had lower albumin and cholesterol concentrations (Bionaz et al., 2007) and grazing dairy cows with severe metritis or having more than one clinical disease after calving presented lower post-partum cholesterol concentrations (Sepúlveda et al., 2015). Few reports on associations of lower prepartum cholesterol or albumin concentrations and postpartum diseases were found (Kaneene et al., 1997; Van Saun, 2004). Also Trevisi et al. (2010) reported lower prepartum cholesterol concentrations in multiparous cows classified by their liver activity index as low, but the authors concluded that the lack of association with udder health was probably due to the limited number of cows included in the study. An earlier prediction of the probabilities for disease may enable the farmer to modify the herd management to improve its health status.

Although there is abundant literature on metabolic markers and peripartum diseases in dairy cows, we have not found field trial reports of the effect of parity (primiparous vs multiparous cows) and health status on metabolic profiles (e.g NEFA, BHB, cholesterol, albumin and calcium concentrations) determined weekly during the transition period. Moreover, the evolution of these metabolic markers during the transition period of healthy and sick cows will allow us to select the strategic moment for bleeding in order to monitor and predict disease risk in dairy herds. Thus, the aims of this study were: a) assess whether the health status (healthy vs sick cows) interacted with parity to affect BCS and metabolic profiles during the transition period and milk production, b) determine and compare the predictive value of metabolic markers (NEFA, BHB, cholesterol, albumin and calcium) to establish disease risk during the transition period in primiparous (PP) and multiparous (MP) Holstein cows.

2. Materials and methods

2.1. Cows and herd management

The study was conducted from October 2014 to September 2015 in a commercial dairy *free stall* (loose-housing system) herd in Rio Grande do Sul, southern Brazil. All procedures were carried out in accordance with regulations of the Animal Experimentation Committee (SIPPEE 10.059.15 CEUA 0522017 UNIPAMPA, Brazil).

Holstein dairy cows (n = 126 PP and n = 182 MP) from a 700-cow herd were selected, with an approximate milk yield of 8000 kg per lactation. The calving seasons were spring/summer (October 2014 to January 2015) and autumn/ winter (May to August 2015). The average temperature-humidity index (THI, Mader et al., 2006) in the autumn calving season was 72, while in spring calving season was 82. Cows were evaluated from 3 weeks before calving, until 4 weeks after calving.

From day −21 until calving, cows were kept on paddock separately by parity without significant pasture allowance and were offered 13.3 kg/d of DM as total mixed ration (TMR) including anionic salts in both calving seasons (2014 y 2015) according to close-up requirements (NRC, 2001) twice a day at 8:00 and 16:30 h (Table 1). After calving, all cows were hosted together in a compost barn until day 3 in milk. They were fed the diet 3 times daily as TMR for ad libitum intake, without access to pasture according to fresh cows requirements (NRC, 2001) in collective feeders, at 5:30, 10:00 and 15:0 h, and milked 2 times daily. The total daily offer of DM was 21.5 kg/d in the spring season (2014) and 28.9 kg/d in the autumn calving season (2015). Close-up and fresh cow diet composition is shown in Table 1. Cows had ad libitum access to water and they received 300 mL of propylene glycol, 250 g of calcium propionate diluted in 1 L water orally and 40 g Calcium gluconate subcutaneously at calving. After day 3 in milk, cows were hosted in a *free-stall* and were fed the same TMR, but milked 3 times daily (DeLaval rotary switch 80 stations). Data for 305-d milk yield were obtained from DairyPlan C-21 software (GEA).

Table 1
Ingredient and nutrient composition of close-up and fresh cow (calving seasons 2014 and 2015) of the total mixed diets (DM basis).

Item	Diet		
	Close-up	2014	2015
Ingredient, %			
Corn silage	57	29	29
Ryegrass haylage	–	11	11
Oat hay/ryegrass	20	6	3
Ground corn grain	–	19	21
Soybeans hull	–	–	18
Solvent-extracted soybean meal	10	14	16
Ground wheat grain	10	–	–
Citrus pulp	–	12	–
Close-up mineral supplement	3	–	–
Fresh cow mineral supplement	–	2	2
Nutrient profile			
Dry mater %	39.84	46.08	56.35
CP %	12.8	13.4	15.2
NDF %	51.8	37.2	39.8
NFC %	26.7	40.6	37.3
Starch %	16.1	21.3	23.7
Ether extract %	3.8	3.4	3.4
NEL, Mcal/kg	1.47	1.68	1.67
DCAD mEq/kg	−50	80	100
Ca %	0.63	0.88	0.63
P %	0.44	0.37	0.37
Mg %	0.35	0.20	0.21
K %	1.27	1.20	1.20
S %	0.32	0.22	0.22
Na %	0.10	0.22	0.16
Cl %	0.74	0.57	0.43

2.2. Study design, diseases recording and sample collection

This study followed a prospective observational cohort study and only healthy cows were enrolled at the beginning of the study. Peripartum diseases were diagnosed by a single trained veterinarian using the following criteria: Clinical hypocalcemia was defined as any recumbent cow within 72 h after parturition exhibiting anorexia, nervous symptoms, staggering, varying degrees of unconsciousness, and good response to intravenously administered calcium (Duffield et al., 1999). Clinical mastitis was characterized by the presence of abnormal milk or by signs of inflammation in 1 or more quarters, evaluated by the milking person from calving to 30 days in milk (DIM) at the start of milking (Duffield et al., 1999). Retained placenta (RP) was defined as failure to expel the placenta within 24 h after parturition (LeBlanc, 2008). Starting at d 3 until d 21 ± 1 after calving, all cows were monitored for metritis twice weekly, every Tuesday and Friday by a veterinarian, using a manual vaginal examination. Presence of abnormal vaginal secretion associated with blood Haptoglobin (Hp) concentration > 1 mg/dL, was used for diagnosis of metritis following Huzzey et al. (2011). Lameness was diagnosed weekly using the scale from 1 to 5, where grade 1 had no alteration in gait and grade 5 the cow was severely limping, without supporting the member on the floor. Cows with lameness were considered those with locomotion score ≥ 3 (Bicalho et al., 2007). Left displaced abomasums (LDA) was diagnosed based on an auscultation of a “ping” during percussion of the left side of the abdomen. During these health checks, presence of any other clinical disease(s) was recorded, and also deaths and culling because of health problems were recorded. Subclinical diseases were diagnosed at the laboratory based on specific metabolite determination. Subclinical

Table 2
Incidence of clinical and subclinical event during the transition period by parity [primiparous (PP), n = 126; multiparous (MP), n = 182] and calving season.

Disease and health status	Spring		Fisher	Autumn		Fisher
	PP	MP		PP	MP	
	n (%)	n (%)	P	n (%)	n (%)	P
Healthy	31 (73.81)	46 (66.67)	NS	38 (45.24)	49 (43.36)	NS
1 event	10 (23.81)	16 (23.19)	NS	29 (34.52)	37 (32.74)	NS
2 events	1 (2.38)	7 (10.14)	NS	17 (20.24)	27 (23.89)	NS
Metritis	2 (4.76)	6 (8.70)	NS	33 (39.29)	41(36.28)	NS
RP	4 (9.52)	10 (14.49)	NS	16 (19.05)	23 (20.35)	NS
Clinical mastitis	3 (7.14)	7 (10.14)	NS	13 (15.48)	24 (21.24)	NS
Others[a]	3 (7.14)	9 (13.0)	NS	1 (2.56)	12 (19.67)	0.01
Subclinical hypocalcemia	6 (14)	27 (39.7)	0.005	6 (7.4)	50 (45)	< 0.0001
Total n of PP and MP	42	69		84	113	

[a] Others include: clinical hypocalcemia, ketosis, DA and lameness.

ketosis was defined by BHB > 1.2 mmol/L (Duffield et al., 2009) and subclinical hypocalcemia by calcium ≤ 2.0 mmol/L (Reinhardt et al., 2011).

Body condition score (BCS) was determined weekly during the transition period, using a 5- point scale (Ferguson et al., 1994). In the same period, blood samples were collected weekly from all cows, from the coccygeal vessel into 10-mL sterile heparinized tubes, centrifuged at 3000 ×*g* for 20 min. Plasma was stored frozen (−20 °C) until further analysis for BHB, NEFA, cholesterol, albumin, Hp and calcium concentrations at the animal endocrine and metabolism laboratory, Veterinary faculty, Montevideo, Uruguay. Metabolites were measured by colorimetric assays on Vitalab Selectra II autoanalyzer (Vital Scientific, Dieren, The Netherlands) using commercial kits: NEFA, Wako NEFA-HR (2), Wako Pure Chemical Industries Ltd., Osaka, Japan; BHB, Randox Laboratories Limited, 55 Diamond Road, Crumlin, Country Antrim, BT29 4QY, United Kingdom. Albumin, calcium and cholesterol: Wiener Laboratories S.A.I.C. Riobamba, Rosario, Argentina. Haptoglobin concentrations (Tridelta Diagnostics Ltd., Morris Plains, Ireland) were measured by ELISA (Thermo, Multiskan EX, USA). The inter-assay coefficient of variation (CV) for all commercial serum controls was less or equal to 10%.

2.3. Statistical analysis

Cows were classified according to their health status in 3 categories as "healthy cows", "1 event" (one clinical event) and "2 events" (more than one clinical event). The associations between parity (MP and PP) and health status, and the individual disease outcomes were analyzed using 2 × 2 contingency tables generated by the PROC FREQ statement in SAS (version 9.2; SAS Institute, 2009). From these tables, the Mantel-Haenszel chi-square test was used to determine the type 1 error risk of the relationship.

Descriptive statistics were calculated for NEFA, BHB, cholesterol, albumin and Ca concentrations by week, during close-up period and further evaluated as continuous outcomes using PROC MIXED, analyzing their differences between health status by period: "close-up" (−2 and −1 week related to calving), "calving" and "fresh cows" (1, 2 and 3 weeks post calving). Each period was considered an independent test because the concentrations of the metabolic markers of interest will change relative to time from calving and their association with health status may likely be influenced by time as reported previously (Huzzey et al., 2011). The model included the fixed effects of parity (PP vs MP), health status (healthy, 1 event or 2 events), week, calving season (spring 2014, autumn 2015) and the interaction (parity × health status, parity × health status × week). Milk production as 305-d milk yield, was also evaluated using PROC MIXED, considering fixed effects of parity, health status, calving season and their interactions in the model.

Data were further evaluated using multivariable logistic regression (MLR) analysis by week (−2, −1 and calving) for health status (healthy vs sick cows) and for individual outcomes: metritis, RP and mastitis, considering parity and calving season in the model. Variables that were not significant ($P > 0.05$) were removed by manual backward stepwise elimination. Those individual outcomes with low incidence were not evaluated, due to their small number. Odds ratio (OR) and their respective 95% confidence interval determined by MLR were used to describe the level of association between the metabolite of interest and the postpartum health outcome, considering the individual outcome as a binary classification; cows with the individual outcome and healthy cows (without any other individual outcome).

For metabolites that remained in the final models, receiver operator characteristic (ROC) curves were constructed to determine area under the curve (AUC). The cutpoint of the metabolites was estimated by Youden Index, and the values of sensitivity, specificity, positive and negative likelihood ratio (LR) were determined using MedCalc V.17.6. (MedCalc®. MedCalc Software. Acacialaan 22. B-8400 Ostend. Belgium). Sensitivity was the proportion of animals diagnosed with metritis, RP or mastitis that were at or above a given metabolite cutpoint, while specificity was the proportion of animals without clinical disease that were below a given cutpoint (Dohoo et al., 2003).

For all statistical analysis $P < 0.05$ was considered a significant effect and $P \leq 0.1$ as a tendency.

3. Results

From the 308 cows evaluated, 46.7% ($n = 144$) had at least one clinical event and 1.9% ($n = 6$) were discarded or died within the first 30 DIM. A greater incidence of sick cows was diagnosed in the autumn calving season compared to the spring season (55.3% vs 30.6%, $P < 0.0001$). No interaction was found among calving season and parity. The number and proportion of cows and their health status (Healthy, 1 event and 2 events), and individual outcomes (metritis, RP, mastitis and subclinical hypocalcemia) stratified by parity and calving season is shown in Table 2. The largest overall incidence was for metritis (26.6%, $n = 82$) followed by retained placenta (17.2%, $n = 53$) and mastitis (15.2%, $n = 47$), with no effect of parity in any disorder. The median time of diagnosis of metritis was 9 DIM (range of 5 to 19 DIM), and of mastitis 10 DIM (range of 1 to 25 DIM). Due to the low proportion of clinical ketosis ($n = 1$), lameness ($n = 13$), displaced abomasums ($n = 6$) and clinical hypocalcemia ($n = 4$), all conditions were considered as "others" with an overall incidence of 8.1% ($n = 25$). Also, subclinical ketosis had a low incidence (3.3%, data not shown). Considering subclinical hypocalcemia, MP presented greater incidence than PP cows, being 43% ($n = 77$) vs 9.5% ($n = 12$) respectively, $P < 0.0001$.

In Table 3, the analysis of BCS and metabolites according to parity, health status, week and their interactions during the close-up period, at

Table 3
Probabilities for fixed effects during close-up (weeks −2 and −1), Calving and Fresh cow (week +1, +2 and +3).

	Effect	Albumin	Cholesterol	Calcium	NEFA	bHB	BCS
Close up	Parity	0.1396	0.3274	0.0697	0.0004	< 0.0001	0.0017
	Health status	0.4507	0.4023	0.8035	0.3447	0.5279	0.6181
	Week	0.6204	0.0012	0.0008	0.3577	0.3758	0.3233
	Calving season	< 0.0001	0.0086	0.1759	< 0.0001	0.1871	0.0334
	Parity vs healthy	0.166	0.4056	0.1668	0.2453	0.4322	0.4465
	Parity vs healthy vs week	0.124	0.726	0.0539	0.0619	0.4258	0.8068
Calving	Parity	0.6268	0.0828	< 0.0001	< 0.0001	< 0.0001	0.0125
	Health status	0.7026	0.7858	0.2183	0.0024	0.0645	0.9051
	Calving season	< 0.0001	< 0.0001	0.0526	< 0.0001	0.3657	0.0748
	Parity vs healthy	0.0775	0.7167	0.0944	0.3433	0.1448	0.6115
Fresh cow	Parity	0.0065	0.568	0.316	0.017	< 0.0001	< 0.0001
	Health status	< 0.0001	< 0.0001	0.1913	0.834	0.1972	0.5719
	Week	0.0086	< 0.0001	< 0.0001	< 0.0001	0.2537	< 0.0001
	Calving season	< 0.0001	0.5844	0.286	< 0.0001	< 0.0001	0.7924
	Parity vs healthy	0.1785	0.6387	0.4932	0.1287	0.7334	0.2148
	Parity vs healthy vs week	0.1332	< 0.0001	0.5265	0.0079	0.0127	0.2353

calving and fresh cow is shown. BCS and concentrations of NEFA and BHB were affected by parity during the entire transition period, while calcium tended to be affected or was affected during the close up or calving respectively. Cholesterol concentrations tended to be affected by parity at calving and albumin was affected by parity but only in fresh cows. Neither BCS nor the concentration of the different metabolites were associated with health status during the close-up period, but health status affected NEFA and BHB concentrations at calving and albumin and cholesterol concentrations in fresh cows (weeks +1, +2 and week +3 for albumin and weeks +2 and +3 for cholesterol).

The evolution of BCS, NEFA and BHB concentrations during the transition period is shown in Fig. 1. Although MP cows presented lower BCS than PP cows, they presented a similar profile, being greatest in the close up period, decreasing until the end of the experiment (week +3), not recovering the initial BCS. During the transition period, the

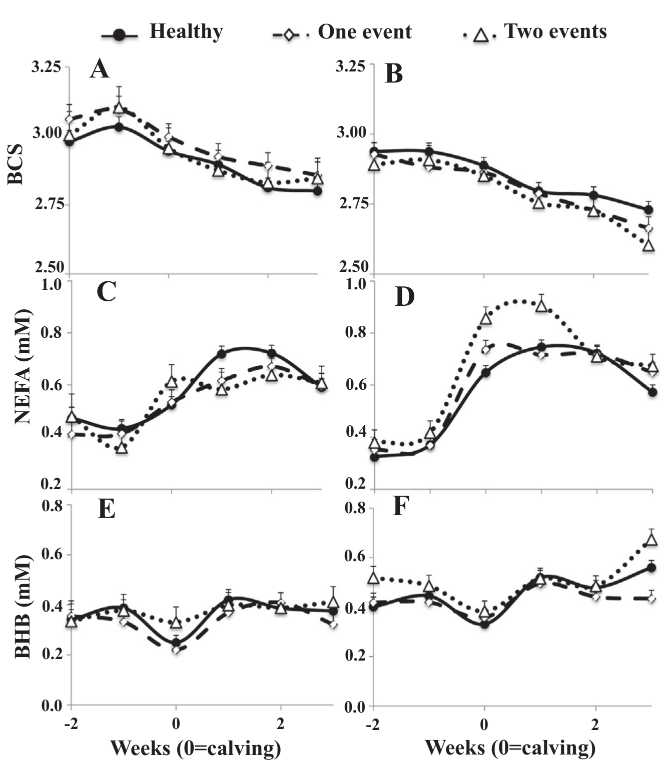

Fig. 1. Body condition score (BCS) evolution (A, B), non sterified fatty acid (C,D) and β hydroxibutirate (E,F) concentrations in primiparous (A, C, E) and multiparous (B, D, F) Holstein cows.

differential NEFA profiles according to health status and parity is shown in Fig. 1 (C and D). All MP cows increased their NEFA concentrations from week −1 to week +1, but sick MP cows presented higher NEFA concentrations than healthy MP cows at calving ($P < 0.05$). Also at week +1, MP cows with 2 events presented higher NEFA concentrations than healthy and 1 event MP cows ($P < 0.05$). In addition, healthy MP cows decreased their NEFA concentrations by week +3, while sick MP cows maintained higher NEFA concentrations ($P < 0.05$). In PP cows, although NEFA concentrations increased from week −1 to week +1, healthy PP cows presented the greatest NEFA concentrations at week +1 in comparison to sick PP ($P < 0.05$) and at week +3 there were no differences between the three health status categories. BHB profiles were similar in both parities during the transition period. While PP cows with 2 events presented higher BHB concentrations than healthy PP and 1 event cows at calving ($P < 0.05$), MP cows with 2 events had greater BHB values at week+3 than healthy MP cows ($P < 0.05$).

The profiles of cholesterol, albumin and calcium concentrations during the transition period are shown in Fig. 2. Cholesterol concentration decreased at calving and increased during postpartum, reaching to greater values than at the start of close-up period, regardless of parity or health status. Healthy cows (MP and PP) presented higher cholesterol concentrations than sick cows (MP and PP) at week +2 and +3 ($P < 0.05$), and healthy MP more cholesterol concentrations than sick MP at week −1 ($P < 0.05$). Healthy MP cows had higher albumin concentrations than sick MP cows during close-up and fresh period ($P < 0.05$, Fig. 2 C and D). Moreover, 1 event MP cows had also greater albumin concentrations than 2 events MP cows ($P < 0.05$) during the fresh period. In PP cows, no differences in albumin concentrations were found between healthy and 1 event PP cows during the experiment, but these two groups had greater albumin concentrations during the fresh period than 2 events PP cows ($P < 0.01$). Calcium concentrations declined sharply at calving in all MP cows, whereas it was not as evident in PP cows, reflecting the effect of parity at this time (Fig. 2 E and F). In spite of the decrease of calcium concentrations at calving in all MP cows, healthy MP cows had higher concentrations than sick MP cows ($P < 0.05$).

The logistic regression analysis including all data in PP and MP cows of both calving seasons showed that albumin concentrations on week −2 was predictive for disease, as the decrease in one unit of albumin was associated with an odd ratio (OR) of 1.27 (95% CI: 1.01 to 1.60, $P = 0.03$) in 2 events cows when compared to healthy cows. Similarly, cholesterol concentrations were predictive for mastitis being the OR 2.24 (95% CI: 1.06 to 4.74, $P = 0.03$).

As calving season and parity affected metabolite concentrations during close-up and calving (Table 3), MLR analysis was performed for

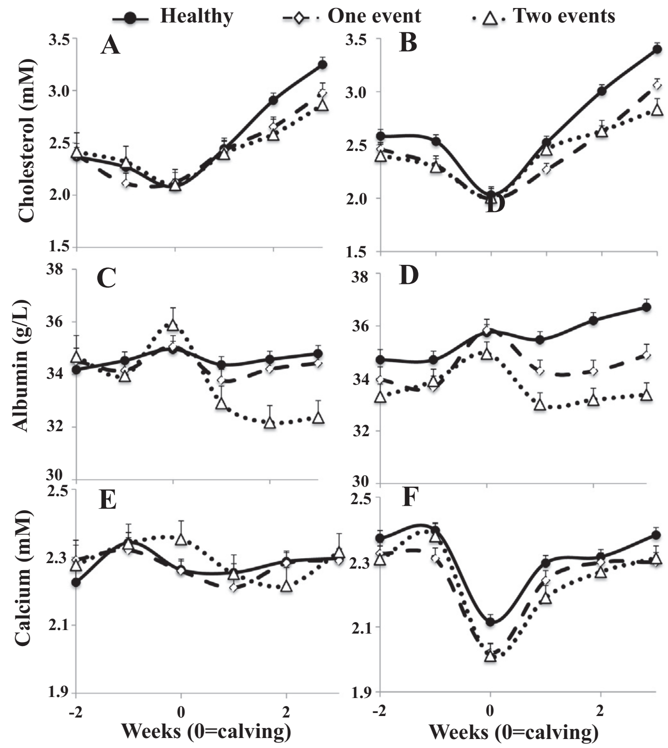

Fig. 2. Cholesterol (A, B), albumin (C,D) and calcium (E,F) concentrations in primiparous (A, C, E) and multiparous (B, D, F) Holstein cows.

each cohort and parity separately. In cohort 2014, there was no metabolite diseases predictor for PP or MP cows. In cohort 2015, significant effects were found only in MP cows. Albumin concentrations were predictive for metritis (OR [IC]); 1.31 [1.05–1.62] ($P < 0.01$) and RP; 1.38 [1.04–1.81] ($P < 0.05$) at week −2 and predictive for RP; 1.26 [1.01–1.57] (P < 0.05) at week −1. Also cholesterol was predictive for mastitis; 4.29 [1.26–14.61] at week −2, 6.32 [1.81–22.08] at week −1 and 9.01 [1.01–42.28] at calving (P < 0.01).

The AUC of the albumin and cholesterol plasma concentrations, as well as values of sensitivity, specificity, positive and negative likelihood ratio (LR) of the cutpoints are shown in Table 4. As shown in Fig. 3, the closer the ROC curve is to the upper left corner, the greater the accuracy of differentiation between cows with and without the heath outcome (metritis, RP or mastitis). Thus, the test is perfect to distinguish between two groups if AUC is 1 and less accurately if AUC is 0.5–0.7, as was the case of the present study.

Milk production was affected by parity, calving season and health status ($P < 0.05$). Primiparous cows produced less milk than MP (6866 ± 272 vs 8341 ± 213 L, $P < 0.0001$) and production was greater in the autumn calving season compared to the spring season (8360 ± 188 vs 6789 ± 278 L, P < 0.0001). Also, healthy cows produced more than sick cows (P < 0.05), but 1 or 2 events cows did not differ in milk production among them (8165 ± 199, 7547 ± 276 and 7098 ± 387 L for healthy, 1 or 2 events cows respectively).

4. Discussion

As far as we know this is the first study reporting that the evolution of metabolic profiles in healthy and sick animals during the transition period varies according to parity. Moreover, prepartum albumin and cholesterol concentrations were predictors for metritis, RP and mastitis but only in MP cows.

The overall incidence of illness in our study (46.4%) was similar to the reported for lactating dairy cows by others (58%, Van Saun, 2004; 30 to 50%, LeBlanc, 2010). A higher incidence of illness was found in the autumn calving season (55.3%) associated with the higher milk production in this calving season. It has been reported that this association is not due to the higher milk production per se, but probably because of a higher rate of acceleration in milk yield (Ingvartsen, 2006). The greater milk production in autumn calving is consistent with the total DM offer (although TMR was offered ad libitum 3 times daily, autumn cows had 7.4 kg DM more than spring cows) associated to the better THI than the spring season, as in the latter the THI exceeded the comfort threshold (> 72) for milk production (Polsky and von Keyserlingk, 2017). In our study, the metritis incidence was 26.6% similar to the reported (21%) by Hammon et al. (2006), been lower than (40%) reported by Giuliodori et al. (2013), but higher than found by others (0.7 to 2.2%, Rajala and Gröhn, 1998; Bruun et al., 2002). Indeed, the incidence of metritis varies among studies and as Huzzey et al. (2007) stated, the diagnostic criteria may be poorly described among studies, which makes it difficult to compare. Overall, incidence of RP in the present study was 17.2%, slightly higher than the reported (3 to 13%) by Ingvartsen et al. (2003). Many risk factors were associated with RP including abortion, dystocia or induced parturition, twins, stillborn calf, milk fever, and increasing age (Correa et al., 1993; Grohn and Rajala-Schultz, 2000) been therefore RP considered multifactorial. The clinical mastitis overall incidence found in this study (15.2%) was in the range (7 to 44%) reported by Ingvartsen et al. (2003) or similar to the reported (11 to 24%) in grazing dairy cows (Bargo et al., 2009; Ribeiro et al., 2013; Sepúlveda et al., 2015).

Clinical hypocalcemia was very low (1.3%), because of this, it was considered together with DA, lameness and clinical ketosis. The incidence of subclinical hypocalcemia and their higher presentation in MP as expected, was similar to the reported by others (Goff, 2006; Reinhardt et al., 2011), and is consistent with the management for hypocalcemia prevention at the herd as described in materials and methods. The very low incidence of clinical, subclinical ketosis and DA (0.3%, 3.3% and 2% respectively) in the present study reflects the preventive management of the herd, as all cows received 300 mL of propylene glycol at calving. Indeed, a high incidence of these diseases has been reported, 8.9 to 34% clinical ketosis (Ingvartsen, 2006;

Table 4
Test performance for albumin and cholesterol to diagnose metritis, RP or mastitis in close-up and calving.

Test performance (CI 95%)								
Outcome	Metabolite	Week	AUC	Cutpoint	Se (%)	Sp (%)	+LR	−LR
Metritis	Albumin (g/L)	−2	0.66 (0.55–0.77)**	≤33.1	42 (24–61)	91 (79–97)	4.72	0.70
RP	Albumin (g/L)	−2	0.70 (0.55–0.80)*	≤34.2	61 (36–83)	80 (65–90)	3.06	0.49
		−1	0.68 (0.54–0.78)*	≤34.0	54 (32–76)	83 (69–92)	3.14	0.55
Mastitis	Cholesterol (mmol/L)	−2	0.71 (0.60–0.77)**	≤2.4	79 (54–94)	60 (44–74)	1.97	0.35
		−1	0.70 (0.60–0.81)**	≤2.3	73 (50–89)	61 (45–75)	1.86	0.45
		0	0.68 (0.56–0.78)*	≤1.7	58 (37–78)	72 (57–83)	2.04	0.58

* P < 0.05.
** P < 0.01.

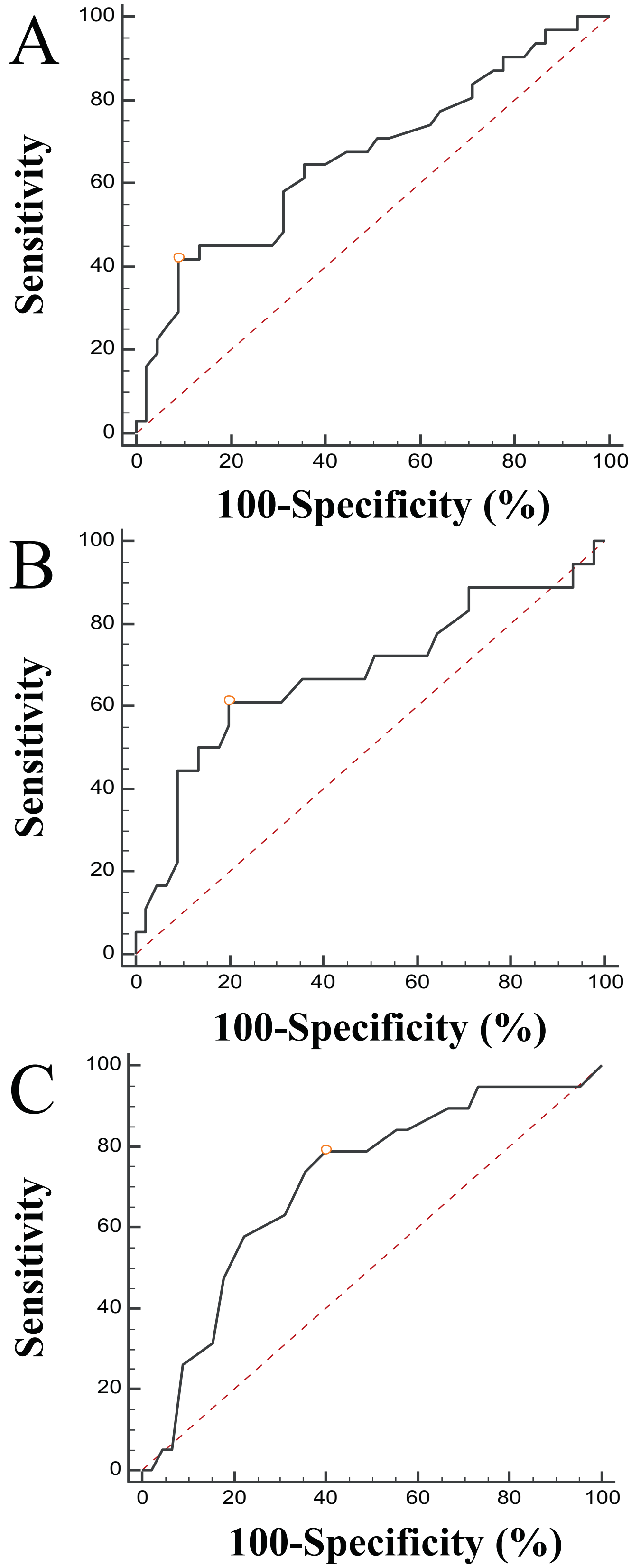

Fig. 3. Receiver operator characteristic (ROC) curve for albumin concentrations as predictor for Metritis (A) and Retained Placenta (B) and cholesterol as predictive for Mastitis (C) at week −2.

Rushen et al., 2008), 30% subclinical ketosis (Duffield et al., 1997) and increasing incidence of left DA (LDA) in the last decade, from between 1 and 2% to 5 to 7% (LeBlanc et al., 2005). Moreover, clinical and subclinical ketosis has been associated with development of LDA (LeBlanc et al., 2005) and thus, our findings agree with this observation, taken into account the low incidence of both diseases. Ingvartsen (2006) reported over conditioning at calving, excessive fat mobilization, low nutrient intake, diet specific factors or management as major factors increasing the risk of ketosis and fatty liver. Also, Seifi et al. (2011) reported in the first and second weeks postpartum, that fat cows were at significantly higher risk of developing clinical ketosis. Even more, elevated prepatum NEFA and postpartum NEFA and BHB concentrations were also used to identify cows at risk of developing diseases (LeBlanc et al., 2005; Ospina et al., 2010a). This was not the case of the present study as NEFA and BHB concentrations during the close-up period were not predictive. This could be the result of the cows being in a comfortable and low-stress environment, as discussed by Drackley et al. (2001) and Ospina et al. (2010b). Therefore, considering the low BHB and NEFA concentrations measured in close-up and low BHB concentrations and good BCS during the transition period, we can suggest a good energy management of transition diet and ketosis prevention at calving, experimenting cows a mild NEB. This may be the reason of the low incidence of ketosis and DA found in the present study and BHB and NEFA variables as not illness predictive in contrast with the cited literature.

Albumin concentrations were predictive in MP cows for metritis and RP at week −2 and for RP at week −1. There are very few reports on this metabolite and disease prediction. The data are consistent with Van Saun (2004) who reported that cows with albumin concentrations < 32.5 g/L during close-up (3 to 21 days prepartum) were 1.46 (95% CI: 1.04–2.04) times more likely to experience a postpartum disease event. Indeed, albumin concentrations reflect a good nutritional early dry and close-up management, as the use of blood chemistries (among others; albumin) in the form of metabolic profiles to determine nutritional status has been advocated (Payne et al., 1970; Van Saun, 2004). Also greater albumin concentrations reflect a healthy liver, as albumin is considered a negative acute phase protein, and decreases in cows with altered hepatic function (Bionaz et al., 2007). The association found among albumin concentrations at week −2 -when all cows were clinically healthy- with pospartum health output, and the fact that albumin determination is an easy and cheap technique, suggest that it could be a relevant tool to monitor herd health status. Also cholesterol concentrations resulted predictive for mastitis, during close-up and at calving, as cholesterol concentrations ≤ than 2.4, 2.3 or 1.8 mmol/L at weeks −2, −1 or calving respectively were 4.3, 6.3 or 9.0 times more likely to experience mastitis as calving approaches. In reference to this, we agree with Kaneene et al. (1997), who reported that cows with lower prepartum cholesterol concentrations are more likely to experience mastitis, metritis or RP postpartum. The decreases in cholesterol concentrations during the close-up period can be explained by the decrease in dry matter intake (DMI) during this time (Janovick Guretzky et al., 2006) and also DMI has been associated with disease development; sick cows consume less feed compared to healthy cows during the transition period (Huzzey et al., 2007). In contrast, Amadori et al. (2015) did not found that cholesterol concentrations were predictive for diseases, while Quiroz-Rocha et al. (2009) reported greater prepartum cholesterol concentrations in cows presenting RP. It should be taken in consideration that the former study included 75 multiparous cows from 26 different herds, while the latter included 1038 (primiparous and multiparous) cows from 20 herds but only RP was registered. Taking into account the performance of albumin and cholesterol to predict metritis, RP or mastitis, although AUC indicated median accuracy (0.66 to 0.71), data of sensitivity, specificity and LR are in the order of other metabolites (Geishauser et al., 1997b; LeBlanc et al., 2005). Indeed, no reports on albumin and cholesterol parameters were found, the sensitivity reported of BHB and NEFA to predict LDA was 48 and 56%

respectively, while specificity was 80 and 78% and LR = 2.4 and 2 respectively (LeBlanc et al., 2005).

The metabolic adaptation to the transition period was strongly influenced by parity as reflected by BCS and all metabolic profiles, as cited by others (Wathes et al., 2007; Adrien et al., 2012). In the present study, PP cows had higher BCS than MP cows, being their respective BCS in the target established as the optimum for both categories as cited by Garnsworthy (2006). Although both categories increased their NEFA concentrations, due to the NEB, MP cows had a greater degree of mobilization probably due to the greater energy demands for milk production as shown in this study. Interestingly, although no differences were found in NEFA concentrations in PP cows, sick MP presented higher NEFA than healthy MP cows at calving, and moreover, NEFA concentrations remained greater in 2 events cows than healthy and 1 event cows in the first week postpartum. Elevated NEFA was associated with immune dysfunction around calving (Kehrli et al., 1989; Hammon et al., 2006).

Cholesterol and albumin profile was affected by health status in both categories until the end of the study, as sick cows presented lower concentrations of both metabolites. This reflects the greater metabolic challenge faced by cows who become sick during the transition, being worse for cows with more than one clinical event reflecting the no recovering of their DMI. This agree with Huzzey et al. (2007) who stated that sick cows consume less feed compared to healthy cows during the transition period. Interestingly, cholesterol and albumin were the disease predictors detected in the close up but only in MP cows. The finding that those clinically healthy MP cows– but not PP cows- during the close up that will become sick later (during fresh period) had lower concentration of these metabolites could be associated to a worse recovery from previous lactation and/or inadequate management during far off dry period. Data is also consistent with NEFA profiles, as sick MP cows – especially cows with two events- had lower albumin and greater NEFA concentrations. These associations have been already reported (Van Saun, 2004). Overall, the worse metabolic profile (NEFA, cholesterol and albumin) found in sick cows during fresh period is consistent with the lower milk production found when compared to healthy cows, as reported by Ospina et al. (2010b).

Parity also affected calcium profile as expected. Reinhardt et al. (2011) reported that the incidence of subclinical and clinical hypocalcemia increases with age. The MP cows had lower calcium levels mainly at calving due to greater milk production and also because the normal homeostatic response to hypocalcemia is decreased with age resulting in greater or prolonged hypocalcemia in older animals (Reinhardt et al., 2011). Also hypocalcemia reduces feed intake so that greater body fat mobilization occurs in early lactation (Goff, 2008). In the present study while no differences were found in sick and healthy PP cows, sick MP cows presented lower Ca and higher NEFA concentrations at calving. Moreover, hypocalcemia significantly increases a cow's susceptibility to RP, metritis and mastitis (Curtis et al., 1983, 1985). Indeed, MP cows with these diseases had lower calcium concentration at calving, but this was not the case in PP cows and we have no obvious explanation for this finding. It should be also taken into account that the activation of the immune system could result in lower calcium levels (Waldron et al., 2003).

5. Conclusion

In summary, prepartum albumin and cholesterol concentrations were predictors for metritis, RP and mastitis in MP cows showing a mild negative energy balance in terms of NEFA, BHB and BCS profiles. Moreover, it is shown that the evolution of metabolic profiles in healthy and sick cows during the transition period varies according to parity and is associated with lower milk production.

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