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RESEARCH ARTICLE

Relationship of BCS Prepartum with Reproductive Performance and Lipomobilization in Holstein Dairy Cows

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ABSTRACT

Body condition score (BCS) changes and non-esterified fatty acids (NEFA) are good indicators for measuring lipomobilization during the transition period. The aim of this study was to establish the possible correlation between cows with a higher BCS during this period and their reproductive performance postpartum. A total of 47 Holstein dairy cows were randomly allotted into two groups according to their prepartum BCS, including: G1 (BCS 3.5 to 3.75, n = 20) and G2 (BCS >3.75, n=27). Moreover, another two groups were established at 14th day postpartum: G3, 37 cows lost more than 0.75 points of BCS and G4, 10 cows lost 0.75 points or less. Blood samples were obtained from 10 to 7 days before expected calving, and 14 days postpartum in all groups and serum concentrations of glucose, β-hydroxybutyrate, NEFA, urea, calcium and hepatic enzymes, and reproductive parameters were determined. NEFA values showed that cows of G2 and G4 had a higher lipomobilization than G1 and G3. Moreover, cows with a BCS of more than 3.75 and NEFA values of more than 495 µmol/L prepartum had a higher risk of non-pregnancy at first AI, and there was an increase in the number of open cows at 150 DIM, this being the reason why the elimination rate due to non-pregnancy increases (20%) and their reproductive lives shorten.

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INTRODUCTION

The most critical period in production of dairy Holstein Friesian cows is from the end of pregnancy to the onset of next lactation. This period is characterized by negative energy balance (NEB), when the dry matter intake (DMI) is decreased and the cow tries to resolve it by mobilizing body reserves. This increased lipolysis is associated with a loss of weight and body condition score (BCS), especially 30–40 days postpartum (Drackley *et al.*, 2001).

Physiologically, high-producing dairy cows lose 0.5 points of BCS in the first 30 days postpartum (Domecq *et al.*, 1997; Duffield *et al.*, 1998). Increased losses of up to 0.75 points are associated with increased chances of problems such as metritis, displaced abomasum, lameness, ketosis and hypocalcaemia (Kim and Suh, 2003); therefore, minimizing loss of BCS in the postpartum period (≤0.5 points) is essential for good herd fertility (Smith *et al.*, 2014). However, high BCS will not necessarily lead to diseases related to metabolic disorders (Vernon, 2005). Obviously, some cows are able to overcome the metabolic adaptation mechanism, while others are not (Herdt, 2000; Drackley *et al.*, 2001; Adrien *et al.*, 2012). Nevertheless, the loss of BCS (ΔBCS) remains a valuable indicator of lipomobilization and, indirectly, a useful indicator to measure lipomobilization during early lactation (Weber *et al.*, 2013).

Serum levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) during the transition period are useful indicators of the capacity of a dairy cow to cope with the NEB during this period (Herdt, 2000). Moderate increases of these metabolites are normal because there is a high energy demand for two weeks before the calving and during early lactation, although an excessive increase of NEFA or BHBA may indicate problems with energy balance and subsequent intensive lipomobilization (González *et al.*, 2011).

Adrien *et al.* (2012) observed that the concentrations of NEFA and BHBA were affected by the BCS. So, cows with high BCS had higher levels of NEFA at calving and the highest concentrations of BHBA in cows were found

at 15 days postpartum. Moreover, an excessive NEB during early lactation, measured in terms of BHBA concentrations, has been associated with decreased reproductive capacity (Walsh *et al.*, 2007a). Thus, low concentrations of glucose and elevated BHBA and NEFA levels affect the ovarian activity and the immune system, which are vital to restore uterine health postpartum (Lucy *et al.*, 2014).

Ospina *et al.* (2010) and Chapinal *et al.* (2012) established limits for BHBA and NEFA; the higher values are associated with decreased reproductive capacity. However, there are a few reports regarding the correlation between BCS>3.75 and the NEFA serum concentrations postpartum with pregnancy rate at 150 DIM (days in milk). Furthermore, other related reproductive parameters, such as elimination rate due to non-pregnancy or the number of inseminations per conception, have not been taken into account until now.

The present study was conducted to determine the relationship of BCS prepartum with reproductive parameters to find out if elevated BCS adversely affects reproductive parameters, and to investigate the correlation between BCS in prepartum period and NEB postpartum.

MATERIALS AND METHODS

Animals: This study was performed at a dairy cattle farm in North-western Spain (Galicia). A total of 47 Hosltein Friesian cows with an average milk production of 9618 Kg per 305-days lactation were used. The cows were housed in free-stalls with a rest area. Handling of cattle during the experimental period was carried out according to European Council Directive 86/609/EEC about the protection of animals used for experimental and other scientific purposes.

BCS was always recorded by the same observer, using a scale according to Ferguson *et al.* (1994). Two groups of cows were established, depending on BCS prepartum period: G1 (n=20), contained animals with BCS: 3.5–3.75; and G2 (n=27) with a BCS more than 3.75. Moreover, another two groups were established 14th day postpartum depending on the loss of BCS during transition period: G3 having 37 cows that lost more than 0.75 points of BCS; and G4 with 10 cows that lost 0.75 points or less.

Blood samples: Antepartum blood samples were taken from 10 to 7 days before the expected date of calving, and postpartum samples were taken at 14 days postpartum. These samples were collected from the coccygeal vein with a sterile vacuum tube without anticoagulant. All samples were processed in the laboratory of Animal Pathology in Lugo (Spain) within 24 h. Samples were centrifuged at 3500 rpm for 10 minutes to obtain serum, which was immediately frozen in Eppendorf tubes at -20ºC until biochemical measurements were carried out.

Samples were analysed by wet biochemical methods, using a spectrophotometer (Clima M-15®). Glucose, urea, ALT and calcium were determined using commercial kits (RAL, Spain). BHBA, NEFA and glutamate dehydrogenase (GLDH) were measured using kits from Randox (Ireland). Gamma glutamyltranspeptidase (GGT) and AST were measured using kits from Spinreact (Spain).

Reproductive data: Transrectal ultrasound examination was carried out 28 days postpartum in order to evaluate uterine involution and reproductive status. The examination was performed using 6.5 MHz rectal transducer (Easi Scan®, BCF Technology Ltd, Bellshill, UK). Insemination was done in animals that exhibited oestrus at least 40 days postpartum. In cows that did not show oestrus up to 60 days postpartum, ovulation was synchronized, using the Ovsynch protocol (Pursley *et al.*, 1995).

Pregnancy diagnosis was carried out 28-30 days after artificial insemination (AI) through transrectal ultrasonography. Non-pregnant animals were resynchronized and inseminated at a fixed time, whereas reproductive data of tested cows were transferred to database Access® to extract the following reproductive parameters: elimination rate due to non-pregnancy, pregnancy rate>150 DIM, number of AI per conception, and percentage of pregnant cows at first AI.

Statistical analysis: All data were analysed using the statistical program SPSS 18.0 (SPSS Inx., Chicago, IL, USA). All normalized data were subjected to ANOVA, considering BCS, ΔBCS and stage of transition period (antepartum or postpartum) as fixed factors and blood parameters as dependent variables. BCS or ΔBCS x stage interactions were checked using a Bonferroni test. Moreover, data from each fixed factor were compared using Student's t test.

Relationships between blood parameters were studied using Pearson's correlation test. Furthermore, Odds Ratio was performed between reproductive parameters and BCS.

RESULTS

Relationship between BCS and energy metabolites: Interaction between BCS or ΔBCS x stage was non-significant, so we have simplified Table 1. Results of this study showed an intensive lipomobilization in the transition period in cows with higher BCS (G2) and with ΔBCS of more than 0.75 points (G4) (Table 1), reviewing biochemical values from markers of energy metabolism (glucose, BHBA and NEFA); specifically, NEFA blood levels were always greater ($P<0.05$), at postpartum versus prepartum period. There were also significant differences in NEFA values between cows with different BCS at prepartum and cows with different ΔBCS ($P<0.05$).

Relationship between BCS and hepatic function, urea and calcium: Among the enzymes of liver function (ALT, GGT, AST and GLDH), activity of AST and GLDH was increased in postpartum than prepartum period and, although G2 had elevated NEFA values, G1 had greater activity of ALT and AST with significant differences ($P=0.006$) (Table 1). The values of urea and calcium showed non-significant differences among groups (Table 1).

Relationship between BCS and reproductive parameters: Results of reproductive parameters are given in Table 2. Animals of G2 showed worse reproductive

performance than G1 during the transition period. Pregnancy rate was higher in G1 than G2 during first 150 DMI (P<0.01). Cows with higher BCS (G2) showed an increased risk of non-pregnancy (OR=11.38) (Table 2; P<0.01). During the whole lactation period, many of these cows were non-pregnant and they were sent to the slaughterhouse. Elimination due to non-pregnancy rate for this group (G2) was 31.6% versus 12.5% in animals with optimal BCS.

The risk of non-pregnancy at first AI was higher in G2 (OR=13.93; Table 2) (P<0.01). The pregnancy rate at first AI was 13.6% in this group versus 68.8% in G1. This fact was reflected in the average number of inseminations per conception, which was significantly lower in this group (G1; 2.50) versus G2 (3.47).

Relationship among different parameters: Correlations among different biochemical parameters are shown in Table 3. A significant negative correlation was observed between glucose and NEFA (r=-0.241; P<0.05) and glucose and AST (r=-0.375; P<0.01). Regarding the two most useful parameters (BHBA and NEFA) for measuring the dairy cows´ capacity to overcome the NEB during the transition period, we observed that both parameters were positively correlated with each other (r=0.485; P<0.01). Also a relative increase of NEFA values implies a rise of other parameters (AST, GGT and calcium), as is demonstrated by a positive significant correlation. No correlation was found between BHBA and liver enzymes.

As regards liver enzymes, significant positive correlations were established between them. Thus, GLDH was positively correlated with AST (r=0.534; P<0.01). Calcium only showed a correlation with GLDH (P<0.01; r=0.345). Urea was correlated positively with AST and ALT activities.

DISCUSSION

Results of our study showed that cows that had a high BCS prepartum and cows with ΔBCS>0.75 points after calving experienced a significant increase in lipomobilization versus cows that had an optimal BCS (3.5–3.75) at calving (Pedron *et al*., 1993; Markusfeld *et al*., 1997) and with ΔBCS ≤0.75 (Table 1). It was found that all cows with high BCS and cows with ΔBCS above 0.75 points during the transition period had higher NEFA than indicated by Ospina *et al.* (2010), which leads to subclinical ketosis. This lipomobilization was a consequence of NEB status, which affected the cows at the beginning of lactation and this was shown by decreased blood glucose levels and increased blood BHBA and NEFA values; as well as increased liver enzymes activities in postpartum period. In our study, we found that activities of AST and GLDH enzymes were increased significantly in postpartum period and the same was true for AST enzyme in G1, probably because this group already had responded to the demands of lactation before calving. However, G2 had higher enzymes activities in postpartum than G1 (data not shown). Liver enzymes (ALT and AST) showed a correlation with calcium and urea, although these parameters were within normal range.

BHBA values at prepartum period (0.57 mmol/L) were lower than those reported by Chapinal *et al.* (2012). Chapinal *et al.* (2012) established that cows with BHBA values≥0.8 mmol/L one week before calving were associated with a decrease in milk production at early lactation and were at a higher risk of displaced abomasum. In our study, BHBA measured a week after calving was 0.71 mmol/L; which was lower than that indicated by Carrier *et al.* (2004) and Djoković *et al.* (2015). Other researchers have demonstrated that cows with BHBA ≥1 mmol/L during the first week after calving were 1.5 times more likely to remain in anoestrus in the following 9 weeks after calving (Walsh *et al.*, 2007b) and extending pregnancy rate (Walsh *et al.*, 2007a). On the other hand, Duffield *et al.* (2009) have shown that cows with BHBA>1.2 at the first and >1.4 mmol/L at the second week after calving had a risk three times higher of suffering metritis. In our study, this metabolite remained within average values (<1 mmol/L) during postpartum, without any sign of clinical or subclinical ketosis, and therefore, possibility of its involvement in reproductive efficiency was excluded. However, BHBA may be considered a good indicator of the nutritional status of dairy cows, as Mouffok *et al.* (2013) reported previously.

Some authors have studied the effect of calcium and urea on pregnancy rate. Blood calcium levels lower than or equal to 2.1 mmol/L a week after calving had OR = 0.07 for pregnancy at first AI (Chapinal *et al.*, 2012). Similarly, several authors have studied effects of urea on pregnancy rate (Schrick *et al.*, 1993; Gilbert *et al.*, 1996). In our work, calcium and urea remained within physiological ranges given by several authors, calcium values remained between 1.25–3 mmol/L (Kaneko *et al.*, 2008; Quiroz-Rocha *et al.*, 2009) and urea, 7.14 - 10.7 mmol/L (Kaneko *et al.*, 2008), thus excluding their effect on reproductive efficiency in our study. Moreover, calcium values remained higher than 2.1 mmol/L in all groups; this value is the cut-off proposed by Chapinal *et al*. (2012) that reduces the probability of pregnancy at first AI.

In our study, higher NEFA values were found in cows with greater loss of BCS (512 µmol/L). These values are similar to those found by Ospina *et al*. (2010), Djoković *et al*. (2015) and Furken *et al*. (2015). Ospina *et al*. (2010) found that 15% of cows with blood NEFA postpartum levels>700 µmol/L are associated with decrease in pregnancy rate, reduction in average milk production and increase in risk of displaced abomasum. NEFA was perhaps the only studied metabolite that was outside the physiological range reported by Ospina *et al.* (2010). So, open cows with BCS>3.75 and NEFA values of 495 µmol/L had OR=11.38 of non-pregnancy at 150 DIM. The total ratio of open cows in this group was 61.9% versus 12.5% in G1. This increase of open cows to 150 DIM only raises elimination rate in G2 (31.6%), reducing the productive life of these animals. Furthermore, OR of non-pregnancy at first AI was 13.93, which was similar to that reported by Ospina *et al.* (2010). These values have resulted in an increase in the number of AI per conception in G2 (3.47) versus G1 (2.5).

Table 1: Biochemical parameters of energy metabolism, liver function, calcium and urea from high-yielding dairy cows depending on Body Condition Score (BCS) and the loss of BCS (Δ BCS) during the transition period

Parameter	BCS at prepartum		Loss of body condition score		Stage		r.m.s.e.	P-Value		
	G1 3.5 – 3.75	G2 > 3.75	G3 ≤ 0.75	G4 > 0.75	Pre	Post		BCS	Δ BCS	Stage
Glucose (mmol/L)	3.01	3.20	3.15	3.15	3.31	2.98	0.43	n.s.	n.s.	n.s.
BHBA (mmol/L)	0.52	0.68	0.58	0.65	0.57	0.71	0.36	n.s.	n.s.	n.s.
NEFA (µmol/L)	451*	495*	353**	512**	383*	584*	200	0.020	0.002	0.015
ALT (U/L)	19.05**	15.31**	17.33	16.13	16.68	15.97	4.25	0.002	n.s.	n.s.
GGT (U/L)	22.39	22.64	23.67	22.36	20.67	24.48	0.14	n.s.	n.s.	n.s.
AST (U/L)	85.02*	68.87*	75.52	72.79	55.64**	90.82**	1.03	0.006	n.s.	0.001
GLDH (U/L)	14.25	11.72	15.69	11.77	5.89**	18.91**	1.21	n.s.	n.s.	0.002
UREA (mmol/L)	9.69	9.14	9.26	9.30	8.55	10.03	0.37	n.s.	n.s.	n.s.
Calcium (mmol/L)	2.24	2.39	2.26	2.29	2.26	2.31	0.15	n.s.	n.s.	n.s.

Significant differences are indicated by asterisk: n.s.: non-significant; *: P<0.05; **: P<0.01; Pre: prepartum period; Post: postpartum period; r.m.s.e.: root mean squared error; BHBA: β-hydroxybutyrate; NEFA: non-esterified fatty acids; ALT: alanine transaminase; GGT: gamma glutamyltranspeptidase; AST: aspartate aminotransferase; GLDH: glutamate dehydrogenase. All data are presented as mean.

Table 2: Reproductive parameters in cows with BCS 3.5 to 3.75 and >3.75 and odds ratio of a lower probability of pregnancy at first artificial insemination and pregnancy>150 DIM

Index/Reproductive parameters	BCS	N	Percentage/ Average±SE	Confidence limits			
				Odds ratio	Lower	Higher	P value
Elimination rate due to non-pregnancy	3.5 – 3.75	18	12.50%				
	> 3.75	22	31.60%				
Number of artificial inseminations	3.5 - 3.75	16	2.50±0.89				
	> 3.75	23	3.48±0.48				
% Pregnancy at 1st AI	3.5 – 3.75	16	68.8%	13.93	2.78	69.88	<0.01
	> 3.75	22	13.6%				
Non pregnancy rate > 150 DIM	3.5 – 3.75	16	12.5%	11.38	2.03	63.75	<0.01
	> 3.75	21	61.9%				

Statistical differences were significant when P<0.05. N: number of cases; BCS: body condition score.

Table 3: Pearson's correlation coefficients among different parameters studied

	BHBA	NEFA	ALT	GGT	AST	GLDH	UREA	Calcium
Glucose	n.s	-0.241*	n.s	n.s	-0.375**	n.s	n.s	n.s
BHBA		0.485**	n.s	n.s	n.s	n.s	n.s	0.331**
NEFA	0.485**		n.s	0.258*	0.313*	n.s	n.s	0.234*
ALT	n.s	n.s		0.151*	n.s	0.165*	0.240*	n.s
GGT	n.s	0.258*	0.151*		0.181*	n.s	n.s	n.s
AST	n.s	0.313*	n.s	0.181*		0.534**	0.286*	n.s
GLDH	n.s	n.s	0.165*	n.s	0.534**		n.s	0.345**
UREA	n.s	n.s	0.240*	n.s	0.286*	n.s		n.s
Calcium	0.331**	0.234*	n.s	n.s	n.s	0.345**	n.s	

**P<0.01 (unilateral correlation); * P<0.05 (unilateral correlation); n.s: not significant. BHBA: β-hydroxybutyrate; NEFA: non-esterified fatty acids; ALT: alanine transaminase; GGT: gamma glutamyltranspeptidase; AST: aspartate aminotransferase; GLDH: glutamate dehydrogenase.

Conclusions: Data showed that cows with BCS higher than 3.75 and loss of BCS higher than 0.75 points suffered a significant increase in lipomobilization versus cows with optimal BCS at calving. Moreover, cows with BCS >3.75 and NEFA values of 495 µmol/L at prepartum had a higher risk of non-pregnancy at first AI, and there was an increase in the number of open cows at 150 DIM, resulting in increased elimination rate due to non-pregnancy and shortened reproductive lives.

Authors contribution: JLB and CC designed the research. Blood sampling and evaluation of the BCS was performed by JMC and the reproductive data was recorded by RM. The data was analysed by JH, VP and JMC and they also prepared tables. The interpretation of results and manuscript was prepared by RM and JMC.

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