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*Original article*

# Dietary energy density in the dry period on the metabolic status of lactating cows

W. Nowak<sup>1</sup>, R. Mięka<sup>1</sup>, E. Pruszyńska-Oszmałek<sup>2</sup>, P. Maćkowiak<sup>2</sup>,  
B. Stefańska<sup>1</sup>, M. Kasproicz-Potocka<sup>1</sup>, A. Frankiewicz<sup>1</sup>, K. Drzazga<sup>1</sup>

<sup>1</sup> Department of Animal Nutrition and Feed Management, Poznań University of Life Sciences, Wołyńska 33, 60-637 Poznań, Poland

<sup>2</sup> Department of Animal Physiology and Biochemistry, Poznań University of Life Sciences, Wołyńska 35, 60-637 Poznań, Poland

## Abstract

The aim of the study was to investigate the effect of different energy concentrations in the isonitrogenous diet fed during the dry period on postpartum health, fertility and blood variables. Forty Holstein multiparous cows were dried 56 days before the expected day of calving and assigned to group (M) with moderate energy concentrations of 0.69 UFL/kg DM or to the low-energy group (L) with energy density of 0.61 UFL/kg DM. From the 7d before the expected day of calving until the 21d of lactation, all the cows were fed the same fresh transition diet (0.82 UFL/kg DM). From the 22d to the 90d of lactation, all the cows received the same highest energy-density lactation diet (0.90 UFL/kg DM). During the dry period the decline of BCS in groups M and L were 0.07 and 0.12 units respectively. The average decrease of BCS from calving to 56 d of lactation were the same in both experimental groups (0.21 BCS). The first-service conception rate tended to be higher in the M group. Insulin-like growth factor-1, glucose,  $\beta$ -hydroxybutyric acid, non-esterified fatty acid, thyroxine serum concentrations prepartum and 3 and 5d postpartum were not significantly affected by the treatment in the dry period.

**Key words:** dairy cows, energy concentration, far-off, close-up, blood indices

## Introduction

The dry period, with the exception of the transition period, is one of the most important for the health, fertility and productivity of lactating cows (Mulligan 2012) and 30-50% of dairy cows are affected by some form of metabolic or infectious disease around the time of calving (Hostens et al. 2012). Shortening or omitting the dry period as a nutrition

strategy has recently been widely discussed (Gumen et al. 2005, Rastani et al. 2005, Winkelman et al. 2008, de Feu et al. 2009). The five-week dry-off period and the three weeks close-up period with feeding concentrates are included in traditional management practice (Kokkonen et al. 2004). Some studies have demonstrated that a single-group, high-forage and low-energy TMR diet in the dry programme may be essential for improving a reproductive success, decreasing

Table 1. Ingredient and nutrient composition of experimental TMR diets.

Ingredients (% of DM)	Dry period diets <sup>1</sup>		Transition diet <sup>2</sup>	Lactation diet <sup>3</sup>
	M	L		
Wheat straw	34.3	51.5		
Soybean meal	4.3	7.7	9.0	9.2
Alfalfa silage	18.9	12.9	12.8	17.6
Maize silage	41.2	26.6	20.0	20.2
Grass silage			11.8	
Maize grain silage			9.7	7.1
Sugar beet pulp silage			5.6	6.7
Brewer's grain silage			7.1	6.2
Hay			6.7	6.4
Barley grain			5.8	8.5
Triticale grain			5.8	8.5
Rapeseed meal				4.0
Glycerol			1.3	
Fat				2.2
Minerals and vitamins	1.3	1.3	4.9	3.6
Nutrient concentration (in 1 kg DM)				
UFL	0.69	0.61	0.82	0.90
PDIN (g)	66	68	98	111
PDIE (g)	69	72	98	105
CP (g)	115	115	165	174
NDF (g)	523	562	353	285
ADF (g)	341	373	203	189
Ca (g)	7.1	7.0	8.7	9.4
P (g)	3.3	3.4	3.6	3.7

<sup>1</sup> from -56d to -8d<sup>2</sup> from -7d to 21d<sup>3</sup> from 22d to 100d

metabolic disorders, and for overall performance (Dann et al. 2006, Janovick and Drackley 2010). Recently, Silva-del-Rio et al. (2010) reported that a moderate-energy diet during the entire dry period, compared to the traditional treatment (the far-off and close-up periods), improved the metabolic status and performance of lactating cows. Feeding in the transition period (close-up) by increasing concentrates may stimulate volatile fatty acid (VFA) absorption by the growth of rumen papillae, which is critical for preventing a decrease in rumen pH in early lactation. However, Kokkonen et al. (2004) suggested that the major effect of rumen epithelial growth cannot be achieved by practical feeding. Douglas et al. (2006) suggested that the duration of energy restriction may be important in eliciting metabolic adaptation in perinatal cows. Drackley (1999) concluded that the duration of the two periods could be critical and continued research to push back the frontiers of cow biology in the transition period. Surprisingly, there is little information on specific nutrient requirements for the single strategy of feeding one diet during the entire dry period for Holstein cows.

Bannink (2010) suggested that modern cows have high and fast adaptive capacity of the rumen wall

tissues, and more experimental trials are required to identify factors affecting the adaptation of rumen function during the transition period. Available data do not clearly support a single strategy for NDF or NFC cow nutrition during the far-off period.

We hypothesised that limiting energy intake by high bulk feeding during the dry period and shortening the close-up period to one week could be beneficial for the metabolism of lactating cows. The aim of the study was to evaluate the effects of a different energy density in a diet fed *ad libitum* for 7 weeks in the dry period, while shortening the close-up period to one week, on postpartum health and blood variables.

## Materials and Methods

All the procedures were approved by Local Ethics Committee No. 10 in Poznan, Poland (64/2007).

Forty Holstein multiparous cows were paired by parity, the expected parturition time and body condition score (BCS), and were randomly assigned to 1 of 2 dietary treatments during the 7-week dry period from -56d to -8d of the expected day of calving. The

Table 2. Effect of dietary treatment in the dry period on BCS.

Group	Days from calving					BCS change			
	-56	-21	0	+14	+56	-56→-21	-21→0	0→14	0→56
M	3.85	3.85	3.78	3.72	3.57	0.00	-0.07	-0.06	-0.21
L	3.80	3.75	3.63	3.51	3.42	-0.05	-0.12	-0.12	-0.21
SEM	0.08	0.04	0.04	0.04	0.04	0.04	0.02	0.19	0.04
P-value	0.48	0.17	0.04	0.02	0.03	0.21	0.48	0.20	1.00

M – 0.69 UFL/kg DM from -56 to -8d

L – 0.61 UFL/kg DM from -56 to -8d

SEM – standard error of the mean

diet in group M was formulated to meet a value of 0.69 UFL in 1 kg DM (52.3% NDF), while in the L group, the energy density was 0.61 UFL/kg DM (56.2% NDF). The both isonitrogenous diets (11.5% CP) were balanced using INRatio ver. 3.3 software and feed nutrition values were estimated using PrevAlim 3.23. In the French energy system, UFL is used as a unit of net energy, which is equivalent to 1 kg standard air-dried barley (Coulon 1989). The diets were based on wheat straw, maize and alfalfa silages and soybean meal, and fed as a total mixed ration (TMR) at 9 a.m. and 2.30 p.m. for the entire experimental period.

From the 7d before the expected date of parturition until the 21d of lactation, all the cows were fed the same fresh, transition diet (0.82 UFL/kg DM, 35.3% NDF, 16.5% CP) to provide adequate energy for a cow with a body weight of 650 kg and daily milk production of 30 kg. From the 22d to the 90d of lactation, all the cows received the same highest energy density lactation diet (0.90 UFL, 28.5% NDF, 17.4% CP). The component and nutrient compositions of the diets are shown in Table 1. In the dry period, the cows were housed in a free-stall barn, and one week before the expected date of calving, they were moved until the 5d after parturition to close-up individual pens. On the 5d of lactation, the cows were transferred to a free-stall barn.

The body condition score (BCS) was assessed according to Edmonson et al. (1989) on -56d and -30d, on the parturition day and on the 14d and 56d of lactation.

Health records were maintained for all the cows. Reproductive performance, such as days to first ovulation, first-service conception rate, services per conception and days open, were also recorded. The first ovulation was identified using an ultrasound scanner equipped with a 7.5 MHz convex transducer. During the study, cow health was monitored and recorded daily by a technician. Weekly forage, concentrate and TMR samples were collected for monthly analyses by wet chemistry for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), calcium (Ca) and phosphorus (P) (AOAC, 2007).

Blood was sampled from the jugular vein 3 hours after morning feeding on -30d and on the 3d, 5d and 28d in lactation. Blood samples were collected into tubes with polystyrene separating granules covered with a clot activator and aliquots were then rotated in a centrifuge, the serum was then frozen and stored (-20°C) for later analyses. The concentrations of glucose, and  $\beta$ -hydroxybutyric acid (BHBA) were analysed with a Pointe Scientific reagent. The concentrations of non-esterified fatty acids (NEFA) were analysed according to Duncombe's colorimetric method (1964). Serum hormone concentrations were analysed by means of radioimmunoassay (RIA): insulin (Millipore Corporation, USA), insulin-like growth factor-1 (Diagnostic Systems Laboratories Inc., USA), thyroxine (T3) and triiodothyronine (T4) (CIS bio international, France).

The results were analysed statistically using Student's t-test with SAS computer software SAS 9.1 (2004) SAS®/STAT and the PROC T-TEST procedure. Significance was declared at  $P \leq 0.05$ , and trends were considered when  $0.05 < P \leq 0.1$ .

## Results

The average duration of the close-up (transition) period was 7.7d ( $\pm 3.2$ ) in low energy L and 6.5d ( $\pm 2.7$ ) in M moderate energy group. The cows fed the L (0.12) diet lost more BCS in the dry period (Table 2) as compared to the M group (0.07). The plateau in BCS during the first week of the transition period before calving was recorded in both groups. Regardless of the diets used in the dry period, the average decrease in BCS on 14d and 56d of lactation was relatively low (0.21) and did not differ significantly between the treatments.

No statistically significant effects ( $P > 0.05$ ) of the treatments during the dry period on the fertility parameters were recorded (Table 3). The average day of first ovulation in the current study was similar in the M (29.7d) and in the L group (30.2d). However, a tendency was found ( $P = 0.08$ ) for cows in the M group

Table 3. Effect of dietary treatment in the dry period on reproductive performance.

Group	Days to first ovulation	First-service conception rate	Services per conception	Days open
M	29.7	0.5	1.6	95.6
L	30.2	0.2	2.2	110.3
SEM	2.09	0.09	0.20	6.47
P-value	0.90	0.08	0.26	0.36

M – 0.69 UFL/kg DM from -56 to -8d

L – 0.61 UFL/kg DM from -56 to -8d

SEM – standard error of the mean

Table 4. Effect of dietary treatment in the dry period on incidence of metabolic disorders.

Item	Dystocia		Displaced abomasum		Hypocalcemia		Mastitis		Metritis		Foot and leg problems	
	M	L	M	L	M	L	M	L	M	L	M	L
Number of cows	2	1	0	0	1	4	2	2	0	0	1	1

Table 5. Effect of dietary treatment in the dry period on serum biochemical indices.

	Group	Prepartum		Postpartum	
		-30d	3d	5d	28d
Glucose (mmol/L)	M	3.15	2.95	2.36	1.97
	L	3.27	2.28	1.87	1.96
	SEM	0.04	0.21	0.19	0.22
	P-value	0.14	0.11	0.20	0.99
Insulin ( $\mu$ U/mL)	M	8.58	4.03	10.11	6.20
	L	6.18	4.66	3.83	5.78
	SEM	0.80	0.55	0.81	0.41
	P-value	0.09	0.58	<0.01	0.62
IGF-1 (ng/mL)	M	374,0	66.3	50.4	134
	L	395,0	84.5	80.1	93.9
	SEM	13.7	8.4	14.9	10.8
	P-value	0.44	0.29	0.33	0.06
BHBA (mmol/L)	M	0.486	0.616	0.718	0.240
	L	0.504	0.704	0.691	0.229
	SEM	0.010	0.051	0.032	0.010
	P-value	0.39	0.35	0.69	0.71
NEFA (mmol/L)	M	0.271	0.853	0.946	0.505
	L	0.272	0.874	0.759	0.327
	SEM	0.011	0.040	0.052	0.030
	P-value	0.97	0.89	0.07	<0.01
T3 (ng/mL)	M	0.85	0.62	0.47	0.75
	L	0.73	0.58	0.49	0.72
	SEM	0.03	0.04	0.02	0.03
	P-value	0.02	0.62	0.70	0.59
T4 (ng/mL)	M	52.90	29.11	32.96	38.69
	L	50.32	26.26	33.40	41.68
	SEM	1.80	1.31	1.53	1.62
	P-value	0.49	0.28	0.88	0.40

M – 0.69 UFL/kg DM from -56 to -8d

L – 0.61 UFL/kg DM from -56 to -8d

SEM – standard error of the mean

to have a higher first-service conception rate (50%) compared to those in the L group (20%). Additionally, there appears to be evidence for a beneficial effect of the diet with a moderate energy content in the dry period concerning the services per conception and days open; however, the high degree of variability and fertility parameters may have prevented the detection of significant differences. The total incidence of health problems after parturition amounted to 7 and 10, respectively, for the M and L group; however, in the M group, more cows suffered from hypocalcemia (Table 4). Concentrations of glucose, insulin, IGF-1, BHBA, T3 and T4 on the 3d, 5d and 28d postpartum were all within the reference range and were not affected by the diets during the far-off period (Table 5). The level of NEFA on the 28d of lactation and concentrations of insulin on the 5d of lactation were significantly higher ( $P < 0.01$ ) in the M group. The glucose concentration tended to be higher ( $P = 0.11$ ) on the 3d of lactation for cows previously fed with the M diet.

## Discussion

In the current study of treatments in the dry period, the mean BCS was not significantly affected; however, as we expected, a decrease in BCS was 0.05 unit lower in the M group than in the L group. Douglas et al. (2006) showed a decrease of 0.5 BCS unit from the dry-off period until approximately 3 weeks before calving during restricted feeding (7.4 kg DM) and an increase of 0.14 BCS unit fed *ad libitum* (14.1 kg DM). In a similar study, Dann et al. (2005) found a lowering of BCS from 3.05 to 2.85 units as an effect of restriction of the daily dry matter intake to 8.16 kg. During the first 56 days of lactation, a decrease in BCS, one of the indicators of the energy status of cow, was similarly low (0.21 BCS) in both experimental groups, suggesting that lipid postpartum mobilisation was not affected by treatment in the dry period. Most of the studies which reported loss of BCS postpartum are negatively associated with days to conception and pregnancy to first service, however, the reasons for reduced pregnancy are not known (Wathes et al. 2007, Roche et al. 2009). Roche et al. (2007) reported a linear decrease in probability of pregnancy at 6 weeks with declining nadir BCS. There was also a negative association between the change of BCS in early lactation, delayed ovarian activity and number of day to first estrus.

There was no statistically significant effect of treatment in the dry period on reproductive performance. The first-service conception rate in spite of similar postpartum BCS change, tended to be higher in the M group compared to the L group. In the current study, lower fertility observed in L cows was not re-

lated to plasma NEFA, insulin, IGF-1 or BHBA concentrations. Lussier et al. (1987) reported that approximately 90 days are required for a follicle to grow from the primary to the pre-ovulatory stage; we believe that low-energy diet in the L group in the far-off period could negatively affect fertility by reducing the competence of the ovulated follicle. However due to the limited number of animals per treatment, it is difficult to come to a definitive conclusion about the benefits of the M diet in the dry period to fertility variables.

Gümen et al. (2005) reported improved reproductive measures (time of the first ovulation, first-service conception rate, days open) in groups fed continuously with a high-energy diet and no planned dry period, compared to the traditional two-phase dry period. Colazo et al. (2009) in spite of increasing the postpartum dry matter intake and a reduction of negative energy balance, demonstrated that restricted nutrition in the last 4 weeks of gestation negatively affected pregnancy at first insemination.

The overall incidence of periparturient health problems was low; however, relatively small number of cows were examined per treatment, the periparturient health data should be interpreted with caution. Health was not significantly affected by the treatment in the far-off period as in the results of other authors (Dann et al. 2005, 2006). Cows overfed during the far-off dry period had a greater negative balance during the first 10 days after calving and might have been at risk of more health problems. Douglas et al. (2006) reported a greater incidence of displaced abomasum in the group fed *ad libitum* as compared to the restricted feeding group, which may reflect the lower prepartum dry matter intake. Additional research on more cows could help interpret the effect of prepartum nutritional management on the health status of lactating cows.

In the current study, pre and postpartum concentration of most of the blood variables were within the normal range (Whitaker 1997, Kawashima et al. 2007) and were not significantly affected by treatment in the dry period. Glucose, insulin and IGF-1 concentrations were highest in the far-off period and decreased during lactation in both groups. Similarly, other authors reported a decrease in glucose and insulin plasma concentrations as calving approached, as a part of adaptation to the mechanism allowing cows to mobilise fat and protein (Grum et al. 1996, Kokkonen et al. 2004, González et al. 2010). Winkelman et al. (2008) demonstrated significant effects of both *ad libitum* and restricted feeding on plasma insulin concentrations, which decreased as parturition approached, reaching the lowest level on the 4d of lactation. However, insulin concentration was greater in *ad libitum*-fed cows on -2d to the expected day of calving.

We found a significant increase in insulin concentration on the 5d of lactation in the M group, without affecting the plasma glucose levels. Holtenius et al. (2003) showed that overfed cows in the dry period may become insulin resistant. Loores et al. (2006) reported that cows fed *ad libitum* had greater concentrations of insulin as compared to cows with restricted feeding during the entire dry period. Douglas et al. (2006) demonstrated a non-significant increase in plasma glucose and insulin concentrations in the prepartum period in the group fed *ad libitum* compared to the group with restricted feeding. Dann et al. (2005) found a significantly higher concentration of plasma insulin in cows fed *ad libitum* compared to cows fed restrictively in the far-off period. Contrary to these findings, van Knegsel et al. (2007) reported a positive relationship between energy retention in body mass and insulin concentration. Rukkamsuk et al. (1998) demonstrated similar glucose concentrations in over-fed cows and cows fed restrictively according to the requirements in the dry period. Grum et al. (1996) found a slower increase in postpartum insulin serum concentrations in cows that were fed high-energy diets in the prepartum period. In spite of higher energy requirements, most plasma metabolites after parturition were not significantly affected, suggesting that long-term energy restriction in the dry period by a moderate bulky diet may invoke favourable metabolic responses in high-yield lactating cows. Bossaert et al. (2008) suggested, that cows respond similarly to glucose load in the dry period and during lactation. Similarly to other research (Grum et al. 1996), we found that the concentration of IGF-1 was not significantly affected by the dry period treatment. As previously reported in a study by Grum et al. (1996) we observed a decrease in IGF-1 concentration after parturition. Beam and Butler (1999) showed that cows with higher concentrations of IGF-1 after parturition need a shorter time to resume oestrous cyclicity. Low plasma IGF-1 adversely affected reproduction by causing a dominant follicle to fail to reach the ovulatory size (Chagas et al. 2006). Konigsson et al. (2008) suggested that IGF-1 is an extremely sensitive signal between metabolism and reproduction, and cows with earlier resumption of postpartum ovarian activity had higher IGF-1 levels in the first two weeks after calving.

We found that serum NEFA and BHBA concentrations did not differ significantly between the treatments in the prepartum period and on the 3d and 5d of the postpartum period. Moreover, the average BHBA concentrations on the 3d and 5d of lactation were lower than 1.0 mmol/L, the cut-off value used for the diagnosis of subclinical ketosis (Duffield et al. 2002). In the present study, in spite of a relatively low BCS decrease during lactation, the NEFA postpartum

serum concentration exceeded in both groups by 0.7 mmol/L, the level recommended by Ospina et al. (2010) but was lower than 1.0 mmol/L as recommended by Seifi et al. (2010). Seifi et al. (2010) found that the probability of culling was 3.6 times greater in cows with serum >1.0 mmol/L in the first week after calving and cows with BHBA > 1.2 mmol/L had a 4.7 times greater risk of developing clinical ketosis. Chapinal et al. (2012) reported that elevated serum concentrations of NEFA (>0.6 mmol/L) in weeks 1 and 2 after calving were associated with longer pregnancy periods and decreased milk production. Silva-del-Rio et al. (2010) found that cows fed moderate energy throughout the entire dry period, compared to those fed a low-energy diet during the far-off period and moderate energy during the close-up period, tended to have greater BHBA plasma concentrations. A marked increase in the plasma NEFA concentrations at parturition was reported earlier in some previous studies (Grum et al. 1996, Douglas et al. 2006). In a study conducted by Douglas et al. (2006) the mean NEFA and BHBA plasma concentrations were lower for cows fed restrictively in the dry period. Murondoti et al. (2004) reported that unrestricted, compared to restricted feed intake in the dry period did not significantly affect NEFA concentrations before parturition; however, BHBA concentrations sharply increased after parturition. Loores et al. (2006) demonstrated that over-feeding in the dry period increased the serum NEFA concentrations during the dry period and decreased it during the first week of lactation. Murondoti et al. (2004) suggested that unrestricted feed intake in the dry period reduces the *de novo* synthesis and oxidation of fatty acids in the liver, which may contribute to the accumulation of triacylglycerol in the liver after parturition. According to van Knegsel et al. (2007) NEFA concentrations do not determine the incomplete oxidation and production of BHBA in periparturient dairy cows. Bossaert et al. (2008) suggested that low insulin stimulates lipolysis and increases NEFA plasma concentration. In the same study, low NEFA concentrations during the dry period positively affected the time of the first ovulation. A high level of NEFA in the last 7 days before parturition has been related to ketosis, displaced abomasum and retained placenta, and could adversely affect the dry matter intake (Cameron 1998, Hayirli et al. 2002). The high NEFA level also reflects a demand for glucose, accompanied by a decrease in glucose, insulin and IGF-1 concentrations (Drackley 1999). Dann et al. (2006) reported that cows with lower energy balance during the far-off period tended to have lower NEFA and BHBA concentrations in the first 10 days of lactation; these two variables were not affected by the close-up treatment. An increase in NEFA prepartum

plasma concentration was observed among cows fed to meet 80% of the requirements, compared to those fed to meet 120% of the requirements, during 40 days of the prepartum period (Dann et al. 2006).

We found that serum T3 and T4 hormone concentrations during lactation were not significantly different between the diets in the dry period. Samanc et al. (2010) reported a downward trend in the concentrations of T3 and T4 from the 30d before calving to the 12d after parturition. A decrease in the plasma concentrations of both thyroid hormones during early lactation among cows with severe fatty liver were reported in other studies (Nikolib et al. 1997, Stojib et al. 2001). Relative to the prepartum period, this can also be seen in healthy cows without fatty liver. Grum et al. (1996) stated that possible effects of the ratio of T3/T4 are not clear; however, Šamanc et al. (2010) suggested that a high T3/T4 ratio during the mid-dry period may be an early indicator of post-partum fatty liver.

In the present study the effect of different energy concentrations in the dry period on metabolic status during lactation was almost non-significant statistically. Both experimental bulky diets (0.69 UFL/kg DM and 0.61 UFL/kg DM) prevented energy overconsumption during the dry period and maintained constant energy balance, resulting in relatively low decreasing BCS in lactation and successful health and fertility. Additionally, there was no negative effect of the abrupt change from the high- to the lower-fibre diet one week before parturition, which confirmed a recent suggestion concerning the high capacity of adaptation of modern cows' to a lower fibre diet in the transition period. We suggest that following high NDF diets for 7 weeks of the dry period, consisting of introducing a fresh lactation diet during the short, one-week close-up period and three weeks after the calving period, could be an alternative feeding strategy in the dry period.

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