Short communication: Effect of dietary manipulation of crude protein content and nonfibrous-to-fibrous-carbohydrate ratio on energy balance in early-lactation dairy cows¹

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ABSTRACT

Disparities between nutrient intake and demand often result in a state of negative energy balance (EB) in the early-lactation dairy cow. Reducing dietary crude protein (CP) content and providing glucogenic nutrients may overcome this issue. This study evaluates whether or not offering a diet lower in CP and higher in nonfiber carbohydrates (LP-NFC) can improve EB and the metabolic status of the early-lactation dairy cow compared with a diet higher in CP and fibrous carbohydrates (HP-FC). Twenty Holstein-Friesian dairy cows were assigned to 1 of 2 dietary treatments in a randomized block design. Diets were isoenergetic (6.57 MJ of net energy for lactation) and formulated to contain 15% CP and 6% starch (HP-FC), or 12% CP and 28% starch (LP-NFC) and were offered for the first 63 d of lactation. Intake and milk yield were determined daily, whereas milk and blood samples, weights, and body condition scores were collected weekly. Intakes (mean $\pm$ standard errors of the mean, SEM) of dry matter (17.4 ± 0.6 kg/d) and energy (113.0 ± 4.6 MJ of net energy for lactation) were not different between treatments. However, the HP-FC group had a higher milk yield (31.8 vs. 28.9 ± 1.4 kg/d) and a lower EB compared with the LP-NFC group. Blood urea N concentration (3.5 vs. 1.8 ± 0.2 mmol/L) was higher, whereas bilirubin (6.0 vs. 6.7 ± 0.2 mmol/L) and β -hydroxybutyrate concentrations (0.7 vs. 0.8 ± 0.05 mmol/L) were lower in the HP-FC group compared with the LP-NFC group. These data suggest that EB can be improved during early lactation through the manipulation of milk output by offering a lower CP, higher NFC diet.

Key words: early lactation, energy balance, dairy cow, carbohydrate

Short Communication

Increases in the genetic potential for milk production have created technical challenges for nutritionists and metabolic challenges for the early-lactation dairy cow. In particular, disparities between energy intake and that required for production can result in a state of negative energy balance (EB) and a metabolic status that predisposes the cow to reduced reproductive performance and clinical and subclinical production diseases cumulating in reduced profitability at the farm level (McArt et al., 2013). Restricting milk production through reducing dietary CP supply may be an effective method of improving EB in early-lactation dairy cows (McCormick et al., 2001; Law et al., 2009). However, these effects are inconsistent in the literature, with some reports showing improved EB (Ørskov et al., 1987) and others showing no effect (Chapa et al., 2001; Law et al., 2009). Improvements in EB and metabolic status of the dairy cow are also possible through altering the ratio of fibrous carbohydrates (FC) to NFC, creating diets that are either lipogenic or glucogenic. Previously, van Knegsel et al. (2007) reported a lower milk fat yield (0.24 kg/d) and improved daily EB by 56 kJ/kg^{0.75} for the glucogenic-type diets.

In the literature, the effect of manipulating dietary CP concentration on EB has been well documented (Chapa et al., 2001; Law et al., 2009). Similarly, the balance of FC and NFC has been studied for effects on EB (van Knegsel et al., 2007). However, with the exception of Broderick (2003), who evaluated the effect of dietary CP and energy content in mid-lactation cows (126 DIM), limited information exists on the simultaneous application of energy type and CP content to improve EB in the early-lactation cow. This experiment aimed to evaluate the effects of offering diets lower in CP and higher in NFC on milk production, EB, and metabolic profile in the early-lactation dairy cow.

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Communities (Amendment of the Cruelty to Animals Act 1876) Regulations 2002 and 2005 (Department of Health and Children, 2005). Twenty multiparous dairy cows (*Bos taurus* strain Holstein-Friesian) were selected from the dairy herd at University College Dublin Lyons Research Farm (Newcastle, Dublin, Ireland; 53°17'56" N, 6°32'18" W). The cows were blocked by parity and calving date and randomly assigned to one of the following isoenergetic diets: high-CP, low-NFC (**HP-FC**) diet or a low-CP, high-NFC (**LP-NFC**) diet (Table 1). Blocks were balanced for previous 305-d lactation milk yield ($6,734 \pm 700$ kg), milk protein yield (230 ± 21 kg), and BCS at calving (3.0 ± 0.11 ; means $\pm$ SE). Dietary treatments were offered once daily from d 1 to 63 postpartum as a TMR. Individual feed intake was facilitated using preprogrammed feed boxes (RIC System; Insentec B.V., Marknesse, the Netherlands). Feed samples were taken daily, pooled on a weekly basis, and analyzed for chemical composition as previously described in Whelan et al. (2011). These data were then entered into the Nutrient Requirements for Dairy Cattle software package (version 1.1.9; NRC, 2012) to calculate NE and MP.

Cows were milked twice daily for the duration of the experiment at 0700 and 1600 h to determine milk yield (**MY**) with weekly sampling for milk constituents (Dairymaster, Kerry, Ireland). Milk samples were analyzed in a commercial milk laboratory (Progressive Genetics, Bluebell, Dublin, Ireland) using infrared analysis (CombiFoss 6000; Foss Analytical A/S, Hillerød, Den-

mark). These data were used to calculate milk energy output according to Tyrrell and Reid (1965). Blood samples were harvested by jugular venipuncture on d 0, 7, 14, 21, 28, 35, and 63 postpartum for determination of glucose, urea, NEFA, BHBA, bilirubin, γ -glutamyl transferase, and glutamate dehydrogenase concentrations. Blood samples were prepared and analyzed as described in Whelan et al. (2012) using Randox reagents and equipment (Randox Laboratories Ltd., Antrim, UK). Rumen fluid was harvested by stomach tubing (Flora Rumen Scoop; Prof-Products, Guelph, ON, Canada) approximately 6 h postfeeding on d 58 to 62 postpartum to determine rumen VFA and $\text{NH}_3\text{-N}$ concentrations. Sample preparation (Whelan et al., 2013) and analyses for VFA (Hart et al., 2009) and $\text{NH}_3\text{-N}$ (Weatherburn, 1967) were as previously described.

Data were checked for adherence to the normal distribution and homogeneity of variance using histograms and formal statistical tests as part of PROC UNIVARIATE of SAS (SAS Institute, 2004). Analysis of data was conducted using PROC MIXED of SAS (SAS Institute, 2004). The model included tests for the fixed effects of treatment, week, parity, and their interactions. Cow within treatment was modeled as a random effect. Statistically significant differences between least squares means were tested using the PDIF command, incorporating the Tukey test for pairwise comparison of treatment means. Where interactions were not significant, these terms were excluded from the model. Statistical significance was assumed at $P < 0.05$ and a

Table 1. Ingredient inclusion rate and chemical composition of TMR used during the experiment

Item	HP-FC TMR ¹	LP-NFC TMR ¹
Inclusion (%)		
Grass silage	46	14
Corn silage	3	41
Barley straw	1	2
NFC-Pellet ²	0	43
FC-Pellet ³	49	0
Composition (% of DM, unless otherwise stated)		
DM (%)	29.3	37.3
NE _L (MJ/kg of DM)	6.57	6.57
CP	14.6	11.9
MP	12.7	8.1
NDF	54.5	39.5
ADF	35.1	22.3
ADL	4.1	2.9
Ash	7.5	5.7
Ether extract	3.0	3.0
NFC	20.4	39.9
Starch	5.7	27.5

¹HP-FC = high-CP, low-starch TMR; LP-NFC = low-CP, high-starch TMR.

²NFC-Pellet = low-CP, high-NFC pellet (48% ground corn, 29% citrus pulp, 19% soybean meal, 1% molasses, and 3% mineral-vitamin premix, on a DM basis).

³FC-Pellet = high-CP, low-NFC pellet [8% ground corn, 14% beet pulp, 16% soybean meal, 55% soy hulls, 2% Megalac (Church & Dwight Co. Inc., Ewing, NJ), 1% molasses, and 4% mineral-vitamin premix, on a DM basis].

Table 2. Effect of dietary treatment on nutrient intake, milk production, and milk composition

Item	HP-FC TMR ¹	LP-NFC TMR ¹	SEM	P-value
Intake (kg/d)				
DM	17.0	17.6	0.56	0.35
Output (kg/d, unless otherwise stated)				
Milk	31.3	29.6	0.60	<0.01
Fat	1.29	1.19	0.05	0.08
Protein	0.92	0.85	0.02	<0.01
Casein	0.70	0.64	0.02	<0.01
Milk composition (% , unless otherwise stated)				
Fat	4.01	3.88	0.15	0.39
Protein	2.92	2.93	0.06	0.89
Casein	2.21	2.21	0.03	0.98
Urea N (mmol/L)	3.57	1.40	0.36	<0.01

¹HP-FC TMR = high-CP, low NFC TMR; LP-NFC TMR = low-CP, high NFC TMR.

tendency toward significance assumed at $0.05 < P < 0.10$.

For all early-postpartum nutritional strategies, optimizing DMI is of paramount importance. In this study, no effect of diet on DMI ($P = 0.35$; Table 2) was detected; thus, intake of starch was higher for animals offered LP-NFC ($P < 0.01$), whereas CP ($P < 0.01$) and NDF ($P < 0.01$) intake was higher for those offered HP-FC. However, animals did not consume their 20 kg of DM allocation at any stage during the 9-wk period and, therefore, the quantity of nutrients ingested was not sufficient to meet production requirements. For example, dietary MP supply was lower than that required for maintenance and production, suggesting that a portion of the AA required for milk production was derived from the mobilization of body tissue. Bauman and Elliot (1983) suggested that the early-lactation dairy cow could mobilize between 5 and 13 kg of body protein without adverse effects on cow health. Our deficit was within the range of 0.2 to 0.3 kg/d that O'Mara (1996) suggested to be tolerable during the first month of lactation using the methodology of Jarrige (1989). That said, N intakes in our experiment were within the range of those reported in Law et al. (2009) for diets of a similar CP content (11.4 vs. 14.4%). However, in the study of Law et al. (2009), animals offered the lower CP diet also had a lower DMI (-1.5 kg/d) and this may have partly attributed to the large difference in MY ($+6.4$ kg/d) between the high- and low-CP groups. In the current study, reducing dietary CP to limit milk production resulted in a 1.7 kg/d reduction in MY, consistent with the predicted response of MY to reduced CP intake by the NRC (2001). Similarly, milk protein yield was reduced where LP-NFC diets were offered, consistent with the reduction in MY and observations of Law et al. (2009). Milk fat, the most energetically demanding component in milk (Tyrrell and Reid, 1965), was also lower in yield where animals were offered HP-FC compared with those offered LP-

NFC ($P = 0.08$). Thus, the difference observed in EB (-21.8 vs. -9.6 MJ NE_L/d for HP-FC vs. LP-NFC, respectively; $P = 0.01$) during this study can be attributed to differences in milk fat yield as well as the other energy-demanding components of milk production: milk protein and lactose.

With the exception of urea, milk composition was unaffected by dietary treatment, suggesting that differences in milk constituent yield observed in this study were as a direct result of the increased MY (Table 2). Urea N equilibrates in body fluid and concentrations in the milk reflect those in the blood (Gustafsson and Palmquist, 1993). In the current study, higher concentrations of urea N in blood and milk were likely a result of the increase in rumen NH₃-N (7.10 vs. 4.24 ± 0.96 mmol/L for HP-FC vs. LP-NFC, respectively; $P < 0.01$) from animals offered HP-FC (Table 3). Lowering blood and milk urea N concentrations is related to reduced urine N excretion (Kauffman and St-Pierre, 2001) and improved reproductive performance in the dairy cow (Butler et al., 1996).

Overall, the differences in EB observed in this experiment were small, with total days in negative EB of 40 and 37 d for HP-FC and LP-NFC, respectively ($P = 0.84$; Figure 1). Similarly, the extent of BCS loss was less than 0.5 units and was, therefore, unlikely to cause a significant metabolic challenge or reduction in reproductive performance in the dairy cow (Buckley et al., 2003; Mulligan et al., 2006). Although EB was more positive for animals offered LP-NFC, serum BHBA concentration was also higher for that treatment (Table 3). This may be a result of increased rumen fermentation of starch to butyrate (Reynolds, 2006), reflected by higher BHBA (11.19 vs. 7.79 ± 0.55 mmol/L; $P < 0.01$) combined with reduced mammary gland uptake of BHBA for de novo synthesis of milk fat (Lemosquet et al., 2010) for animals offered LP-NFC. That said, concentrations of BHBA and NEFA observed in our study were below those reported in Mills et al. (1986) for cows

Table 3. Effect of dietary treatment on energy balance, BCS, rumen fermentation, and selected blood metabolites

Item ¹	HP-FC TMR ²	LP-NFC TMR ²	SEM	P-value
NE intake (MJ/d)	109.2	116.7	4.60	0.12
Energy output ³ (MJ/d)	138.5	128.9	5.56	0.03
Energy balance (MJ/d)	-21.8	-9.6	4.02	0.01
BCS	2.77	2.80	0.11	0.47
Change in BCS	-0.20	-0.06	0.11	0.36
Weight	605	587	30	0.54
Blood metabolite (mmol/L, unless otherwise stated)				
Urea N	3.52	1.76	0.21	<0.01
GGT (IU/L)	15.01	18.90	2.29	0.11
GLDH (IU/L)	14.16	16.01	2.71	0.37
Glucose	3.19	3.11	0.08	0.36
Bilirubin	6.02	6.69	0.24	0.01
NEFA	0.40	0.36	0.07	0.54
BHBA	0.68	0.79	0.05	0.03

¹NE = net energy; BCS = average BCS for the duration of the trial on a scale of 1 to 5 in 0.25 increments (Edmonson et al., 1989); GGT = γ -glutamyl transferase; GLDH = glutamate dehydrogenase.

²HP-FC TMR = high-CP, low-NFC TMR; LP-NFC TMR = low-CP, high-NFC TMR.

³Energy output was calculated as $NE_M + NE_L$, where $NE_M = 0.33 \text{ MJ} \times \text{weight}^{0.75}$ (NRC, 2001).

confirmed with ketosis (4.1 and 0.81 mmol/L for BHBA and NEFA, respectively), reflecting the low levels of body tissue mobilization observed during the current study. Similarly, bilirubin, although higher for animals offered the LP-NFC treatment ($P = 0.01$), was within the normal range (0.7 to 14 mmol/L) reported for dairy cows (Boyd, 1984). Additionally, the concentrations of the liver enzymes glutamate dehydrogenase (>25 IU/L) and γ -glutamyl transferase (>40 IU/L) were below the thresholds indicative of fatty liver (West, 1997). Interestingly, glucose levels were not elevated where animals were offered the LP-NFC diet, despite starch supply being almost 4 kg/d higher and milk lactose yield be-

ing 0.09 kg/d lower. However, glucose levels are tightly regulated in the body and the provision of extra starch in our experiment may have encouraged glucose uptake by peripheral tissue via increased insulin (van Knegsel et al., 2007; Garnsworthy et al., 2008). The mammary gland also displays flexibility in its choice of nutrients for the synthesis of lactose, reducing its requirement for glucose. Bequette et al. (2006) demonstrated that 46 to 86% of galactose was synthesized from non-glucose sources, such as NEAA, BHBA, and acetate. In the current experiment, glucose levels may have been maintained through a combination of increased uptake by peripheral tissues in animals offered the LP-NFC TMR

Figure 1. Effect of dietary treatment on energy balance (MJ/d) over the first 9 wk of lactation. The error bars represent the SEM for each week's LSM. Color version available in the online PDF.

and reduced mammary uptake in animals offered the HP-FC TMR.

The results of this experiment indicate that altering the ratio of dietary fibrous carbohydrates to NFC and CP content improves EB during early lactation associated with a reduction in MY. Further research is required in this area to determine the effects of such dietary strategies on fertility parameters and lifetime production capabilities of the dairy cow. This will ultimately aid the producer in determining the feasibility of applying a postpartum nutritional strategy such as that reported herein.

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