Effect of supplemental concentrate type on milk production and metabolic status in early-lactation dairy cows grazing perennial ryegrass-based pasture¹

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ABSTRACT

Forty-four early-lactation dairy cows of mixed parity were used to examine the effect of 4 supplemental concentrate types ($n = 11$) on milk production and metabolic status. Animals were blocked by parity and calving date, and blocks were balanced for previous milk yield and milk protein yield. Cows received grazed pasture plus 5.17 kg of DM/d of 1 of the following isoenergetic (1.1 units of energy for lactation) concentrates: 1) high crude protein (CP) with rolled barley (HP, 19% CP); 2) low CP with rolled barley (LP, 15% CP); 3) low CP with barley and a supplemental methionine hydroxy analog (HMBi; LP + HMBi, 15% CP); and 4) low CP with ground corn (LP-corn, 15% CP). Milk yield was recorded from d 1 to 100 postpartum, with weekly milk sampling, body weight, and body condition score (BCS) measurements. Blood and rumen sampling were conducted weekly from wk 2 to 6 postpartum. Milk yield was lower for cows in the LP treatment compared with those offered other concentrate types (25.2 vs. 27.5 ± 0.39 kg/d). Animals in the HP group had a higher milk yield than those in the LP + HMBi group (28.2 vs. 26.8 ± 0.39 kg/d). Milk fat yield was lower from animals in the LP-corn group compared with those in the LP + HMBi group (0.94 vs. 1.03 ± 0.03 kg/d). Milk protein yield was lower in the LP group compared with those in the HP group (0.88 vs. 0.97 ± 0.02 kg/d). Animal body weight, BCS, and BCS loss were not affected by concentrate type. However, nonesterified fatty acids were higher from animals in the HP group than for those in the LP + HMBi group (0.41 vs. 0.33 ± 0.03 mmol/L), and β -hydroxy butyric acid was higher from animals in the HP group than for those in the other treatments (0.71 vs. $0.59 \pm$

0.03 mmol/L). Glucose was higher from animals in the LP-corn group than for those in the HP and LP groups (3.3 vs. 3.2 ± 0.05 mmol/L). Blood urea-N was higher from animals offered HP compared with those offered the other treatments ($5.49.6$ vs. 4.21 ± 0.44 mmol/L). However, rumen $\text{NH}_3\text{-N}$ and volatile fatty acid concentration in the rumen were not affected by supplemental concentrate type. Reducing supplemental concentrate CP reduced milk yield. However, milk fat production and energy-corrected milk were not different, reducing the likelihood of an improved energy balance or a more favorable blood metabolic profile in early-lactation dairy cows grazing perennial ryegrass. Offering HMBi with low-CP concentrates or replacing rolled barley with ground maize improves milk production relative to low-CP concentrates and metabolic status relative to high-CP concentrates.

Key words: dairy cow, grazing, metabolite, concentrate

INTRODUCTION

Increased milk production and a reduced nutrient intake make early lactation a challenging period for the dairy cow. Imbalances between nutrient intake and those required for production often result in a state of severe negative energy balance (NEB) and a metabolic status that predisposes the cow to reduced reproductive performance and clinical and subclinical production diseases (Patton et al., 2006; Mulligan and Doherty, 2008). In Ireland, as well as other northern European countries, grazed pasture forms the main component in the diet of the dairy cow (Rath and Peel, 2005). However, pasture alone cannot meet the requirements of the high-yielding, early-lactation dairy cow (Schor and Gagliostro, 2001). Therefore, concentrate amount and type of supplement may be an important component in the nutrition, reproduction, and health of the high-producing, grazing dairy cow in early lactation.

Improvements in milk production require the use of high-CP feeds to ensure that sufficient protein is available postpartum to support maximal milk production

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(Law et al., 2009). However, grazed pasture can contain high levels of CP (Van Vuuren et al., 1990), and further supplementing the animal with high-CP concentrates will increase N loss from the rumen and increase blood urea-N concentration, which is energetically costly (McDonald et al., 2002) and can reduce fertility in the dairy cow (Tamminga, 2006). Additionally, high levels of CP intake are associated with increased environmental N loss because of increased urinary N excretion (Mulligan et al., 2004). Reducing CP intake has been shown to reduce milk yield (Broderick, 2003) and improve the metabolic energy status (Law et al., 2009) in early-lactation dairy cows offered TMR. Where grazed pasture was offered, reducing concentrate CP had little effect on milk production (Mulligan et al., 2004). However, this experiment was conducted on animals averaging 92 DIM; therefore, it is necessary to evaluate supplemental concentrate CP in early lactation when DMI is often too low to meet the cow's requirements for production.

Where CP is reduced, methionine may limit milk production (NRC, 2001). In several feeding situations, supplemental methionine improved milk yield and milk constituent yield (Broderick et al., 2008; Phipps et al., 2008) relative to unsupplemented diets. In addition, methionine is important for the synthesis of apoproteins and other lipid transporters and may play a role in the prevention of fatty liver associated with NEB (Bobe et al., 2004). However, improving milk energy output without a concurrent improvement in energy supply may further increase the degree of NEB experienced by the dairy cow. Therefore, it is of interest to examine the effects of offering a methionine source supplementary to low-CP concentrates on milk production and energy balance in early-lactating dairy cows offered grazed pasture.

Altering the starch source through replacing rolled barley with ground corn may also have positive effects on milk production and metabolic status. Corn contains more starch with a higher rumen resistance than barley (Herrera-Saldana et al., 1990), and when digested in the small intestine, it yields glucose (Ørskov, 1986). Improving the glucose status of the dairy cow may improve the energy supply to the mammary gland and peripheral tissues (Lemosquet et al., 2009), thus reducing the need for gluconeogenesis. Additionally, increasing circulating glucose and insulin concentrations improved AA supply to the mammary gland (Rulquin et al., 2004) and milk protein synthesis in midlactation (160 DIM) cows (Rius et al., 2010). In early lactation, a low DMI reduces concentrations of glucose and insulin; improving the supply of glucogenic nutrients can reduce the mobilization of fat and, therefore, the production of NEFA and BHBA (van Kneysel et al., 2007). It is of interest to investigate the effect of offering ground

corn rather than rolled barley-based concentrates on milk production and blood metabolites in pasture-fed, early-lactation dairy cows.

The objective of this experiment was to assess the effects of (1) reducing supplemental concentrate CP; (2) including a supplemental methionine hydroxyl analog (**HMBi**) in low-CP concentrates; and (3) replacing rolled barley with ground corn in low-CP concentrates on milk production and metabolic status in early-lactation dairy cows offered grazed pasture.

MATERIALS AND METHODS

Animals and Management

All procedures described in this experiment were approved by the animal research ethics committee at University College Dublin and were conducted under experimental license from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of the Cruelty to Animals Act 1876) Regulations, 2002 and 2005 (S.I.613; Department of Health and Children, 2005).

Twenty primiparous and 24 multiparous dairy cows (*Bos taurus* strain Holstein-Friesian) were selected from the spring-calving dairy herd at University College Dublin Lyons Research Farm, Newcastle, Dublin, Ireland (53°17'56" N, 6°32'18" W). Animals were blocked by parity and calving date (mean calving date November 3, 2009 $\pm$ 30 d) and assigned to 1 of 4 pasture-based dietary treatments in a randomized complete block design ($n = 11$). Treatment groups were balanced for previous lactation milk yield (7432 $\pm$ 844 kg) and milk protein yield (253 $\pm$ 30 kg) in the multiparous animals. The experiment was conducted from d 1 to 100 postpartum (**PP**).

Treatments consisted of a perennial ryegrass-based pasture and 1 of 4 supplemental concentrates. These were 1) a high-protein (19% CP) concentrate (**HP**); 2) a low-protein (15% CP) concentrate (**LP**); 3) a low-protein (15% CP) concentrate plus supplemental HMBi (**LP + HMBi**); and 4) a low-protein (15% CP) concentrate containing ground corn (**LP-corn**). The ingredient inclusion rates of concentrates offered during the experiment are presented in Table 1. Crude protein was reduced by replacing soybean meal with cereal grains and digestible fiber. Rolled barley was the main starch source in the HP, LP, and LP + HMBi treatments, whereas in the LP-corn treatment, rolled barley was replaced with ground corn (Gain Feeds, Glanbia PLC, Kilkenny, Ireland). Concentrates were isoenergetic (1.1 units of energy for milk production/kg of DM; Table 2). All animals received the concentrate portion of their

Table 1. Ingredient composition of concentrates used during the experiment¹

Ingredient (% of DM)	HP	LP	LP + HMBi	LP-corn
Barley grain	47.5	50.0	50.0	0
Corn grain	0	0	0	50.0
Beet pulp	11.1	23.1	23.6	11.7
Citrus pulp	6.8	4.6	3.8	4.2
Soybean hulls	0	0	0	8.3
Soybean meal	25.1	12.6	12.1	16.2
MetaSmart ²	0	0	0.8	0
CPO coater ³	0.6	0.6	0.6	0.6
Milk solids	5.0	5.0	5.0	5.0
Monocalcium diphosphate	1.1	1.4	1.4	1.3
Limestone flour	0.7	0.6	0.6	0.6
Salt	0.6	0.6	0.6	0.6
Calcined magnesite	0.9	0.9	0.9	0.9
Trace elements + vitamins ⁴	0.6	0.6	0.6	0.6

¹HP = high-CP concentrate; LP = low-CP concentrate; LP + HMBi = low-CP concentrate containing supplemental 2-hydroxy-4-methylthio butanoic acid (HMBi); LP-corn = low-CP concentrate with corn replacing rolled barley.

²MetaSmart (Adisseo France SAS, Antony, France) consists of not less than 57% monomer esters of HMBi, 4% maximum humidity.

³CPO coater = crude palm oil coater used in the formation of the concentrate pellet.

⁴Trace elements + vitamins contained the following: 2.5 mg/kg of Co; 10 mg/kg of I; 6.5 mg/kg of Se; 37 mg/kg of Cu; 50 mg/kg of Mn; 125 mg/kg of Zn; 10 IU/kg of vitamin E; 8,000 IU/kg of vitamin A, and 2,000 IU/kg of vitamin D₃.

diets twice daily during milking at a rate of 2.58 kg of DM/animal (total of 5.17 kg of DM/animal per day).

Animals were grazed together and were offered fresh allocations (10 kg of DM/animal) of pasture twice daily (20 kg of DM/animal per day). Pregrazing herbage mass was determined using the quadrant and shears method. Briefly, an area (0.25 m²) was cut using a hand-held shears (Gardena Accu 90; Gardena GmbH, Ulm,

Germany) to a height of 4 cm at 6 random locations throughout the paddock. Each 0.25 m² of grass was then collected and weighed; a sample of pasture was taken for determination of DM and routine chemical analysis. Average pregrazing herbage mass was 1,966 ± 602 kg of DM/ha, whereas postgrazing herbage mass was 520 ± 216 kg of DM/ha. The chemical composition of the pasture offered during this experiment is presented in

Table 2. Chemical composition of supplemental concentrates¹ and pasture offered during the experiment

Item	HP	LP	LP + HMBi	LP-corn	Pasture
DM (%)	86.7	85.8	86.2	85.9	20.7
Energy (UFL ² /kg of DM)	1.12	1.10	1.10	1.14	0.98
Chemical composition ³ (%)					
DMD	88.1	87.9	89.2	89.2	86.5
OMD	88.9	88.7	90.0	90.1	87.1
CP	19.2	15.0	14.5	15.7	18.8
PDIA	7.2	5.2	5.2	7.4	3.9
PDIN	14.5	10.8	10.8	12.2	10.7
PDIE	13.9	12.0	12.0	13.3	9.8
NDF	21.9	21.8	22.2	22.3	46.0
ADF	9.1	10.6	11.1	11.5	16.1
ADL	0.7	1.0	1.2	0.7	5.3
Ash	8.7	8.1	8.0	7.9	4.9
Ether extract	1.6	1.8	1.8	1.7	1.7
WSC	—	—	—	—	18.8
Starch	29.6	30.3	30.7	34.4	—

¹HP = high-CP concentrate; LP = low-CP concentrate; LP + HMBi = low-CP concentrate containing supplemental 2-hydroxy-4-methylthio butanoic acid (HMBi); LP-corn = low-CP concentrate with corn replacing rolled barley.

²UFL = units of energy for lactation.

³DMD = digestibility of DM; OMD = digestibility of OM; PDIA = feed protein truly digested in the small intestine; PDIN = protein truly digested in the small intestine, where N is limiting microbial protein synthesis; PDIE = protein truly digested in the small intestine, where energy is limiting microbial protein synthesis; WSC = water-soluble carbohydrates.

Table 2. The pasture offered during this experiment was estimated to contain 97% perennial ryegrass (*Lolium perenne* L.) and 3% white clover (*Trifolium repens* L.). Pastures received chemical N in February (29 kg/ha), March (58 kg/ha), and April (58 kg/ha).

Data and Sample Collection

Samples of pasture were collected daily and pooled weekly, whereas concentrate samples were collected weekly and pooled on a monthly basis. Pasture and concentrate samples were dried at 55°C in a forced-air oven to determine DM. Animals were milked twice daily at 0700 and 1600 h. Milk output and milk sampling were facilitated by using a milk metering and sampling system (Weighall; Dairy Master, Kerry, Ireland). Samples of milk were taken weekly during consecutive a.m. and p.m. milkings, pooled according to production, and stored at -20°C pending analysis. Animal BW was determined following the a.m. milking once weekly. Body condition score was also determined at this time using a scale of 1 to 5 in 0.25 increments (Edmonson et al., 1989).

Blood samples were harvested by jugular venipuncture on d 14, 21, 28, and 35 postpartum for determination of glucose, urea, NEFA, BHBA, bilirubin, γ -glutamyl transferase (GGT), and glutamate dehydrogenase (GLDH). Blood samples for glucose were harvested into a 4-mL Vacutainer tube containing NaF (6 mg) and Na₂EDTA (12 mg; Ref. No. 368521; BD, Plymouth, UK) and centrifuged at $2,100 \times g$ for 20 min at 4°C for extraction of plasma. These samples were stored at -20°C pending analysis. Blood samples for other analytes were harvested into a 10-mL Vacutainer (Ref. No. 8303209; BD) and allowed to clot for 16 h at 4°C before centrifuging at $2,100 \times g$ for 20 min at 4°C for extraction of serum. These samples were also stored at -20°C pending analysis.

Rumen fluid was harvested by stomach tube (Flora Rumen Scoop; Prof-Products, Guelph, ON, Canada) after the a.m. milking on d 14, 21, 28, and 35 postpartum. Samples were strained through 4 layers of cheesecloth. An 8-mL sample was drawn off, mixed with 2 mL of 50% (wt/vol) TCA, and stored at -20°C pending determination of VFA and NH₃-N concentrations.

Sample Analyses

Dried pasture and concentrate samples were ground in a hammer mill fitted with a 1-mm screen (Lab Mill; Christy Turner, Suffolk, UK). Neutral detergent fiber and ADF were determined sequentially using the method of Van Soest et al. (1991) adapted for use in the Ankom 220 Fiber Analyzer (Ankom Technology, Fairport,

NY). Samples were not subjected to combustion after fiber extraction; therefore, the values reported include ash. No Na₂SO₄ was used in the procedure. However, a thermostable α -amylase (FAA, 17,400 Liquefon Units/mL; Ankom Technology) was included when concentrate NDF was determined. Acid detergent lignin was determined after ADF determination by soaking the sample in 72% H₂SO₄ for 3 h and then triple rinsing with 80°C distilled H₂O before drying at 104°C for 3 h. Ash was determined after combustion in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 550°C for 5 h. Starch was determined using the Megazyme Total Starch Assay Procedure (Product No. K-TSTA; Megazyme International Ireland Ltd., Wicklow, Ireland). Gross energy was determined by bomb calorimetry (Parr 1281 Bomb Calorimeter, Parr Instrument Company, Moline, IL). Ether extract was determined using Soxtec instruments (Tecator, Höganäs, Sweden) and light petroleum ether. The N contents of pasture and concentrate samples were determined by combustion (FP 528 Analyzer, Leco Corp., St. Joseph, MI). In vitro digestibility of DM of pasture and concentrates offered was determined using a modification of the method of Tilley and Terry (1963) for use in the Ankom Daisy Incubator (Ankom Technology).

Concentrations of milk fat, protein, and lactose were determined in a commercial milk laboratory (Progressive Genetics, Dublin, Ireland) using infrared analysis (CombiFoss 5000, Foss Analytical A/S, Hillerød, Denmark). Blood samples were analyzed for GLDH (Kit No. GL441), urea (Kit No. UR3825), NEFA (Kit No. FA115), and BHBA (Kit No. RB1008) using enzymatic tests. Glucose (Kit No. GL3816) was analyzed using the hexokinase test, whereas GGT (Kit No. GT3817) and bilirubin (Kit No. BR3859) were analyzed using colorimetric tests. All test kits were sourced from Randox Laboratories Ltd. (Antrim, Northern Ireland). All blood analyses were carried out using a clinical blood analyzer (RX imola; Randox Laboratories Ltd.).

Rumen fluid was allowed to thaw for 16 h to 4°C, and then centrifuged at $2,100 \times g$ for 10 min at 4°C. One milliliter of supernatant was drawn off and diluted 1:5 with distilled H₂O, and then centrifuged at $1,600 \times g$ for 15 min at 4°C, and 200 μ L of supernatant was drawn off and used to determine NH₃-N concentrations according to the phenol-hypochlorite method of Weatherburn (1967). Rumen fluid was prepared for VFA analysis by diluting 250 μ L of the original undiluted rumen fluid with 3.75 mL of distilled H₂O, to which 1 mL of internal standard solution (0.5 g of 3-methylvaleric acid in 1 L of 0.15 M oxalic acid) was added. The resulting solution was centrifuged at $1,600 \times g$ for 10 min at 16°C and filtered through a syringe tip filter (polytetrafluoroethylene, 13-mm diameter, 0.45 μ m) into 2-mL

gas chromatography vials. Concentrations of VFA were determined using a gas chromatograph (Varian 3800 GCL; Varian Inc., Palo Alto, CA) fitted with a 15-m capillary column with an i.d. of 0.53 mm coated with 1.20 μm of acid-modified polyethylene glycol (EC-1000; Grace Davison Discovery Sciences, Lancashire, UK).

Statistical Analyses

Data were checked for adherence to the normal distribution and homogeneity of variance using histograms and formal statistical tests as part of the Univariate procedure of SAS (2004). Blood NEFA and GLDH were transformed using natural logarithms because preliminary analysis showed these data to be positively skewed; untransformed values are reported for clarity. However, transformed values were used to determine significance (Al Ibrahim et al., 2010). Analysis of data was conducted using the repeated measures function in Proc Mixed of SAS (2004), where cow was the repeatedly studied subject. The model included tests for the fixed effects of treatment, week, and parity and their interactions. Statistically significant differences between least squares means were tested using the PDIFF command incorporating the Tukey test for pairwise comparison of treatment means. When interactions were not significant, this term was excluded from the model. Statistical significance was assumed at a value of $P < 0.05$, and a tendency toward significance was assumed at a value of $P > 0.05$ but $P < 0.10$.

RESULTS

Milk Production, Milk Composition, Animal BW, and BCS

Table 3 shows the effect of supplemental concentrate type on milk production, milk composition, animal BW, and BCS. Average daily milk yield for the duration of the experiment is presented in Figure 1. Animals offered LP had lower ($P < 0.01$) milk yields compared with those offered other concentrate types; milk yield was greater ($P = 0.03$) in the HP treatment compared with the LP + HMBi treatment. However, LP-corn had a milk yield similar ($P > 0.10$) to those of the HP and LP + HMBi treatments. Animals in the HP treatment had a higher milk yield during the first 3 wk of lactation compared with those in the LP and LP-corn treatments. Energy-corrected milk was higher ($P = 0.07$) from animals offered the LP + HMBi diet compared with those offered the LP diet. Animals in the LP + HMBi treatment had a greater ($P < 0.01$) milk fat yield compared with those fed LP-corn. Milk protein yield was higher ($P < 0.01$) from animals offered the HP diet

compared with those offered the LP diet, whereas lactose yields were lower from animals offered the LP diet compared with those offered the HP ($P < 0.01$), LP + HMBi ($P = 0.02$), and LP-corn ($P < 0.01$) diets. Milk fat concentration was higher ($P < 0.01$) for animals in the LP and LP + HMBi treatments compared with those in the HP and LP-corn treatments. Similarly, milk protein concentration was higher ($P < 0.01$) for animals in the LP treatment compared with those in the HP and LP-corn treatments. Lactose concentration in the milk was higher for animals in the LP-corn treatment compared with those in the HP ($P = 0.02$), LP ($P < 0.01$), and LP + HMBi ($P = 0.06$) treatments. Somatic cell count, animal BW, BCS, and the change in BCS up to d 42 were not affected ($P > 0.10$) by type of supplemental concentrate.

Blood Metabolites and Rumen Fermentation

Table 4 shows the effect of supplemental concentrate type on blood metabolites and rumen fermentation. Blood glucose concentrations were higher for animals in the LP-corn treatment compared with those in the HP ($P < 0.01$) and LP ($P = 0.03$) treatments. Plasma BHBA was higher in the blood from animals in the HP treatment compared with those in the LP ($P = 0.04$), LP + HMBi ($P < 0.01$), and LP-corn ($P < 0.01$) treatments. Animals in the HP treatment also had higher ($P = 0.04$) NEFA compared with those in the LP + HMBi treatment. Activity of GGT was higher in animals in the LP + HMBi treatment compared with those in the HP ($P < 0.01$) and LP ($P = 0.05$) treatments. However, GLDH and bilirubin were not affected by concentrate type. Concentrations of blood urea-N were higher from animals in the HP treatment compared with those in the LP ($P < 0.01$), LP + HMBi ($P = 0.03$), and LP-corn ($P = 0.04$) treatments. No effect of concentrate type was observed on rumen fermentation measurements.

DISCUSSION

Milk Production, Milk Composition, Animal BW, and BCS

Limiting MP availability during early lactation has been shown to limit milk yield and, as a result, may improve the metabolic status of the dairy cow (Law et al., 2009). In the present study, animals offered the LP diet had lower milk production than those offered the HP diet, consistent with previous observations in which dietary CP was reduced (Broderick, 2003; Law et al., 2009). These results suggest that milk yield may have been limited by available AA when concentrate

Table 3. Effect of supplemental concentrate type¹ on milk production, milk composition, animal BW, and body condition

Item	HP	LP	LP + HMBi	LP-corn	SED ²
Output (kg/d)					
Milk	28.2 ^a	25.2 ^c	26.8 ^b	27.5 ^{ab}	0.39
ECM ³	29.3 ^{xy}	27.7 ^y	29.5 ^x	28.6 ^{xy}	0.81
Fat	0.98 ^{ab}	0.96 ^{ab}	1.03 ^a	0.94 ^b	0.029
Protein	0.97 ^a	0.88 ^b	0.94 ^{ab}	0.94 ^{ab}	0.017
Lactose	1.35 ^a	1.21 ^b	1.31 ^a	1.35 ^a	0.023
Milk composition (% unless stated)					
Fat	3.49 ^b	3.76 ^a	3.79 ^a	3.40 ^b	0.089
Protein	3.31 ^b	3.44 ^a	3.38 ^{ab}	3.32 ^b	0.026
Lactose	4.68 ^b	4.68 ^b	4.70 ^{b,y}	4.75 ^{a,x}	0.019
SCC (cells/mL)	82.47	90.96	88.75	81.64	16.46
Animal variable					
BW (kg)	508	482	502	509	16.8
BCS ⁴	3.01	3.04	2.91	3.04	0.082
BCS loss (to d 42)	-0.15	-0.38	-0.04	-0.24	0.224

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

^{x,y}Means within a row with different superscripts differ ($P > 0.05$, but $P < 0.10$).

¹HP = diet based on grazed pasture plus 5.17 kg of DM/d of a high-CP concentrate; LP = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate; LP + HMBi = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate containing supplemental 2-hydroxy-4-methylthio butanoic acid isopropyl ester (HMBi); LP-corn = LP = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate with corn grain replacing barley grain as the starch source.

²SED = SE of the difference.

³ECM = $(0.327 \times \text{milk, kg/d}) + (12.69 \times \text{fat, kg/d}) + (7.65 \times \text{protein, kg/d})$ (Eslamizad et al., 2010).

⁴BCS was on a scale of 1 to 5 in 0.25 increments (Edmonson et al., 1989).

CP was reduced. Additionally, improved circulating glucose may have supported increased lactose synthesis, and therefore milk yield, because lactose is the osmotic regulator for the mammary gland uptake of water (Rigout et al., 2002). In contrast, milk fat yield and ECM were not reduced by reducing the supplemental concentrate CP in the current study. This result is important where milk is purchased on a component-based pricing system (Breen et al., 2007) because dairy producers can reduce concentrate CP without reducing the milk price received. However, milk fat synthesis has a high demand for energy (Tyrrell and Reid, 1965), and failure to reduce milk fat yield would reduce the potential for supplemental concentrate CP to improve energy balance.

In the current study, supplementing the low-CP concentrate with HMBi improved milk production to levels comparable with those of animals offered HP, suggesting that available methionine may limit production in early-lactation dairy cows offered pasture and low-CP supplements. The results from this study and those of others using TMR-based diets (Noftsgger et al., 2005; Broderick et al., 2008; Phipps et al., 2008) highlight the importance of methionine in milk protein synthesis and also the synthesis of apoproteins for the transport of FA to support milk fat synthesis (Pullen et al., 1989).

Including ground corn in the concentrate at the expense of rolled barley also increased milk yield, protein

yield, and lactose yield to levels comparable with those of cows fed HP. Corn contains more starch and has a higher supply of digestible protein to the small intestine and balance of methionine than barley (O'Mara, 1996). Additionally, the provision of a postruminal supply of starch may reduce AA utilization for gluconeogenesis, thus improving the AA supply to the mammary gland (Reynolds, 2006). The combination of an improved supply of digestible protein to the small intestine and increased circulating glucose (Table 4) may have resulted in an improved mammary gland AA supply and milk protein synthesis (Bequette et al., 2001; Rius et al., 2010) when LP-corn was offered. In contrast, animals offered LP-corn had the lowest yield and concentration of milk fat. Previously, Rigout et al. (2002) demonstrated that increased circulating glucose levels reduced milk fat yield (-234 g/d) through reduced circulating precursors for milk fat synthesis. In this study, animals offered LP-corn had the highest glucose levels and numerically lower BHBA, NEFA, and rumen acetic acid concentrations, the combined effects of which may have contributed to a reduction in milk fat synthesis.

Altering concentrate type did not affect the quantity of BCS loss to d 42 despite previous suggestions that increased CP could accelerate (Ørskov et al., 1987) and increased starch (LP-corn) could reduce (Lemosquet et al., 1997) rates of body tissue mobilization. However, in this experiment, only small differences (maximum

Figure 1. Effect of supplemental concentrate type on milk yield (kg/d) over the duration of the study. HP = diet based on grazed pasture plus 5.17 kg of DM/d of a high-CP concentrate; LP = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate; LP + HMBi = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate containing supplemental 2-hydroxy-4-methylthio butanoic acid isopropyl ester (HMBi); LP-corn = LP diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate with corn grain replacing barley grain as the starch source. Color version available in the online PDF.

1.8 ± 0.8 kg/d) in ECM were observed between concentrate types. The similarities between energy requirements may have reduced the potential for differences in energy balance, and therefore BCS loss, in this study.

Blood Metabolites and Rumen Fermentation

Measurement of blood metabolites allows for the determination of more discrete differences in energy status (Al Ibrahim et al., 2010). In this experiment, reducing concentrate CP did not alter circulating glucose despite the higher requirements for lactose production when HP was offered. The increase in urea in the blood when HP was offered indicates that glucose levels may have been maintained through increased use of nonessential AA for gluconeogenesis (Lemosquet et al., 2009). Although differences in BCS change were not observed in this study, mobilization of visceral adipose tissue cannot be ruled out as a source of energy in early lactation (Reynolds et al., 2004).

As expected, reducing concentrate CP (HP vs. other treatments) reduced blood urea-N. Lower blood urea-N

was most likely caused by a reduction in AA available for degradation in the liver because rumen-derived $\text{NH}_3\text{-N}$ was not different among treatments. Reduced blood urea-N has been associated with reduced urinary N excretion (Kauffman and St-Pierre, 2001), consistent with observations made in a companion study (Whelan et al., 2011). Additionally, a higher uterine pH and increased progesterone levels associated with reduced urea-N concentrations can improve the reproductive performance in lactating dairy cows (Butler et al., 1996).

Providing supplemental methionine to low-CP concentrates reduced circulating BHBA and NEFA, despite milk production from these animals being similar to that of cows offered HP. Previously, Rulquin et al. (2006) and Socha et al. (2008) observed reductions in NEFA with an increasing supply of methionine, suggesting improved lipid transport of FA to peripheral tissues. The improvement in milk fat yield in this study suggests that FA were transported to the mammary gland to support milk fat synthesis. In contrast, GGT activity was higher in animals offered LP + HMBi than

Table 4. Effect of supplemental concentrate type¹ on blood metabolites and rumen fermentation

Item	HP	LP	LP + HMBi	LP-corn	SED ²
Blood metabolite					
Glucose (mmol/L)	3.15 ^b	3.18 ^b	3.22 ^{ab}	3.34 ^a	0.053
NEFA ³ (mmol/L)	0.41 ^a	0.40 ^{ab}	0.33 ^b	0.37 ^{ab}	0.032
BHBA (mmol/L)	0.71 ^a	0.63 ^b	0.56 ^b	0.59 ^b	0.028
GGT ⁴ (u/L)	11.35 ^b	12.20 ^b	13.89 ^a	12.57 ^{ab}	0.659
GLDH ⁵ (u/L)	15.88	13.16	16.99	15.86	2.038
Bilirubin (μmol/L)	5.76	5.84	5.70	5.42	0.284
Urea-N (mmol/L)	5.49 ^a	4.00 ^b	4.30 ^b	4.34 ^b	0.436
Rumen fermentation (mmol/L)					
Acetic	52.53	49.32	51.71	49.85	1.722
Propionic	16.75	16.13	15.93	16.01	0.531
Isobutyric	0.57	0.58	0.64	0.64	0.031
Butyric	9.43	8.48	8.94	8.49	0.406
Isovaleric	0.86	0.83	0.90	0.95	0.039
Valeric	1.17	1.21	1.15	1.13	0.063
Total VFA	79.69	76.84	79.75	77.13	2.495
Acetic:propionic	3.12	3.09	3.20	3.13	0.079
Ammonia-N	6.46	5.83	6.77	5.94	0.741

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹HP = diet based on grazed pasture plus 5.17 kg of DM/d of a high-CP concentrate; LP = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate; LP + HMBi = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate containing supplemental 2-hydroxy-4-methylthio butanoic acid isopropyl ester (HMBi); LP-corn = LP = diet based on grazed pasture plus 5.17 kg of DM/d of a low-CP concentrate with corn grain replacing barley grain as the starch source.

²SED = SE of the difference.

³Level of significance corresponds to the transformed variable.

⁴GGT = γ -glutamyl transferase.

⁵GLDH = glutamate dehydrogenase (level of significance corresponds to the transformed variable).

those offered HP and LP. However, across all dietary treatments, GGT (<40 IU/L), GLHD (<25 IU/L), and bilirubin (<14 μmol/L) concentrations were not high enough to indicate abnormalities in liver function (Boyd, 1984; West, 1997). When ground corn was included at the expense of rolled barley in low-CP concentrates, blood glucose increased. This increase reflects the higher starch content in LP-corn compared with LP. Because neither total VFA nor propionate differed between treatments, it is likely that this additional starch was either digested slowly in the rumen or digested postruminally. Increasing postruminal digestion of starch can have a sparing effect on endogenously produced glucose (Nocek and Tamminga, 1991). Thus, glucose supply to the peripheral tissue is improved and circulating BHBA is reduced, consistent with the observations of the current experiment and those of Lemosquet et al. (1997).

CONCLUSIONS

Reducing supplemental concentrate CP offered to early-lactation dairy cows grazing perennial ryegrass-based pasture can reduce milk yield and protein yield. However, BHBA was also reduced, indicating a more favorable metabolic status could be achieved with low-CP concentrates. Replacing barley with corn, providing

a supplemental methionine source in low-CP concentrates, or both ameliorated the effects of reduced milk production without negatively affecting the metabolic status of the early-lactation dairy cow.

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