Body condition score, some nutritional parameters in plasma, and subsequent reproductive performance of Montbéliarde cows in Algeria

A Kalem, C Hanzen[1], A Abdelli and R Kaidi

Institut des Sciences Vétérinaires, Université Saad Dahlab- Blida1. Laboratoire des Biotechnologies liées à la Reproduction Animale (LBRA), Algérie.
abdelliamine@hotmail.fr
[1]Service de thériogénologie des animaux de production, Faculté de Médecine vétérinaire, Université de Liège, Liège, Belgique.

Abstract

The objective of this study was to investigate the relationships among body condition score (BCS) and plasma metabolite concentrations, and subsequent reproductive performance of Montbéliarde cows in Algeria. The study was conducted in two commercial dairy farms in Tizi-Ouzou area. Blood samples were collected from 50 Montbéliarde dairy cows at 2, 4, 6 and 8 weeks postpartum to measure serum non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), glucose, total cholesterol, urea nitrogen, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γGT), calcium, magnesium, potassium, phosphorus, sodium, and progesterone. Body condition score (BCS) was assessed at calving and at each time when blood samples were taken. Resumption of postpartum cyclicity was evaluated by progesterone concentrations (≥1 ng/mL) at 4, 6, and 8 weeks postpartum.

Increased BHBA, NEFA and glucose concentrations were associated with a lower probability of ovarian activity resumption (ROA) and pregnancy at first insemination (P/1-AI). However, concentrations of plasma cholesterol, AST, ALT, TG and total protein were positively associated with ROA. In addition, increased BHBA and sodium were associated with increased of time to conception. Moreover, decreased Mg concentrations were associated with increasing of time from calving to first insemination. No significant effect was seen between BCS at calving and BCS loss on P/1-AI. So balanced nutrition and reproduction management can ameliorate reproductive performance.

***Key words**: BCS, dairy cow, fertility, nutritional parameter, plasma metabolite, progesterone, post-partum.*

Note d'état corporel, quelques paramètres plasmatiques et performances de reproduction ultérieures de vaches Montbéliardes en Algérie

Résumé

L'objectif de l'étude est d'explorer les relations entre l'état corporel (BCS), les concentrations de certains métabolites du plasma, et les performances de reproduction de vaches Montbéliardes en Algérie. L'étude a été menée dans 2 fermes laitières privées situées dans la wilaya de Tizi-Ouzou. Des échantillons de sang ont été prélevés sur 50 vaches laitières de race Montbéliarde à 2, 4, 6 et 8 semaines post-partum, afin de mesurer les acides gras non estérifiés (AGNE), le β-hydroxybutyrate (BHBA), le glucose, le cholestérol total, l'urée, les protéines totales, l'aspartate aminotransférase (AST), l'alanine aminotransférase (ALT), la γ-glutamyltransférase (γGT), le calcium, le magnésium (Mg), le potassium (K), le phosphore (P), le sodium (Na) et la progestérone (P4). La note d'état corporel (NEC) a été estimée au vêlage ainsi qu'aux moments des prélèvements de sang. La reprise de la cyclicité post-partum a été évaluée par le dosage de la progestéronémie (≥1 ng / ml) dans les semaines 4, 6 et 8 post-partum.

L'augmentation des teneurs en BHBA, AGNE et du glucose ont été associés à une probabilité plus faible de la reprise de l'activité ovarienne (RAO) et de la gestation à la première insémination (P / 1-AI). Cependant, les concentrations du cholestérol plasmatique, AST, ALT, TG et des protéines totales ont été positivement associées à la RAO. En outre, l'augmentation du BHBA et du sodium ont été associés à une augmentation de l'intervalle de temps pour avoir une conception, alors qu'une diminution des concentrations en Mg a été associée à l'augmentation de l'intervalle entre le vêlage et la première insémination. Aucun effet significatif n'a été observé entre la NEC au vêlage ou la perte de NEC et P / 1-AI. Ainsi, une alimentation équilibrée et une bonne gestion de la reproduction peuvent améliorer les performances de reproduction.

Mots-clés : *fertilité, métabolite du plasma, paramètre nutritionnel, progestérone, vache laitière.*

Introduction

In Algeria, livestock policies requested to reduce reliance on milk imports, based on importing heifers of dairy cows especially Montbéliarde breed. This breed had been imported from France (Madani et al 2008) and seem most adapted to Algerian climate and farming conditions especially in the north of the country (Kadi et al 2007). However, the dairy productions as well as the reproductive performances of these imported cows are still far less than those obtained in their country of origin (Madani et al 2008; Miroud et al 2014). For example, the pregnancy rate at first AI (P/IA) in Algeria was 40.5% (Abdelli et al 2015) vs 54% in France (Barbat et al 2010). The most important factor of this poor reproductive performance is the nutrition (Mouffok et al 2011). Previous research confirmed that nutrition played an important role in reproduction. Consequently, partitioning of tremendous demand for nutrients to milk production early in lactation, at the expense of reproduction, has shaped a conceptual framework to address the effect of nutritional imbalances on future reproductive performance (Walsh et al 2007), as measured by days to resumption of ovarian activity (ROA), probability of pregnancy after first AI (P/AI) and time to conception. The concept of prioritization of energy and other nutrients, also known as homeorhesis (or teleophoresis), was described in 1980 (Bauman and Currie 1980) and has set the foundation for the study of negative energy balance (NEB) using different methods, namely body condition score (BCS) measurements and assays on plasma metabolites, enzymes or hormones (Tillard et al 2008; Ribeiro et al 2013; Rutherford et al 2016). Recently, there have been some reports on the relationships among the resumption of ovarian cyclicity postpartum and nutritional end points, such as body condition score (BCS) and several plasma metabolites in dairy cows

(Shrestha et al 2005; Tillard et al 2008; Damptey et al 2014; Jeong et al 2015). They have reported associations of prolonged intervals to first postpartum ovulation with some plasma metabolites, such as non-esterified fatty acids (NEFAs), 3-β-hydroxybutyrate (BHBA), total-cholesterol (T-cholesterol), aspartate aminotransferase (AST), γ-glutamyltransferase (γGT), alanine aminotransferase (ALT), urea nitrogen, total protein, calcium, magnesium, and phosphorus in plasma (Shrestha et al 2005; Tillard et al 2008; Damptey et al 2014; Jeong et al 2015). These blood metabolites with BCS changes may help establish strategies for dairy reproductive management (Jeong et al 2015). The objective of this study was to determine the relationship of blood metabolites, BCS and subsequent reproductive performance of Montbéliarde cows in Algeria.

Materials and methods

Animals and herds

The study was conducted in two commercial dairy farms (A and B) in Tizi-Ouzou willaya (longitude 36° and latitude 4°), Algeria. The area has a Mediterranean climate with two distinct seasons, with peak summer temperatures reaching 38°C and winter with a minimum temperature below freezing. Fifty Montbéliarde dairy cows, 25 primiparous and 25 multiparous equally distributed on each farm, were followed throughout lactation. Their daily average milk production was 25 kg/d. During the period of study, the cows received green fodder, clover in cold season and meadow fodder in warm season with vetch oats hay. Based on the production, basic ration was individually supplemented with commercial concentrate (18% digestible raw protein), as well as roughly crushed maize grains, soybean meal, barley and vitamin-mineral mixture.

Sample collection and blood analysis

Blood samples were collected from the coccygeal vein into two 10 ml vacutainers, one containing lithium heparin and the other containing no anticoagulant. Samples were taken before the morning feeding at four times (wk 2, 4, 6, and 8 postpartum). At these time points, blood samples were carried out simultaneously. Samples were kept chilled and allowed to clot (for the ones containing no anticoagulant). Samples were centrifuged at 1,400 × g for 10 min to collect serum within 5 h of blood collection and were frozen at −20°C until analyzed. Heparinized plasma was used for the determination of NEFAs, BHBA, glucose, total cholesterol, triglycerides (TG), urea nitrogen, total protein (PT), aspartate aminotransferase (AST), γ-glutamyltransferase (γGT), alanine aminotransferase (ALT), calcium, magnesium, sodium, potassium, and phosphorus levels. All blood metabolites except NEFAs and BHBA were determined with enzymatic method by spectrophotometric assay in an autoanalyzer (Cobas 6000, Roche Hitachi, Mannheim, Germany) in a commercial laboratory, using commercial kits. The intra and interassay coefficients of variation were < 5% for each assay. Serum BHBA concentration (mmol/L) was measured using a hand-held meter (Precision Xceed, Abbott Laboratories, Abbott Park, IL) at room temperature (Iwersen et al 2009; McArt et al 2013). OptiumXceed is a hand-held device used to test blood BHBA concentrations; his sensitivity and specificity was 85 to 90% and 94 to 98%, respectively (Voyvoda and Erdogan 2010). Because there were not sufficient reagents for NEFAs, plasma NEFAs concentration was measured one time (at wk 4) using the DVM- NEFA test (Veterinary Diagnostics, Newburg, Wisconsin, USA). The sensitivity and specificity of the DVM-NEFA test were 84% and 96%, respectively (Leslie et al 2003). Progesterone (P4) was

quantified by ELISA (Elecsys 2010, Roche Diagnostics GmBH, Mannheim, Germany) using progesterone ECL kit. These kits can be used to measure P4 in plasma bovine (Ayad et al 2014).

Fatness assessment

A body condition score (BCS), on a five-point scale scored to the nearest half-point, was used to assess the individual level of body fatness and its variation during the postpartum period (Edmondson et al 1989). Cows were scored five times before eating, at fortnightly intervals, from calving to 52 days in milk (DIM). All BCS measurements were done by a trained single operator for a given herd. Two variables of fatness were of peculiar importance: at calving (BCS-calv) and the difference from calving to wk 4 postpartum (dBCS).

Determination of estrous cyclicity and pregnancy diagnosis

Pregnancy was diagnosed in all cows on d 30 after AI via transrectal ultrasonography of the uterus and its contents and characterized by visualization of a live embryo. Cows diagnosed as pregnant on d 30 were reexamined by transrectal palpation 35 d later. Progesterone data were dichotomized using a threshold of 1 ng/ml for indicating the presence of an active corpus luteum (Stevenson et al 2006). Ovulation was considered to have occurred 5 days before the first progesterone measurement >1 ng/ml and was followed by another consecutive sample of luteal concentrations. Resumption of ovarian activity was calculated at 52 DIM.

Statistical analysis

Statistical analyses were performed with SAS (Version 9.1.3; SAS Institute Inc, Cary, NC). Postpartum plasma concentrations of metabolites were reported as continuous variables. Each variable of these metabolites was tested for normal distribution using the Proc Univariate (SAS Inst. Inc.). If the variable does not fit the normal distribution, adjustments such as logarithmic, squared, square root transformations are possible tools to normalize the data to calculate valid descriptive statistics (Farver 1997). Fertility responses of interest were estrous cyclicity on day 52 postpartum, P/AI, time from calving to first insemination and time to pregnancy.

Because serum metabolites were measured over time, blood metabolite concentrations, except NEFAs, were analyzed by fitting the fixed effects of day, cycling, or pregnancy status and the interaction of day with cycling or pregnancy status in a repeated measures variance analysis using a PROC MIXED models (SAS Inst. Inc.). The model included assessment factors (group, time), individual variability (within farm) and the group* time. The layout of our model can be summarized as follows:

$Y_{ijklm} = \mu + G_i + T_k + GT_{ik} + B_{l(im)} + \varepsilon_{iklmn}$;

where:

Y_{iklm} = m-th observation of the l-th cow Bl within the i-th group
Gi* farm Fm, at the k-thtimeTk;

μ = total average;

G_i = effect of the i-th group [two groups (ER/LR and Pregnant/not pregnant)];
T_k = effect of the k-th time to calving
GT_{ik} effect of the interaction between the i-th group and the kth time to calving;
Bl(im) = effect of the l-th cow within the i-thgroup* m-th
farm;
ε_{iklm} = random effect or error.

Covariance structure used (compound symmetry) was chosen based on the Akaike information criterion. The analysis was carried out using compound symmetry for covariance structure.
The effect of plasma metabolites at each week and peripartum disease on probability of pregnancy at first insemination (P/IA) and resumption of postpartum: activity (ORA) was assessed using mixed-effects multivariable (PROC GENMOD), using a normal distribution and cow as a random affect. Cow level variables offered to the model included parity, season of calving, initial BCS (BCS-calv), change in BCS (dBCS), season of calving. A manual backward stepwise regression was used in all models, and elimination was performed on the basis of the Wald statistic criterion when $P > 0.10$ and biologically not important. No interaction terms were tested. Cows were considered as random effects to account for the correlation between observations of the same cow and correlations between cows in the seam farm. Odds ratios (OR) and 95% confidence intervals (95% CI) were determined by logistic regression. Cow parity was grouped as either primiparous or multiparous, whereas calving season was grouped as cold season (September to March; 24 cows) or warm season (May to August; 26 cows).

Time to first insemination and conception were analyzed with multivariable survival analysis using Cox's proportional hazards regression accounting for clustering of cows within farms with Proc PHREG (SAS Inst. Inc.). A Stacked line plot of BCS changes was generated using Prism 6.07 (GraphPad Software, Inc. La Jolla, CA USA).

Results

Ovarian resumption

Two groups were formed based on differences in onset of postpartum resumption of ovarian activity. Early responders (ER, n = 28/50 or 56% of cows), showed first ovulation between 15 d and 52 d after parturition. Late responders (LR, n = 22/50 or 44%) were cows with a first ovulation between 53 and 87 d postpartum.

There was no effect of group (early responders/ late responders) and interaction between group and sampling time ($P > 0.05$) on all plasma metabolites, but there were significant effects of sampling time ($P < 0.0001$) on BHBA, Cholesterol, ALT and Mg (Table 1). For BCS, there was no effect of group and sampling time ($P > 0.05$), but there were significant effects of interaction between group and sampling time ($P < 0.05$, figure 1).

For the results of the final multivariable logistic regression as shown in Table 2, only 6 variables were retained for each week (wk 2 and 4). Within individual weeks after calving, an association between plasma Glucose (OR=0.52; P =0.01), TG (OR=1.27; P =0.03), AST (OR=3.17; P =0.01) and Ca (OR=0.38; P =0.04) concentrations and the ORA was identified, accounting for the effect of correlation of cows within a farm in week 2. In week 4, an

association between plasma BHBA (OR=0.54; P <0.0001), Cholesterol (OR=1.68; P =0.02), PT (OR=3.39; P =0.01) and ALT (OR=2.05; P <0.0001) concentrations and the ORA was identified. However, there was no effect (p>0.05) of parity on ORA in both times, BHBA in wk2 and NEFAs in wk4.

Table 1. Comparison of means and standard deviation (SD) of serum constituents and body condition score (BCS) between cows' group (G) early responders (ER) and late responders (LR) at days 0, 15, 30, 41 and 52 relating parturition.

metabolites	G	Day 0	Day 15	Day 30	Day 41	Day 52	P*		
							G	Time	Time*G
BHBA	ER		0.63±0.35	0.77±0.40	0.85±0.32	0.70±0.32	0.03	<0.0001	0.85
(mmol/l)	LR	-	0.66±0.28	1.06±0.32	1.14±0.49	0.82±0.24			
Glucose	ER	-	0.54±0.11	0.59±0.09	0.62±0.16	0.67±0.14	0.81	0.04	0.99
(g/l)	LR	-	0.63±0.13	0.63±0.10	0.69±.15	0.65±0.09			
Chol (g/l)	ER	-	1.07±0.26	1.37±0.30	1.47±0.34	1.54±0.39	0.40	<0.0001	0.92
	LR	-	0.99±0.37	1.33±0.34	1.48±0.40	1.55±0.38			
TG (g/l)	ER	-	0.24±0.27	0.21±0.14	0.20±0.09	0.19±0.07	0.24	0.32	0.28
	LR		0.15±0.06	0.17±0.06	0.16±0.06	0.18±0.06			
Urea (g/l)	ER	-	0.25±0.08	0.27±0.12	0.24±0.11	0.25±0.13	0.71	0.38	0.68
	LR		0.19±0.09	0.25±0.12	0.23±0.11	0.27±0.12			
PT (g/l)	ER	-	73.7±10.8	78.5±9.34	75.3±12.7	78.5±9.98	0.57	0.33	0.77
	LR		74.1±12.5	77.3±8.43	78.9±10.1	78.1±9.33			
AST (UI)	ER	-	94.8±16.3	92.4±18.9	89.7±23.6	90.2±20.1	0.21	0.79	0.78
	LR		85.1±14.1	84.6±19.1	87.4±18.3	100±46.2			
ALT (UI)	ER	-	29.4±8.72	34.2±11.1	35.1±13.9	37.2±12.8	0.83	<0.0001	0.91
	LR		23.6±6.75	27.1±8.68	33.2±10.9	31.3±8.94			
γGT (UI)	ER	-	22.3±15.5	21.3±10.7	22.3±8.73	22.7±7.53	0.17	0.45	0.97
	LR		19.8±4.99	20.4±4.27	20.2±3.82	28.6±37.3			
Ca (mg/l)	ER	-	83.6±9.88	87.7±8.22	84.6±13.6	85.4±10.6	0.53	0.50	0.85
	LR		84.9±11.1	87.5±11.1	88±9.60	86.1±11.3			
P (mg/l)	ER	-	56.5±13.6	55.7±13.7	53.2±15.1	59.7±19.1	0.37	0.83	0.57
	LR		55.8±10.4	60.4±16	58.1±18.9	54.7±16.6			
Mg (mg/l)	ER	-	18.2±4.32	18.9±3.67	18.1±3.93	18.8±4.17	0.11	<0.0001	0.99
	LR		16.8±4.37	18.3±2.81	19.9±5.06	18.3±3.35			
Na (Meq/l)	ER	-	138±7.05	137.±7.05	134±7.62	139±6.62	0.34	0.91	0.65
	LR		140±10.05	136.±5.33	138±6.56	138±5.58			
K (Meq/l)	ER	-	4.27±0.46	4.36±0.57	4.49±0.85	4.50±0.52	0.03	0.93	0.75
	LR		4.47±0.43	4.49±0.37	4.40±0.54	4.38±0.48			
BCS	ER	2.86±0.30	2.52±0.29	2.38±0.22	2.27±0.25	2.27±0.32	0.06	0.13	0.0005
	LR	2.93±0.36	2.61±0.21	2.27±0.25	2.11±0.21	2.07±0.18			

* Significant difference was considered at the level of P < 0.05.

Table 2. Final logistic regression model of the association between parity, glucose, triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, serum non-esterified fatty acid (NEFA), 3-β-hydroxybutyrate (BHBA), total protein (PT) and calcium (Ca) concentrations in the second and fourth week postpartum with the risk of resumption of ovarian activity at 52 days in milk (DIM).

Variable	Estimate	SE	P-value	OR	95% CI
Week 2					
Parity	0.05	0.14	0.89	1.02	0.77-1.34
BHBA (mmol/l)	-0.30	0.16	0.05	0.74	0.54-1.01
Glucose (g/l)	-0.65	0.27	0.01	0.52	0.30-0.89
TG (g/l)	0.24	0.11	0.03	1.27	1.02-1.59
AST (UI)	1.15	0.45	0.01	3.17	1.31-7.67
Calcium (mg/l)	-0.97	0.49	0.04	0.38	0.14-0.99
Week 4					
Parity	-0.02	0.13	0.89	0.98	0.76-1.27
NEFAs (mmol/l)	-0.22	0.20	0.26	0.79	0.53-1.18

BHBA (mmol/l)	-0.61	0.15	<0.0001	0.54	0.39-0.73
Cholesterol (g/l)	0.52	0.22	0.02	1.68	1.08-2.61
PT (g/l)	1.22	0.48	0.01	3.39	1.30-8.79
ALT (UI)	0.71	0.48	<0.0001	2.05	1.46-2.86

Pregnancy at the first AI

Thirty-four percent of the cows were pregnant after first AI. There was no effect of interaction between group P/NP cows and sampling time ($P > 0.05$) on all plasma metabolites, but there were significant effects of sampling time ($P < 0.0001$) on BHBA, Cholesterol, ALT and MG. The BHBA and K concentrations in the pregnant cows group tended to be higher ($P < 0.05$) compared to the non-pregnant cows group (Table 3). Also, there were significant effects of interaction between examined groups and sampling time ($P < 0.001$) only on BCS (figure 1).

The results of the multivariate prediction models within individual weeks after calving are shown in Table 4. From these models, cows with high BHBA concentration in wk 2 were significantly less likely to be diagnosed pregnant after first insemination (OR=0.65; P=0.001). In week 4 after calving, an association between plasma BHBA (OR=0.75; P= 0.03), NEFAs (OR=0.52; P =0.002), glucose (OR=0.53; P =0.01), AST (OR=0.41; P <0.0001) and phosphorus (OR=0.61; P= 0.01) concentrations and the P/IA was identified.

However, P/AI did not differ significantly among cows with different BCS-Calv even if cows with higher BCS-Calv tended ($p = 0.078$) to be more frequently pregnant. Likewise, no significant association was found between P/IA and dBCS (P=0.63).

Figure 1. Body condition score (BCS) (Mean ± SD) from calving to 52 days postpartum in dairy cows that either ovulated (early responders) or had not ovulated (late responders) (A) at 52 days in milk (DIM) or were either pregnant or not pregnant after first insemination (B).

Table 3. Comparison of means and standard deviation (SD) of serum constituents and BCS between cows group (G): pregnant (P) and not pregnant (NP) after first AI at days 0, 15, 30, 41 and 52 relating parturition.

PR/IA	G	Day 0	Day 15	Day 30	Day 41	Day 52	*P		
							G	Time	Time*G
BHBA	P	-	0.51±0.21	0.68±0.26	0.75±0.19	0.67±0.35	0.05	<0.0001	0.35
(mmol/l)	NP	-	0.71±0.34	1.01±0.40	1.09±0.47	0.80±0.25			
Glucose	P	-	0.57±0.12	0.61±0.11	0.63±0.09	0.66±0.14	0.85	0.01	0.18
(g/l)	NP	-	0.59±0.13	0.61±0.10	0.66±0.18	0.66±0.11			
Chol (g/l)	P	-	1.01±0.21	1.29±0.29	1.45±0.37	1.46±0.32		<0.0001	0.7461
	NP	-	1.05±0.36	1.39±0.33	1.49±0.37	1.60±0.41	0.890		
TG (g/l)	P	-	0.23±0.25	0.21±0.17	0.19±0.09	0.17±0.05	0.58	0.69	0.26
	NP	-	0.19±0.18	0.18±0.07	0.18±0.08	0.19±0.07			
UREA	P	-	0.23±0.10	0.21±0.10	0.23±0.12	0.25±0.13	0.60	0.43	0.19
(g/l)	NP	-	0.22±0.08	0.28±0.13	0.24±0.11	0.27±0.13			

PT (g/l)	P	-	73.2±13.2	78.9±7.89	76.5±12.3	79.6±10.3	0.94	0.03	0.51
	NP	-	74.3±10.7	77.5±9.43	77.1±11.6	77.8±9.31			
AST (UI)	P	-	89.3±16.4	84.3±15.1	82.7±19.8	86.5±19.3	0.53	0.63	0.22
	NP	-	91.2±15.9	91.3±20.8	91.8±21.6	98.9±39.3			
ALT (UI)	P	-	27.1±8.79	30.9±13.2	33.8±13.2	36.1±14.5	0.11	<0.0001	0.14
	NP	-	26.8±8.28	31.1±9.30	34.5±12.5	33.9±9.81			
γGT (UI)	P	-	19.5±8.81	18.3±5.03	19.5±4.39	20.7±6.35	0.76	0.28	0.96
	NP	-	22.1±13.4	22.2±9.54	22.1±7.94	27.7±30.6			
Ca (mg/l)	P	-	81.9±12.6	83.9±10.9	83.7±15.2	84.9±12.1	0.82	0.56	0.82
	NP	-	85.3±8.98	89.6±8.15	87.3±10.1	86.2±10.2			
P (mg/l)	P	-	55.7±13.5	53.3±11.1	49.8±13.9	54.1±11.9	0.42	0.65	0.14
	NP	-	56.8±11.6	60.1±16.1	58.2±17.7	59.2±20.5			
Mg (mg/l)	P	-	17.1±3.38	18.2±3.21	17±3.57	17.3±3.26	0.48	<0.0001	0.95
	NP	-	17.9±4.80	18.8±3.38	19.9±4.67	19.3±3.95			
Na (Meq/l)	P	-	138±9.58	135±7.90	133±9.10	138±6.58	0.94	0.99	0.39
	NP	-	139±7.98	138±4.89	137±5.95	139±5.97			
K (Meq/l)	P	-	4.19±0.44	4.25±0.55	4.36±0.77	4.31±0.48	0.67	0.99	0.16
	NP	-	4.45±0.45	4.50±0.44	4.50±0.70	4.52±0.51			
BCS	P	2.82±0.25	2.56±0.24	2.41±0.20	2.32±0.25	2.35±0.29	0.24	0.99	0.0005
	NP	2.92±0.36	2.56±0.27	2.29±0.25	2.14±0.23	2.09±0.23			

* Significant difference was considered at the level of $P < 0.05$.

Table 4. Final logistic regression model of the association between Parity, glucose, Triglycerides (TG), aspartate aminotransferase (AST), non-esterified fatty acid (NEFA), 3-β-hydroxybutyrate (BHBA), Phosphorus, BCS at calving (BCS-calv) and BCS loss (dBCS); and pregnancy at first insemination.

Variable	Estimate	SE	P-value	OR	95% CI
Week 2					
Parity	0.22	0.13	0.10	1.24	0.95-1.62
BHBA (mmol/l)	-0.43	0.13	0.001	0.65	0.50-0.84
Week 4					
Parity	0.36	0.11	0.001	1.43	1.15-1.78
BHBA (mmol/l)	-0.28	-0.28	0.03	0.75	0.58-0.98
NEFAs (mmol/l)	-0.64	0.20	0.002	0.52	0.35- 0.78
Glucose (g/l)	-0.62	0.25	0.01	0.53	0.33-0.87
AST (UI)	-0.89	0.22	<0.0001	0.41	0.27-0.63
Phosphorus (mg/l)	-0.49	0.19	0.01	0.61	0.42-0.88
BCS-calv	0.12	0.07	0.08	1.13	0.98-1.30
dBCS	-0.45	0.25	0.07	0.63	0.39-1.03

Time to first insemination and to conception

We analyzed the factors that affected the hazard of resumption of cyclicity by 12 weeks postpartum (Table 5). Only K and Mg concentrations in second week affected [the hazard ratio (HR) for K=0.45 and it was 1.22 for Mg], whereas calving season did not (P>0.05). Days to conception within 150 DIM affected SC, BHBA and Na plasma concentration of the 4th week with a HR=0.22, 0.11 and 0.68 respectively, whereas parity did not (table 6).

Table 5. Cox proportional hazards model for the effect of Season of calving, K and Mg on time from calving to first insemination.

Variable	Estimate	SE	P value	HR	95% CI
Season of calving	-0.79	0.47	0.09	0.45	0.18-1.13
K* (Meq/l)	-0.80	0.31	0.01	0.45	0.24-0.83
Mg* (mg/l)	0.19	0.06	0.001	1.22	1.08-1.37

*Metabolites measured at wk2

Table 6. Cox proportional hazards model for the effect of parity, Season of calving, BHBA and sodium on time to conception.

Variable	Estimate	SE	P value	HR	95% CI
Parity	-2.01	1.24	0.10	0.13	0.01-1.54
Season of calving	-1.47	0.51	0.004	0.22	0.08-0.62
BHBA* (mmol/l)	-2.24	0.70	0.001	0.11	0.03-0.42
Sodium* (Meq/l)	-0.38	0.18	0.03	0.68	0.47-0.97

*Metabolites measured at wk4

Discussion

This study evaluated the relationship between the concentrations of certain nutrient-sensitive blood metabolites and the resumption of ovarian cyclicity, pregnancy at first insemination, time to first insemination and to conception in postpartum Monbéliarde cows. The ROA seemed relatively higher in the present study (56%) than some earlier report (32.6%) from the same breed in France (Pires et al 2015). However, overall mean P/AI at first insemination was 34%, much lower than reported by Barbat et al (2010) which was 54%.

Negative associations between nutritional status in early lactation and subsequent reproductive performance have been reported in a number of previous studies (Beam et Butler 1999; Boland and Lonergan 2003; Konigsson et al 2008). Metabolic profiles are frequently used to assess energy status and it influences, among other factors, dairy cow fertility (Wathes et al 2007).

Contrary to comparable studies, the results of our study clearly demonstrate no relationship between post-partum metabolic profiles and the resumption of ovarian cyclicity. There was a relationship between post-partum metabolic profiles of BHBA, K and odds of pregnancy at the first AI. However, no significant differences were detected in the whole period (interaction with time) of observation in the circulating metabolites concentration between cows grouped according to their ovarian activity and pregnancy state. The effect of postpartum circulating metabolites concentration on P/1-AI, time to beginning of luteal activity has been described later in lactation with variable results (Canfield and Butler 1990; Taylor et al 2003; Shrestha et al 2005). Their effect depends an adaptational system to NEB (Butler 2000). Given the complexity of this adaptational system and the number of metabolites involved, it seems impossible to expect whether this metabolic adaptation is, at a certain point in time, successful or not (LeBlanc 2010). Furthermore, it is interesting to note that there is no interaction with time for NEFAs, because it was measured one time only (at 30 DIM). Blood NEFA concentrations have been found to be a more accurate measure of NEB (Ospina et al 2010).

Only BCS was affected by both ROA groups and P/1-AI groups x time interaction. However, there was not a relationship between post-partum BCS profile and P/1AI or ORA. Change in BCS highly correlates with cumulative negative energy balance (NEB) and reflects total energy deficit. The effect of NEB on reproductive performances in dairy cows is well known (Konigsson et al 2008). Both the duration and magnitude of negative energy balance are associated with reduced reproductive performance (Walsh et al 2007). Consequently, prolonged mobilisation of body reserves during early lactation can have significant deleterious effects on resumption of ovarian activity postpartum, conception rate and infertility (Domecq et al 1997; Boland and Lonergan 2003). The magnitude of BCS change may be a more important predictor of reproductive performance.

Within individual weeks, as hypothesized, factors associated with OAR and P/1-AI included indicators of nutritional status, parity and season of calving. As expected, energetic metabolite had most effect on both ROA and P/1-AI. Ovarian resumption risk was not significantly affected by BHBA concentrations in wk 2 and by NEFAs concentrations in wk 4. However, there was a strong association between elevated circulating ketone concentrations in the fourth week postpartum and risk of pregnancy at first insemination. Furthermore, the probability of pregnancy after first AI decreased with increasing circulating BHBA concentrations in wk 2 and with increasing circulating BHBA and NEFAs concentrations in wk 4. Both elevated serum concentrations of NEFAs and BHBA have been identified as a priori risk of decreasing ROA (Dubuc et al 2012; Reberio et al 2013; Shin et al 2015) and P/1-AI (Mcart et al 2012; Garverick et al 2013).

Circulating concentrations of NEFAs and β-hydroxybutyrate (BHBA) measure aspects of the success of adaptation to negative energy balance (LeBlanc 2010), their elevated level may be indicative of decreased DMI and greater NEB (McArt et al 2013). Increased severity of negative energy balance and increased time to its nadir decreased the probability of ovulation and pregnancy (Colazo et al 2009). NEB acting through the combined metabolic signaling of low IGF-I, GH, insulin and glucose (Hammon et al 2009). Surprisingly, negative effect of plasma glucose concentrations on ROA and P/1-AI were unexpected, as glucose levels usually had a positive effect on ROA and P/1-AI (Garverick et al 2013; Shin et al 2015). This finding may be explained by the variation of glucose measurement in ruminant. Concentrations of plasma glucose can vary under the influence of numerous factors (Bowden 1971). Diurnal variation, stress, sampling time post-feeding, diet composition, and intake can affect glucose concentrations in dairy cow (Herbein et al 1985). Their measurements are more accurate when insulin is measured. AST and ALT were used to assess liver function associated with hepatic lipidosis during postpartum (Bobe et al 2004). We found a negative relationship between AST, ALT and ORA and ALT at wk 4 and risk of pregnancy at first insemination. However, Samarütel et al (2008) reported that higher AST concentrations could be related to the delayed first ovulation. Similarly, passive effect of plasma TG, cholesterol concentrations on ROA were observed. Plasma cholesterol increased during early lactation in that study and was partly attributed to the hepatic re-esterification of NEFA as TG (Bjerre-Harpøth et al 2012). The patterns of effect of cholesterol and triglyceride concentrations on fertility in dairy cows were reported in previous study (Guédon et al 1999). Likewise, total protein was associated with ovarian cycles postpartum in dairy cows according to previous study (Meikle et al 2004). Moreover, Shrestha et al (2005) didn't found an evidence relationship. This may be explained by the typical mobilization of body fat and protein to meet the requirements for milk production and maintenance during early lactation (Goff and Horst 1997). These proteins have been converted to provide substrate for gluconeogenesis and the breakdown of triglycerides from adipose tissue and the provision of glycerol as a substrate for gluconeogenesis (Van Dorland et al 2009).

It has been reported that reduced blood calcium concentration increases the incidence of postpartum reproductive disorders and fertility (Chapinal et al 2011; Reberio et al 2013) although decreasing phosphorus may affect ovarian activity (Jeong et al 2015), and conception rate (Costa et al 2015). Our results are not consistent with these findings and they rather agree with others that failed to detect positive relationship between postpartum blood calcium and ROA and between postpartum blood calcium, phosphorus and P/1-AI. Contrary to our hypothesis, we found no relationship between BCS at calving and dBCSs concentrations around calving and the odds of pregnancy at the first AI which has been shown by other studies (Ospina et al 2010; Garverick et al 2013). Conversely, other studies show an

association between BCS at calving BCS loss and pregnancy at first insemination (Roche et al 2007; Chapinal et al 2012).

It is known that time from calving to first insemination is a reflection of management practice regarding the voluntary waiting period and estrus detection efficiency (Walsh et al 2007). In our results, only blood magnesium concentrations at wk 2 was positively related to time from calving to first insemination, though, blood potassium had a negative association. This finding may be explained with DMI, because magnesium and potassium are not stored in the body. Jeong et al (2015) reported that a lower level of magnesium in ration may affect reproduction performance. Furthermore, previous study suggests that feeding high levels of K may delay the onset of puberty and ovulation, impair corpus luteum development and increase the incidence of anestrus in heifers (Smith and Chase 1985). Season of calving was not significantly associated with time from calving to first insemination. However, contrary to other report (Ospina et al 2010), season was significantly associated with time to conception. In other words, cows calved at worm season had 1.47 more time to become pregnant comparing with cows calved at cold season. Postpartum BHBA concentrations had a negative effect on time to conception, contrary to results found by Ospina et al (2010). Sodium is the most important extracellularly cation, it is indispensable for several functions in the organism. Though, in this study we found negative effect of postpartum sodium concentrations on time to conception.

Conclusion

- The present study demonstrated that the increased NEFA, BHBA, glucose, phosphorus and calcium concentrations, decreased cholesterol, AST, ALT, TG and protein total were associated with decreased ROA and P/1-AI. However, only increased BHBA (among energetic metabolites) was associated with increasing of time from calving to conception. Increased potassium and decreased magnesium were associated with increasing of time from calving to first insemination. These findings indicate that balanced nutrition and reproduction management should be emphasized to ameliorate reproductive performance.

Conflict of interest statement

The authors declare that none of them have any conflict of interest to declare.

References

Abdelli A, Belabdi I, Souames S and Iguer-Ouada M 2015 Facteurs affectant la réussite de la première insémination artificielle dans des exploitations laitières de la région de Tizi-Ouzou, Algérie. Renc. Rech. Ruminants. 2, 215.

Ayad A, Iguer-Ouada M and Benbarek H, 2014 Electrochemiluminescence immunoassay for progesterone by using a heterologous system in plasma bovine. Vet. World. 7, 610-613.

Barbat A, Le Mézec P, Ducrocq V, Mattalia S, Fritz S, Boichard D, Ponsart C and Humblot P 2010 Female Fertility in French Dairy Breeds: Current Situation and Strategies for Improvement. J. of Reprod and Development. 56, S15-S21.

Bauman D E and Currie W B 1980 Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and hemeorhesis. J. Dairy Sci. 63, 1514–1529.

Beam S W and Butler W R 1998 Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J Dairy Sci 81,121-131.

Bjerre-Harpøth V, Friggens N C, Thorup V M, Larsen T, Damgaard B M, Ingvartsen K L and Moyes K M 2012 Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. J. Dairy Sci. 95, 2362–2380.

Bobe G, Young J W and Beitz D C 2004 Invited Review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87, 3105–3124.

Boland M P and Lonergan P 2003 Effects of Nutrition on Fertility in Dairy Cows. Advances in Dairy Technology. 15, 19-32.

Bowden D M 1971 Non-esterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: a review. Canadian Journal of Animal Science 51, 1-13.

Butler W R 2000 Nutritional interactions with reproductive performance in dairy cattle. Anim Repro Sci. 60-61, 449-457.

Canfield R W and Butler W R 1990 Energy balance and pulsatile LH secretion in early postpartum dairy cattle. Domestic Animal Endocrinology. 7, 323-330.

Chapinal N, Carson M, Duffield T.F, Capel M, Godden S, Overton M, Santos J E P and Le Blanc S J 2011 The association of serum metabolites with clinical disease during the transition period. J Dairy Sci. 94, 4897–4903.

Chapinal N, Carson M E, LeBlanc S J, Leslie K E, Godden S, Capel M, Santos J E P, Overton M W, Duffield T F 2012 The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95,1301–1309.

Colazo M G, Hayirli A, Doepel L and Ambrose D J 2009 Reproductive performance of dairy cows is influenced by prepartum feed restriction and dietary fatty acid source. J. Dairy Sci. 92, 2562– 2571.

Damptey J K, Obese F Y, Aboagye G S, Ayim-Akonor M and Ayizanga R A 2014 Blood metabolite concentrations and postpartum resumption of ovarian cyclicity in Sanga cows. S. Afr. J. Anim. Sci. 44, 10-17.

Domecq J J, Skidmore A L, Lloyd J W and Kaneene J B 1997 Relationship between body condition scores and conception at first artificial insemination in a large dairy herd of high yielding Holstein cows. J. Dairy Sci, 80, 113-120.

Dubuc J, Duffield T F, Leslie K E, Walton J S and Le Blanc S J 2012 Risk factors and effects of postpartum anovulation in dairy cows. J. Dairy Sci. 95: 1845–1854.

Edmonson A J, Lean I J, Weaver L D, Farver T and Webster G A Body condition scoring chart for Holstein dairy cows. J Dairy Sci. 72, 68–78.

Farver T B Concepts of normality in clinical biochemistry. In: Kaneko J J, Harvey J W and Bruss ML Clinical Biochemistry of Domestic Animals. 5th ed. San Diego: Academic Pr; 1997. pp. 1–19.

Garverick H A, Harris M N, Vogel-Bluel R, Sampson J D, Bader J, Lamberson W R, Spain J N, Lucy M C and Youngquist R S 2013 Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. J. Dairy Sci. 96, 181–188.

Goff J P and Horst R L1997 Effects of the addition of potassium or sodium, but not calcium, to prepartum ratios on milk fever in dairy cows. J Dairy Sci. 80,176-186.

Guedon L, Saumande J, Dupron F, Couquet C and Desbals B 1999 Serum cholesterol and triglycerides in postpartum beef cows and their relationship to the resumption of ovulation. Theriogenology. 51, 1405–1415.

Hammon H M, Stürmer G, Schneider F, Tuchscherer A, Blum H, Engelhard f T, Genzel A, Staufenbiel R and Kanitz W 2009 Performance and metabolic and endocrine changes with emphasis on glucose metabolism in high-yielding dairy cows with high and low fat content in liver after calving. J. Dairy Sci. 92, 1554-1566.

Herbein J H, Aiello R J, Eckler L I, Pearson R E and Akers R M 1985 Glucagon, Insulin, Growth Hormone, And Glucose concentrations in blood plasma of lactating dairy cows. J. Dairy Sci. 68, 320-325.

Iwersen M, Falkenberg U, Voigtsberger R, Forderung D and Heuwieser W 2009 Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. Journal of Dairy Science 92, 2618–2624.

Jeong J K, Choi I S, Kang H G, Hur T Y, Jung Y H and Kim I H 2015 Relationship between serum metabolites, body condition, peri- and postpartum health and resumption of postpartum cyclicity in dairy cows. Livestock Sci. S1871-1413 (article in press).

Kadi S A, Djellal F et Berchiche M 2007a Caractérisation de la conduite alimentaire des vaches laitières dans la région de Tizi-Ouzou, Algérie. Livestock Research for Rural Development. 19 *Article* #51. http://www.lrrd.org/lrrd19/4/kadi19051.htm

Konigsson K, Savoini G, Govoni N, Invernizzi G, Prandi A, Kindahl H and Veronesi M C 2008 Energy balance, leptin, NEFA and IGF-I plasma concentrations and resumption of postpartum ovarian activity in swedish red and white breed cows. Acta Vet Scand. 50, 1–7.

Le Blanc S 2010 Monitoring metabolic health of dairy cattle in the transition period. Journal of Reproduction and Development. 56S, S29-S35.

Leslie K, Duffield T and Le Blanc S 2003 Monitoring and managing energy balance in the transition dairy cow. J Dairy Sci 86, 101-107.

Madani T, Yakhlef H and Marie M 2008 Effect of age at first calving on lactation and reproduction of dairy cows reared in semi-arid region of Algeria. Livestock Research for Rural Development 20 *Article #6*. http://www.lrrd.org/lrrd20/6/mada20092.htm

McArt J A A, Nydam D V and Oetzel G R 2012 Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95, 5056–5066.

McArt J A A, Nydam D V, Oetzel G R, Overton T R and Ospina P A 2013 Elevated non-esterified fatty acids and b-hydroxybutyrate and their association with transition dairy cow performance. The Veterinary Journal. 198, 560–570.

Meikle A, Kulcsar M, Chilliard Y, Febel H, Delavaud C, Cavestany D and Chilibroste P 2004 Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction.127, 727-737.

Miroud K, Hadef A, Khelef D, Ismail S et Kaidi R 2014 Bilan de reproduction de la vache laitière dans le nord-est de l'Algérie. Livestock Research for Rural Development 26 *Article #6*. http://www.lrrd.org/lrrd26/6/miro26107.htm

Mouffok C, Madani T, Smara L, Baitiche M, Allouche L and Belkasmi F 2011 Relationship between body condition score, body weight, some nutritional metabolites changes in blood and reproduction in Algerian Montbeliarde cows. Vet. World. 4, 461-466.

Ospina P A, Nydam D V, Stokol T and Overton T R 2010 Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93, 1596–1603.

Pires J A A, Chilliard Y, Delavaud C, Rouel J, Pomiès D and Blan F 2015 Physiological adaptations and ovarian cyclicity of Holstein and Montbéliarde cows under two low-input production systems. Animal. 9,121. 986–1995.

Ribeiro E S, Lima F S, Greco L F, Bisinotto R S, Monteiro A P A, Favoreto M, Ayres H, Marsola R S, Martinez N, Thatcher W W and Santos J E P 2013 Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. J. Dairy Sci. 96, 1–16.

Roche J R, Macdonald K A, Burke C R, Lee J M and Berry D P 2007 Associations Among Body Condition Score, Body Weight, and Reproductive Performance in Seasonal-Calving Dairy Cattle. J DairySci. 90, 376–391.

Rutherford A J, Oikonomou G and Smith R F 2016 The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle. J. Dairy Sci. 99,1–8. Article in press.

Samarütel J, Ling K, Waldmann A, Jaakson H, Kaart T and Leesmäe A Field trial on progesterone cycles, metabolic profiles, body condition score and their relation to fertility in Estonian Holstein dairy cows. Reprod Domest Anim. 43, 457–63.

Shin E K, Jeong J K, Choi I S, Kang H G, Hur T Y, Jung Y G and Kim I H 2015 Relationships among ketosis, serum metabolites, body condition, and reproductive outcomes in dairy cows. Theriogenology. 84, 252–260.

Shrestha H K, Nakao T, Suzuki T, Akita M and Higaki T 2005 Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high producing dairy cows in a subtropical region in Japan. Theriogenology 64, 855–866.

Smith R D and Chase L E 1985 Nutrition and reproduction. In: Animal Science Mimeo, vol. 88. Dept. of Animal Science Cornell University Ithaca, NY.

Taylor V J, Beever D E, Bryant M J and Wathes DC 2003 Metabolic profiles and progesterone cycles in first lactation dairy cows. Theriogenology. 59, 1661–1677.

Tillard E, Humblot P, Faye B, Lecomte P, Dohoo I and Bocquier F 2008 Postcalving factors affecting conception risk in Holstein dairy cows in tropical and sub-tropical conditions. Theriogenology. 69, 443–457.

Van Dorland H A, Richter S, Morel I, Doherr M G, Castro N and Bruckmaier R M 2009 Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. J Dairy Sci. 92, 1924–1940.

Voyvoda H and Erdogan H 2010 Use of a hand-held meter for detecting subclinical ketosis in dairy cows. Research in Veterinary Sci. 89, 344–351.

Walsh R B, Walton J S, Kelton D F, Le Blanc J S, Leslie K E and Duffield T F 2007 The Effect of Subclinical Ketosis in Early Lactation on Reproductive Performance of Postpartum Dairy Cows. J. Dairy Sci. 90, 2788–2796.

Wathes D C, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris D G, Kenny D, Murphy J and Fitzpatrick R 2007 Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology. 68s, 232-241.

Weng X, Zhao W, NeethirajanS and Duffield T F 2015 Microfluidic biosensor for β-Hydroxybutyrate (βHBA) determination of subclinical ketosis diagnosis. J. of Nanobiotechnology. 13, 1-8.

Zhang Z, Liu G, Wang H, Li X and Wang Z 2012 Detection of Subclinical Ketosis in Dairy Cows. Pak Vet J. 32, 156-16

Received	Accepted	Published
14 October 2016	15 November 2016	1 January 2017

Go to top