Effect of different levels of rapidly degradable carbohydrates calculated by a simple rumen model on performance of lactating dairy cows

J. Doorenbos,^{*1} J. Martín-Tereso,^{*} J. Dijkstra,[†] and H. van Laar^{*}

^{*}Trouw Nutrition R&D, PO Box 220, 5830 AE Boxmeer, the Netherlands

[†]Animal Nutrition Group, Wageningen University and Research, PO Box 338, 6700 AH Wageningen, the Netherlands

ABSTRACT

Aggregating rumen degradation characteristics of different carbohydrate components into the term modeled rapidly degradable carbohydrates (mRDC) can simplify diet formulation by accounting for differences in rate and extent of carbohydrate degradation within and between feedstuffs. This study sought to evaluate responses of lactating dairy cows to diets formulated with increasing levels of mRDC, keeping the supply of other nutrients as constant as possible. The mRDC content of feedstuffs was calculated based on a simple rumen model including soluble, washable, and non-washable but potentially degradable fractions, as well as the fractional degradation and passage rates, of sugar, starch, neutral detergent fiber, and other carbohydrates. The mRDC term effectively represents the total amount of carbohydrates degraded in the rumen within 2 h after ingestion. Fifty-two lactating Holstein cows (of which 4 were rumen fistulated) were assigned to 4 treatments in a 4 × 4 Latin square design. Treatments were fed as a total mixed ration consisting of 25.4% corn silage, 23.1% grass silage, 11.6% grass hay, and 39.9% concentrate on a dry matter basis. Differences in mRDC were created by exchanging nonforage neutral detergent fiber-rich ingredients (mainly sugar beet pulp) with starch-rich ingredients (mainly wheat) and by exchanging corn (slowly degradable starch) with wheat (rapidly degradable starch) in the concentrate, resulting in 4 treatments that varied in dietary mRDC level of 167, 181, 194, or 208 g/kg of dry matter. Level of mRDC did not affect dry matter intake. Fat- and protein-corrected milk production and milk fat and lactose yield were greatest at 181 mRDC and decreased with further increases in mRDC. Milk protein yield and concentration increased with increasing mRDC level. Mean rumen pH and diurnal variation in ruminal pH did not differ between treatments. Total daily meal time and number of visits per meal were smaller at 181

and 194 mRDC. Despite milk production responses, increasing dietary mRDC levels, while maintaining net energy and intestinal digestible protein as well as other nutrients at similar levels, did not influence rumen pH parameter estimates and had minor effects on feeding behavior. These results indicate that aggregating rapidly degradable carbohydrate content into one term may be a simple way to further improve predictability of production responses in practical diet formulation for lactating dairy cows.

Key words: rapidly degradable carbohydrate, rumen model, milk production, dairy cow

INTRODUCTION

Milk production and composition are of major importance to the economics of a dairy farm and to a large extent depend on energy and protein intake of the cow (Huhtanen and Nisinen, 2012; Daniel et al., 2016). Several systems calculate ME or NE_L and MP values for individual feedstuffs based on estimates of rumen fermentation and intestinal or total-tract digestibility of nutrients (Tylutki et al., 2008; Volden, 2011; CVB, 2012). Besides energy and MP supply, dairy cow performance is influenced by rumen function, the supply of rumen degradable substrates, and the profile of nutrients available in the postabsorptive metabolism (Dijkstra et al., 2012; Huhtanen and Nisinen, 2012).

Rumen degradable substrates can be subdivided into carbohydrates and protein. Rumen degradable carbohydrates consist of different chemical components, the primary constituents being starch, NDF, sugar, and pectin. Each component has distinct ruminal degradation and fermentation characteristics. Although total VFA production is related to the intake of rumen fermentable OM, components like NDF or starch result in different proportions of individual VFA produced in the rumen (Nozière et al., 2011). Diet composition can also affect VFA proportions through interconversions between the major VFA (acetate, propionate, and butyrate; Sutton et al., 2003). These VFA have different effects on milk production and composition (Thomas and Martin, 1988). Therefore, each fraction of rumen

Received November 8, 2016.

Accepted March 7, 2017.

¹Corresponding author: Jeroen.doorenbos@trouwnutrition.com

degradable carbohydrates can have different effects on milk production and composition, even when energy or MP supply does not differ. Replacing starch for NDF or changing the starch source (i.e., corn vs. barley or wheat) will affect VFA molar proportions, intestinal digestibility, and metabolic responses, although not always resulting in a response in production performance (Lechartier and Peyraud, 2011; Ferraretto et al., 2013; Piccioli-Cappelli et al., 2014).

The major mechanisms to control ruminal pH are buffering by saliva, removal of VFA by ruminal absorption, or passage to the small intestine (Dijkstra et al., 2012). Higher ruminal VFA production through increased carbohydrate supply and fermentation rate not compensated by increased ruminal VFA absorption or buffering will lower average ruminal pH or increase diurnal variation in ruminal pH. This change in pH dynamics can affect nutrient availability and thus affect production performance (Dijkstra et al., 2012).

Meeting the animal and microbial nutrient requirements in diet formulation will optimize cost-effective animal performance while minimizing nutrient excretion into the environment. Inclusion of several formulation bounds, required to account for dietary carbohydrate characteristics, including levels of various sources and differences in rate of degradation between and within feedstuffs, increases the complexity of diet formulation. The amount of rumen degradable carbohydrates depends on the rumen soluble, washable, and nonwashable but potentially degradable fractions, as well as the fractional degradation rate (k_d) and fractional passage rate (Van Duinkerken et al., 2011). As k_d is a nonlinear characteristic representing the fraction of substrate being degraded per unit of time, this value cannot be used directly in linear programming for diet formulation (Volden, 2011). Aggregating rumen degradation characteristics of the different carbohydrate components per feedstuff into terms such as modeled rapidly degradable carbohydrates (**mRDC**) and modeled total degradable carbohydrates (**mTDC**) can simplify diet formulation and still capture the effect of total carbohydrate supply as well as rate of degradation. In the present study, mRDC and mTDC are calculated for individual feedstuffs based on a simple rumen model.

The objective of our study was to evaluate production responses of lactating dairy cows to diets that were formulated to increasing mRDC levels, keeping the supply of other nutrients as constant as possible.

MATERIALS AND METHODS

The experiment was conducted at the Trouw Nutrition Dairy Research Facility (Kempenshof) in Boxmeer, the Netherlands. The experiment was conducted under

approval of the Dutch Animal Ethics Committee according to the Experiments on Animals Act, 1977.

Animals and Experimental Design

Fifty-two lactating Holstein dairy cows (8 primiparous and 44 multiparous) averaging 144 ± 62.7 DIM, 22.0 ± 3.15 kg of DM/d and 31.3 ± 6.13 kg of milk/d at the start of the experiment were used. Four of the multiparous cows had a rumen fistula (10 cm i.d., Bar-Diamond, Parma, ID). Animals were housed in a slatted-floor freestall barn and had free access to water at all times.

The experiment was set up as a 4×4 Latin square design with 4 treatments and 4 periods. Each period consisted of 2 wk of adaptation and 1 wk of measurements. Cows were assigned to a block consisting of 4 animals, according to milk yield, DMI, and DIM, based on the average value of these parameters obtained in the last 4 wk before start of the experiment. The rumen-fistulated animals formed one block. Within each block, cows were randomly assigned to 1 of 4 different treatment sequences.

Body weights were recorded automatically 4 times per week (twice after the morning milking and twice after the evening milking).

Dietary Treatments and Feeding

The 4 treatments consisted of increasing levels of mRDC at 167, 181, 194, and 208 g of mRDC/kg of DM. Concentrate ingredient composition was used to create the differences in mRDC level for the lowest and highest mRDC diets (Table 1; low mRDC and high mRDC concentrate, respectively). The basal forage mixture consisted of grass silage, corn silage, and grass hay and was identical for all treatments (Table 2). Animals were fed ad libitum TMR, which consisted of the forage mixture supplemented with different amounts of the low mRDC concentrate or the high mRDC concentrate to create the differences between treatments (Table 3). The TMR were formulated to be iso-energetic based on NE_L (Van Es, 1978), and iso-nitrogenous based on intestinal digestible protein (**DVE**) and degraded protein balance in the rumen (Tamminga et al., 1994). The TMR provided adequate net energy and intestinal digestible protein for a cow consuming 22 kg of DM/d and producing 32 kg of milk/d.

The DM content of grass silage and corn silage was analyzed weekly in a forced-air oven at 60°C for 48 h to ensure a constant composition of the TMR and DM supply to the animals. The TMR were produced daily and administered once per day in electronic forage feeders (Insentec, Marknesse, the Netherlands) after removal of

refusals. The 13 cows per treatment were given access to 7 feeders via electronic ear transponders, allowing for individual feed intake registration per cow.

Simple Rumen Carbohydrate Model for mRDC Calculation

Rumen degradable carbohydrates are defined as carbohydrates degraded in the rumen within 2 h after

ingestion. The dietary mRDC content was calculated for individual feedstuffs using the simple model defined below. The nutrients comprising the carbohydrate fraction are NDF, starch, sugar, and residual OM [calculated as DM minus ash, CP, NDF, starch, sugar, crude fat, and fermentation acids (the latter in silages only)]. For each feedstuff and nutrient, the amount of mRDC (g/kg of DM) was calculated using feedstuff-specific in situ degradation parameters, namely, washable (W), soluble

Table 1. Ingredient composition, analyzed chemical composition, and calculated nutrient value of low and high modeled rapidly degradable carbohydrate (mRDC) concentrates

Item	Concentrate	
	Low mRDC	High mRDC
Ingredient composition (g/kg of DM)		
Corn	157	38
Wheat	—	387
Bypass soybean meal	102	—
Palm kernel expeller	154	—
Rapeseed meal	167	155
Bypass rapeseed meal	—	144
Sunflower seed meal	—	103
Sugar beet pulp	301	—
Citrus pulp	34	—
Soybean hulls	—	78
Cane molasses	12	—
Beet molasses	—	9
Dextrose	—	21
Urea	20	8
Palm oil fatty acids	17	7
Hydrogenated palm oil	—	16
Limestone	—	10
Salt	—	7
Magnesium oxide	4	3
Monocalcium phosphate	17	8
Sodium bicarbonate	10	—
Vitamin and mineral premix ¹	7	7
Analyzed chemical composition (g/kg of DM unless stated otherwise) ²		
DM (g/kg)	898 (0.4)	884 (0.8)
OM	916 (0.6)	931 (0.4)
CP	243 (1.2)	244 (1.3)
NDF	325 (2.9)	234 (3.3)
Starch	119 (6.8)	305 (2.3)
Sugar	76 (0.5)	76 (1.4)
Crude fat	49 (3.1)	49 (3.6)
Calculated nutrient value (based on ingredient composition; g/kg of DM unless stated otherwise)		
NE _L ³ (MJ/kg of DM)	7.50	7.49
DVE ⁴	141	137
OEB ⁵	45	47
mRDC ⁶	167	271
mTDC ⁶	411	448
Bypass starch ⁶	50	43

¹Contained per kilogram of premix: 84 g of Ca, 125 mg of Co, 250 mg of I, 2,500 mg of Cu, 3,333 mg of Mn, 110 mg of Se, 8,333 mg of Zn, 1,333,300 IU of vitamin A, 333,300 IU of vitamin D₃, and 25,906 IU of vitamin E.

²Mean ± SE; SE based on composited sample per period (n = 4).

³Calculated NE_L (Van Es, 1978).

⁴Calculated intestinal digestible protein (Tamminga et al., 1994).

⁵Calculated degraded protein balance in the rumen (Tamminga et al., 1994).

⁶mRDC = modeled rapidly degradable carbohydrates; mTDC = modeled total degradable carbohydrates. For calculation of mRDC, mTDC, and bypass starch, see Materials and Methods, simple rumen carbohydrate model for mRDC calculation.

Table 2. Analyzed chemical composition (g/kg of DM unless stated otherwise) and calculated nutrient value (g/kg of DM unless stated otherwise) of forages [mean $\pm$ SE; SE based on composited sample per period (n = 4)]

Item	Corn silage	Grass silage	Grass hay
Analyzed chemical composition			
DM (g/kg as fed)	330 (6.6)	446 (8.9)	852 (5.3)
OM	962 (0.2)	867 (4.7)	942 (4.9)
CP	77 (1.5)	170 (4.1)	74 (6.5)
NDF	375 (2.9)	490 (10.3)	688 (7.9)
Starch	344 (4.4)	NA ¹	NA
Sugar	10 (0.4)	26 (2.2)	84 (7.8)
Crude fat	34 (0.8)	38 (0.8)	16 (1.1)
OM digestibility (%)	77.1 (0.54)	74.7 (0.47)	55.8 (0.61)
Calculated nutrient value			
NE _L ² (MJ/kg of DM)	6.89	5.83	4.16
DVE ³	53	68	38
OEB ⁴	-29	54	-39
mRDC ⁵	219	137	110
mTDC ⁵	495	391	343
Bypass starch ⁵	68	0	0

¹NA = not analyzed.²Calculated NE_L (Van Es, 1978).³Calculated intestinal digestible protein (Tamminga et al., 1994).⁴Calculated degraded protein balance in the rumen (Tamminga et al., 1994).⁵mRDC = modeled rapidly degradable carbohydrates; mTDC = modeled total degradable carbohydrates. For calculation of mRDC, mTDC, and bypass starch, see Materials and Methods, simple rumen carbohydrate model for mRDC calculation.

(S), and nonwashable but potentially degradable (D) fraction (all fractions in g/kg per nutrient), with WS (washable but insoluble fraction; g/kg of nutrient) calculated as $W - S$, together with the fractional rate of degradation (k_d ; /h) and fractional rate of passage (k_p ; /h) of each fraction according to the formula of Ørskov and McDonald (1979) using a time after ingestion of 2 h:

$$\begin{aligned} &\text{Rapidly degradable nutrient} = \text{Nutrient} \\ &\times (S \times k_d S / (k_d S + k_p S) \times \{1 - \exp[-(k_p S + k_d S) \\ &\times \text{time}]\} + WS \times k_d WS / (k_d WS + k_p WS) \\ &\times \{1 - \exp[-(k_p WS + k_d WS) \times \text{time}]\} + D \times k_d D / \\ &(k_d D + k_p D) \times \{1 - \exp[-(k_p D + k_d D) \times \text{time}]\}). \end{aligned}$$

The degradation parameter values and fractional degradation and passage rates for concentrate ingredients and grass hay used in these calculations were according to Van Duinkerken et al. (2011) with minor modifications (Appendix Table A1). Degradation parameters and fractional degradation rates of grass silage and corn silage were calculated using regression equations that were derived from a data set of in situ incubations according to the methodology described by Van Laar

and Doorenbos (2014). Fractional passage rates of grass and corn silage are presented in Appendix Table A1.

Modeled total degradable carbohydrate content was calculated similar to mRDC, in this case with time after ingestion to be infinite. Bypass starch was calculated as dietary starch minus rumen degraded starch, also as estimated by in situ degradability and passage rates.

Milk Yield and Composition

Cows were milked twice daily at 0500 and 1700 h. Milk yield was recorded automatically at each milking. Milk was sampled every week at Tuesday and Thursday morning and Monday and Wednesday evening milking events to represent the full week. Samples were collected in tubes containing sodium azide and bronopol as preservatives and stored at 4°C. Morning samples as well as evening samples were pooled and analyzed for fat, CP, lactose, and urea (Qlip, Deventer, the Netherlands) using a Foss Milkoscan FT6000 (ISO, 2013). Somatic cell count was analyzed in the same sample using a Fossomatic FC/5000 (ISO, 2006a). Fat- and protein-corrected milk yield (**FPCM**, corrected to 4.0% fat and 3.3% protein; kg/d) was calculated as milk yield (kg/d) $\times [0.377 + 0.116 \times \text{fat} (\%) + 0.06 \times \text{protein} (\%)]$. Feed efficiency was expressed as kilogram of milk or FPCM produced per kilogram of DMI.

Table 3. Ingredient composition, calculated chemical composition, and calculated nutrient value, for the 4 dietary treatments differing in modeled rapidly degradable carbohydrate (mRDC) level

Item	Treatment ¹			
	mRDC 167	mRDC 181	mRDC 194	mRDC 208
Ingredient composition (g/kg of DM)				
Grass silage	231	231	231	231
Corn silage	254	254	254	254
Grass hay	116	116	116	116
Concentrate low mRDC	399	266	133	—
Concentrate high mRDC	—	133	266	399
Calculated chemical composition ² (g/kg of DM)				
OM	918	919	921	922
CP	164	164	164	165
NDF	410	399	387	376
Starch	134	156	179	201
Sugar	50	50	51	51
Crude fat	41	40	39	38
Calculated nutrient value ² (g/kg of DM unless stated otherwise)				
NE _L ³ (MJ/kg of DM)	6.57	6.57	6.57	6.57
DVE ⁴	90	89	89	88
OEB ⁵	18	19	19	19
mRDC ⁶	167	181	194	208
mTDC ⁶	420	425	430	435
Bypass starch ⁶	37	36	35	35

¹Treatments had dietary mRDC levels of 167, 181, 194, or 208 g/kg of DM.

²Based on the calculated concentrate composition and the chemical analysis of the individual forages in the TMR.

³Calculated NE_L (Van Es, 1978).

⁴Calculated intestinal digestible protein (Tamminga et al., 1994).

⁵Calculated degraded protein balance in the rumen (Tamminga et al., 1994).

⁶mRDC = modeled rapidly degradable carbohydrates; mTDC = modeled total degradable carbohydrates. For calculation of mRDC, mTDC, and bypass starch, see Materials and Methods, simple rumen carbohydrate model for mRDC calculation.

Rumen pH

Rumen pH was recorded every 2 min with a pH logger (LRCpH T7 logger, Dascor, Escondido, CA). Loggers were inserted in the rumen of 4 fistulated animals the day before the start of the last week of each period and removed on the last day of that same week. At the beginning and end of the measuring period, loggers were calibrated in pH 4 and pH 7 buffers and recordings were corrected for drift in pH over time. The pH data were analyzed according to the method described by Colman et al. (2012). In brief, pH data were recalculated to the minutes per day that pH was below a certain value, ranging from pH 5.0 to 7.4, with steps of 0.1. This results in a cumulative time below pH graph, with pH on the x-axis and minutes per day below a certain pH on the y-axis. For each cow and week, a logistic curve was fitted through the data points with PROC NLIN of SAS version 9.4 (SAS Institute Inc., Cary, NC) according to the model: $T = 1,440 / \{1 + \exp[-B_0 \times (pH - B_1)]\}$, where T is the cumulative time below pH (min/d). The parameters describing the curve are B₀ (slope at the inflection point) and B₁ (inflection point).

B₀ reflects the diurnal variation in ruminal pH and B₁ is the median rumen pH.

Feeding Behavior Data Collection and Analysis

For every visit of a cow to a feeder, the start and end time of the visit as well as the start and end weight of the feeder were recorded. Visits without intake (12% of total visits), with a rate of intake less than 0.005 kg of DM/min (0.1%; animals spending much time at the feeder without actual intake) or with a rate of intake larger than 1.5 kg of DM/min (0.2%; expected to be caused by feed wastage) were removed from the data set.

Feeding behavior was analyzed according to Yeates et al. (2001). Time interval (in seconds) between 2 consecutive visits was transformed with a natural logarithm. For every cow a 2-population model (Gaussian-Weibull; **GW**) and a 3-population model (Gaussian-Gaussian-Weibull; **GGW**) was fitted through the individual transformed time intervals using the finite mixture models (FMM) procedure of SAS 9.4 (SAS Institute Inc.). In the GW model, the 2 populations describe

the time intervals within meals and the time intervals between meals, respectively. In the GGW model, the 2 Gaussian distributions describe within-meal intervals and the Weibull distribution describes the between-meal interval. Per cow and treatment, the relevance of adding a second G distribution was determined based on the likelihood ratio test (Yeates et al., 2001). A significant lower $-2\log$ -likelihood value indicates that the GGW model improved the goodness of fit of the data as compared with the GW model, and the GGW model was selected. Otherwise the GW model was selected. For each selected model, a meal criterion was determined as the intersection between the Gaussian and the Weibull curve (GW model) or the intersection between the second Gaussian and the Weibull curve (GGW model). This meal criterion is used to describe the longest interval between 2 consecutive visits that are still part of the same meal. Visits were clustered into meals per day by using the meal criterion and total daily meal time was calculated. Total daily eating time was calculated as total daily meal time minus intervals within meals. Per meal, the number of visits, mean meal duration, eating time, DMI, and rate of DMI [DMI (kg) per meal, divided by eating time (min) per meal] were calculated (Abrahamse et al., 2008).

Sampling and Chemical Analyses

During every third week of each period, concentrates and forages were sampled and stored at -18°C until further analysis. Concentrates were sent to Masterlab (Boxmeer, the Netherlands) and forages to AgroXpertus (Wageningen, the Netherlands). Concentrates were analyzed for DM, ash, CP, crude fat, NDF, starch, and sugar after grinding to 0.75 mm using a Retsch ZM200 mill (Retsch, Haan, Germany). Dry matter was determined after drying for 4 h at 103°C (EC 152/2009; EC, 2009). Ash was determined after incineration at 550°C (EC 152/2009; EC, 2009). Crude protein was determined according to the Dumas principle (ISO 16634-1; ISO, 2008). Crude fat was determined by treating the sample with hydrochloric acid and subsequent extracting with petroleum (EC 152/2009; EC, 2009). Amylase-treated NDF was determined using heat stable α -amylase and expressed without residual ash (ISO 16472; ISO, 2006b). Starch was determined enzymatically using amyloglucosidase (ISO 15914; ISO, 2004). Sugar was determined according to the Luff-Schoorl method and expressed as glucose (71/250/EEC; EC, 1971).

Forages were analyzed for DM, ash, CP, crude fat, NDF, starch, sugar, and OM digestibility. Samples were dried for 16 h at 70°C and ground at 1 mm (ISO 16498;

ISO, 2012). Dry matter, ash, crude fat, and starch were analyzed using the same methods as for concentrates. Crude protein was analyzed according to the Kjeldahl principle (EC 152/2009; EC, 2009). Amylase-treated NDF was determined using heat-stable α -amylase and expressed without residual ash (AOAC International, 2002). Sugar was determined according to the Luff-Schoorl method and expressed as glucose (EC 152/2009; EC, 2009). Organic matter digestibility was determined after 48 h incubation in rumen fluid and subsequently 48 h incubation in a pepsin-HCl solution according to Tilley and Terry (1963).

Statistical Analyses

The results of the third experimental period had to be removed from the data set due to problems with feed preparation. Data of 5 cows were excluded because occurrence of mastitis (2 cows), leg problems (2 cows), and general health problems (1 cow) that led to nonrepresentative data. As a result, the number of replicates used in the statistical analysis was 36 for treatment 1, 2, and 3, and 34 for treatment 4. For the analysis of the pH variables, 3 replicates per treatment were used. For individual cows, all data were averaged into weekly results per period. Feed intake, milk and component yield, milk composition, feed efficiency, BW change, pH variables, and feeding behavior characteristics were analyzed as repeated measurements with PROC MIXED of SAS 9.4 (SAS Institute Inc.) according to the following model:

$$Y_{ijk} = \mu + \text{mRDC}_i + \text{Square}_j + \text{Period}_k + \varepsilon_{ijk},$$

where Y is the dependent variable, μ is the overall mean, and ε_{ijk} is the residual error. The model included the fixed effects of level of mRDC (mRDC_i ; $i = 1$ to 4), square (Square_j ; $j = 1$ to 4; not for pH variables), and period (Period_k ; $k = 1$ to 3). Block was used as a random effect. Period was the repeated effect, with cow being the random subject. The covariance structure chosen was based on Akaike information criterion and Bayesian information criterion best fit. Covariance structure was autoregressive (1) for milk fat concentration, milk protein concentration, milk urea, diet and FPCM efficiency, BW change, all pH parameters, meals per day, visits per meal, eating time per meal, DMI per meal, and rate of DMI per meal. Toeplitz covariance structure was used for total daily meal time and total daily eating time, and ante (1) covariance structure was used for all other parameters. Orthogonal contrasts were used to test for linear, quadratic, or cubic effects of mRDC level. Linear, quadratic, or cubic effects of

Table 4. Dry matter intake and production performance of lactating dairy cows offered diets with different levels of modeled rapidly degradable carbohydrates (mRDC)

Item	Treatment ¹				SEM	P-value ²		
	mRDC 167	mRDC 181	mRDC 194	mRDC 208		L	Q	C
DMI (kg/d)	22.6	22.7	23.3	23.1	0.6	0.06	0.65	0.05
Yield (kg/d)								
Milk	27.8	28.6	28.0	27.7	1.4	0.42	0.07	0.06
FPCM ³	29.2	30.4	29.6	29.4	1.4	0.79	0.03	0.02
Milk fat	1.22	1.27	1.23	1.21	0.06	0.32	0.06	0.03
Milk protein	0.96	1.00	0.99	0.99	0.04	0.04	0.09	0.08
Milk lactose	1.25	1.29	1.27	1.25	0.07	0.73	0.02	0.15
Milk composition (%)								
Milk fat	4.40	4.46	4.43	4.34	0.07	0.22	0.07	0.69
Milk protein	3.47	3.55	3.55	3.59	0.04	<0.01	0.28	0.07
Milk lactose	4.47	4.48	4.50	4.48	0.04	0.30	0.20	0.48
Milk urea (mg/dL)	27.0	28.1	26.4	27.2	0.6	0.51	0.68	<0.01
SCC (10 ³ /mL)	228	176	148	163	40	0.09	0.73	0.86
Efficiency (kg/kg)								
Milk/DMI	1.23	1.25	1.21	1.20	0.06	0.13	0.29	0.23
FPCM/DMI	1.29	1.33	1.29	1.26	0.06	0.14	0.18	0.23
BW change (kg/d)	0.8	0.1	0.6	0.7	0.3	0.86	0.19	0.25

¹Treatments had dietary mRDC levels of 167, 181, 194, or 208 g/kg of DM.

²L = linear; Q = quadratic; C = cubic.

³FPCM = fat- and protein-corrected milk yield.

treatments were declared significant at $P < 0.05$ and as trends at $0.05 \leq P < 0.10$. Results are presented as least squares means.

RESULTS

Forage supply was identical across treatments, and therefore dietary mRDC contrasts between the treatments originated exclusively from concentrate composition. Dietary mRDC level tended to have a linear ($P = 0.06$) and cubic effect on DMI ($P = 0.05$; Table 4). Milk production tended to respond quadratically ($P = 0.07$) and cubically ($P = 0.06$) to mRDC, whereas FPCM showed quadratic ($P = 0.03$) and cubic ($P = 0.02$) responses to mRDC level. Milk fat yield responded cubically to mRDC level ($P = 0.03$) and tended to respond quadratically ($P = 0.06$). Milk protein yield increased linearly with increasing mRDC level ($P = 0.04$) and

tended to respond in a quadratic ($P = 0.09$) and cubic manner ($P = 0.08$) to mRDC level. Milk lactose yield showed a quadratic response to mRDC level ($P = 0.02$). Milk fat concentration tended ($P = 0.07$) to respond quadratically. Increasing mRDC level linearly increased milk protein concentration ($P < 0.01$) and tended to affect milk protein concentration cubically ($P = 0.07$). Milk urea showed a cubic response ($P < 0.01$) to mRDC level. The SCC tended to decrease linearly with increasing mRDC ($P = 0.09$). Dietary mRDC level did not affect milk lactose concentration, efficiency (kg of milk/kg of DMI and kg of FPCM/kg of DMI), or BW change.

Rumen pH, described by the parameters B_0 (slope) and B_1 (inflection point) of the logistic curve of time spent below a certain pH, was not affected by mRDC level (Table 5, Figure 1). In agreement with this observation, measured mean pH and cumulative time

Table 5. Rumen pH data of lactating dairy cows offered diets with different levels of modeled rapidly degradable carbohydrates (mRDC)¹

Item	Treatment ²				SEM	P-value ³		
	mRDC 167	mRDC 181	mRDC 194	mRDC 208		L	Q	C
B_0 (slope)	6.8	6.8	6.0	5.7	1.1	0.40	0.87	0.82
B_1 (inflection point)	6.53	6.53	6.57	6.49	0.11	0.86	0.72	0.77
Measured mean pH	6.52	6.53	6.56	6.49	0.05	0.76	0.39	0.50
Measured cumulative time pH <5.8 (min/d)	20	9	23	46	26	0.47	0.53	0.90

¹Based on data of 4 rumen-fistulated animals per period.

²Treatments had dietary mRDC levels of 167, 181, 194, or 208 g/kg of DM.

³L = linear; Q = quadratic; C = cubic.

Table 6. Feeding behavior of lactating dairy cows offered diets with different levels of modeled rapidly degradable carbohydrates (mRDC)

Item	Treatment ¹				SEM	P-value ²		
	mRDC 167	mRDC 181	mRDC 194	mRDC 208		L	Q	C
Meal criterion (min)	32.5	31.8	24.4	35.3	3.4	0.94	0.07	0.07
Per day								
Meals (no.)	5.5	5.6	5.8	5.7	0.2	0.31	0.43	0.79
Total daily meal time (min)	266	252	251	269	9	0.83	0.02	0.82
Total daily eating time (min)	220	214	223	220	9	0.57	0.53	0.10
Per meal								
Visits (no.)	3.8	3.4	3.5	4.1	0.2	0.19	<0.01	0.91
Meal duration (min)	49.7	48.4	45.9	50.8	2.8	0.93	0.19	0.40
Eating time (min)	42.2	40.1	39.5	40.4	1.9	0.34	0.28	0.99
DMI (kg)	4.1	4.0	4.1	4.1	0.2	0.97	0.73	0.73
Rate of DMI (g/min eating time)	103	107	107	104	5	0.70	0.19	0.90

¹Treatments had dietary mRDC levels of 167, 181, 194, or 208 g/kg of DM.

²L = linear; Q = quadratic; C = cubic.

(min/d) of pH below 5.8 did not differ between treatments.

Although meal criterion tended to be affected quadratically and cubically by mRDC level ($P = 0.07$), the number of meals per day was not different (Table 6). Total daily meal time responded quadratically to mRDC level ($P = 0.02$), but total daily eating time was not affected. The number of visits per meal responded quadratically to mRDC level ($P < 0.01$). Meal duration, eating time, DMI, and rate of DMI per meal were not affected by mRDC level.

DISCUSSION

Diets formulated to be iso-energetic and iso-nitrogenous with increasing levels of mRDC resulted in significant production responses in this study. Milk production tended to be responsive to mRDC, and milk fat yield and FPCM were significantly influenced by mRDC, either quadratically or cubically. Additionally, milk protein yield and milk protein concentration linearly increased with increasing level of mRDC. Because calculated bypass starch and sugar content were almost

Figure 1. Effect of dietary modeled rapidly degradable carbohydrate (mRDC) level on measured time below a certain rumen pH ($\square$: 167 g of mRDC/kg of DM; $\blacklozenge$: 181 g of mRDC/kg of DM; $\bullet$: 194 g of mRDC/kg of DM; $\blacktriangle$: 208 g of mRDC/kg of DM) or described by a logistic curve (solid line: 167 g of mRDC/kg of DM, $B_0 = 6.8$, $B_1 = 6.53$; long-dash line: 181 g of mRDC/kg of DM, $B_0 = 6.8$, $B_1 = 6.53$; short-dash line: 194 g of mRDC/kg of DM, $B_0 = 6.0$, $B_1 = 6.57$; dotted line: 208 g of mRDC/kg of DM, $B_0 = 5.7$, $B_1 = 6.49$). The parameters describing the logistic curve are B_0 (indicating slope at the inflection point) and B_1 (indicating the inflection point), respectively. B_0 and B_1 are unaffected by the mRDC level ($P > 0.05$).

identical between treatments and mTDC only slightly increased with increasing mRDC, these results can mainly be attributed to differences in mRDC. Theoretically, iso-energetic and iso-nitrogenous diets should not result in differences in milk production response. However, the observed responses in our study can potentially be explained by (1) potential inaccuracies in the calculation of NE_L and MP supply, or (2) biological effects (such as effects on DMI, rumen degradation and fermentation, or metabolic effects) that are not captured by the concept of an accurately calculated NE_L and MP.

Potential Inaccuracies in the Calculation of NE_L and MP Supply

The NE_L system used for diet formulation is based on total-tract digestibility of ingested nutrients and does not take into account differences in rumen degradation and fermentation or the site of digestion between distinct carbohydrate fractions along the gastrointestinal tract. Diets in our study were formulated to have comparable bypass starch levels between treatments, and thus are not expected to result in differences in small intestinal glucose supply. Ruminal VFA concentrations were not determined in our study, but are expected to differ between treatments due to differences in ruminal carbohydrate degradation characteristics with changes in mRDC (Nozière et al., 2011). This in turn can affect milk production and composition (Thomas and Martin, 1988) even when the calculated NE_L content of diets is similar. For our study, this could possibly be an explanation for the differences observed especially in milk, FPCM, and milk fat production.

Observed effects on the increase in milk protein yield and concentration with increasing mRDC could be related to changes in microbial protein production. The DVE system of Tamminga et al. (1994) used for diet formulation assumes a fixed efficiency of microbial protein production per gram of fermented OM. However, Van Duinkerken et al. (2011) applied varying efficiencies for microbial protein production depending on the type of substrate degraded in the rumen. Although diets were formulated to similar DVE level, increasing starch at the expense of NDF in the diets with increasing mRDC might have improved the efficiency of microbial protein synthesis (Fanchone et al., 2013), resulting in an increased flow of microbial protein and AA to the small intestine.

Metabolic Effects

It can be expected that other mechanisms in addition to total energy or MP supply play a role in

lactating dairy cow performance. Rumen degradation and fermentation differ between carbohydrate fractions, resulting in changes in available energy and MP yielding nutrients, as well as influencing rumen health, metabolic pathways, and hormone levels (Nozière et al., 2011; Dijkstra et al., 2012; Arriola Apelo et al., 2014).

Arriola Apelo et al. (2014) reported that blood flow, mammary nutrient uptake, and subsequent milk protein synthesis are influenced by certain specific AA, energy substrates, and hormones. Similar to our own findings, milk protein yield and concentration increased in high-starch compared with high-NDF diets, as described by Piccioli-Cappelli et al. (2014) and Cantalapiedra-Hijar et al. (2015). These authors reported increased blood insulin concentration with high-starch feeding, which, together with branched-chain AA, may regulate mammalian target of rapamycin complex 1 (mTORC1) and milk protein synthesis (Arriola Apelo et al., 2014). Another possible explanation for the observed effects on milk protein is the observation by Cantalapiedra-Hijar et al. (2014) that starch-rich diets can result in a lower energy requirement of the portal-drained viscera, thereby saving AA for other purposes. Data on the AA sparing effect due to glucose availability in the small intestine, which in turn could result in higher milk protein yield, are controversial (Cantalapiedra-Hijar et al., 2014; Piccioli-Cappelli et al., 2014). For our study this is less relevant as calculated bypass starch content was comparable between diets.

As discussed by Cantalapiedra-Hijar et al. (2014), carbohydrate composition can change the use of N for milk protein production even when energy and N intake do not differ. Similarly, Rigout et al. (2003) concluded that differences exist in milk fat production and possibly in milk protein production between propionate and glucose, and that the nature of energy yielding nutrients should be taken into account in diet formulation. Huhtanen and Nousiainen (2012) demonstrated a quadratic effect of increased NFC content on milk and milk protein production, although the authors mentioned that the relevance of the response, even though significant, was small. However, NFC does not take into account differences in carbohydrate components in terms of rumen degradation, fermentation, and subsequent metabolites. In the overview of Huhtanen and Nousiainen (2012), the maximum response for milk protein yield occurred at higher NFC levels than for milk yield. This indicates that milk protein concentration continues to increase with higher NFC levels where milk yield already starts to decrease, which is in line with the increase in milk protein concentration and milk protein yield with increasing mRDC levels in our experiment.

Rumen pH Effects

Besides metabolic effects or potential inaccuracies in the calculation of NE_L and MP, differences in production performance can also be caused by changes in pH dynamics (Dijkstra et al., 2012). The level of mRDC did not affect rumen pH parameter estimates in our study. Average pH measured as mean pH or as the inflection point of the logistic curve was relatively high and well above the pH threshold of 6.0 to 6.3 given by Dijkstra et al. (2012), below which rumen fiber fermentation may be impaired. The slope of the logistic curve represents diurnal variation in pH. A higher slope indicates a more stable rumen fermentation process with less time in which pH is low or high (Colman et al., 2012). In our study, forage fiber was identical across treatments and NDF levels were relatively high. Forage NDF of the diets (228 g of NDF from forage per total diet DM) is well above the NRC (2001) recommendation of 190 g/kg of DM to support rumen health. Feeding increasing levels of mRDC up to 208 g/kg of DM, as in our study, should not challenge rumen stability. Based on the rumen pH parameter estimates, it is not expected that milk fat depression occurred due to changes in rumen fermentation. The increase in mRDC could have led to a lower concentration of acetate (Krause et al., 2003; Lechartier and Peyraud, 2011) or a higher formation of several *trans*-fatty acids in the rumen (Bauman and Griinari, 2003), which are associated with a lower milk fat production, which could potentially be an explanation for the observed decrease in milk fat yield and concentration with increasing mRDC from 181 mRDC onward.

DMI and Feeding Behavior

A decrease in pH due to increased diet fermentability could be compensated by a change in feeding behavior, resulting in more stable rumen conditions, as was shown by Krause et al. (2003). This compensation did not occur in our study as the number of meals, total daily eating time or eating time per meal were not affected by mRDC. Feeding behavior and DMI in lactating dairy cows can be affected by several dietary characteristics such as (forage) NDF content, particle size, or (ruminal degradable) starch content (Allen et al., 2009; Schadt et al., 2012; Ferraretto et al., 2013). Exchanging starch for nonforage fiber sources can result in an increased DMI (Abrahamse et al., 2008; Arndt et al., 2014; Boerman et al., 2015), decreased DMI (Lechartier and Peyraud, 2011), or no DMI response (Dann et al., 2014, 2015). Assuming that rumen fill was not limiting, a decreased NDF intake with increasing mRDC might be the reason

why DMI tended to linearly increase with increasing mRDC.

Eating time, as a component of feeding behavior, can be affected by particle size of ingested feed (mainly forage) because particles need to be masticated to a certain length before they can be swallowed (Schadt et al., 2012). As the forage base in our study was identical between treatments this is not expected to have influenced feeding behavior or DMI. Concentrates in our study were ground at 3 mm before pelleting so it can be expected that particle size was below the threshold given by Schadt et al. (2012) and that eventual differences between treatments were small, thereby not influencing eating time.

Propionate infused in the rumen of lactating dairy cows has shown different responses on DMI (Raggio et al., 2006; Stocks and Allen, 2012, 2014). Although DMI decreased in the studies of Stocks and Allen (2012, 2014), neither study observed effects on feeding behavior such as number of meals per day, meal duration, or DMI per meal. Propionate production or proportion in the rumen was not determined in our study, but it is likely that differences between treatments were not large enough to exert an effect on feeding behavior or DMI.

Applicability of mRDC in Diet Formulation

The observed responses to mRDC in our study, despite the fact that treatments were calculated to be iso-energetic and iso-nitrogenous, indicate that the amount of mRDC can have additional value next to NE_L and ME for controlling dairy cow performance. The model proposed for mRDC is relatively simple as compared with more complex rumen models such as Dijkstra et al. (1992). However, the present simple model still uses several fractional degradation and passage rate values. When formulating simple diets, an estimation of rumen degradable starch could lead to similar results as mRDC. However, when diets are more complex with several sources of carbohydrates [e.g., NDF from different feedstuffs (sugar beet pulp or soy hulls vs. forage fiber)] or high-sugar diets, just using degradable starch would not be sufficient. For the use of mRDC in diet formulation, rumen fractional degradation and passage rates, which are already available in feed matrices, can be used without the need for the user to adapt them. These rates can be fixed values or can be calculated with feedstuff-specific equations that use analyzed proximate nutrients as input, depending on the type of feedstuff or characteristic, as described by Van Duinkerken et al. (2011) and Volden (2011). With this approach, farm-specific feedstuffs only require an

analysis of the proximate nutrients to drive the model. This analysis can be done by laboratories that have developed NIRS calibrations specifically for this purpose.

CONCLUSIONS

Increasing dietary levels of mRDC without changes in other dietary characteristics, which include commonly used feed evaluation values such as NE_L and DVE, but also mTDC and bypass starch, showed significant effects on FPCM, milk fat and protein yield, and milk protein concentration. No effect on rumen pH parameter estimates and minor effects on feeding behavior were observed. Our results suggest that aggregating rapidly degradable carbohydrate content into one term mRDC may result in a simple formulation tool to improve predictability of production response in practical diet formulation.

ACKNOWLEDGMENTS

The authors acknowledge the staff at the dairy research farm Kempenshof (Boxmeer, the Netherlands) and J. Bikker (MS student, Wageningen University & Research, Wageningen, the Netherlands) for their contribution in the execution of the study and J. Doelman (Trouw Nutrition, Boxmeer, the Netherlands) for correcting the English version of the manuscript. The study was funded by Trouw Nutrition (Boxmeer, the Netherlands).

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APPENDIX

Table A1. For the calculation of modeled rapidly degradable carbohydrates, the contribution of rapidly degradable material per nutrient was calculated using fractional degradation and passage rates according to Van Duinkerken et al. (2011)¹

Parameter ² (/h)	Study	NDF	Starch	Sugar	ROM ³
k _d S	Present	NA ⁴	NA	3.0	NA
	Van Duinkerken	NA	NA	2.0	NA
k _p S	Present	NA	NA	0.12	NA
	Van Duinkerken	NA	NA	0.11	NA
k _d WS	Present	NA	0.375 + k _d D	NA	= 2.5 × k _d D
	Van Duinkerken	= k _d D	0.375 + 2 × k _d D	NA	= 2.5 × k _d D
k _p WS	Present	NA	0.09	NA	0.09
	Van Duinkerken	0.08	0.08	NA	0.08
k _p D concentrate	Present	0.03	0.06	NA	0.06
	Van Duinkerken	0.01855 + 0.1775 × k _d D	0.06	NA	0.01855 + 0.1775 × k _d D
k _p D corn silage	Present	0.0175	0.045	NA	0.045
	Van Duinkerken	0.0139 + 0.1775 × k _d D	0.045	NA	0.0139 + 0.1775 × k _d D
k _p D grass silage	Present	0.02	NA	NA	0.035
	Van Duinkerken	0.0139 + 0.1775 × k _d D	NA	NA	0.0139 + 0.1775 × k _d D
k _p D grass hay	Present	0.025	NA	NA	0.035
	Van Duinkerken	0.0139 + 0.1775 × k _d D	NA	NA	0.0139 + 0.1775 × k _d D

¹Minor modifications to some of these values have been made, and for comparison fractional rates used in the present study as well as fractional rates used by Van Duinkerken et al. (2011) are presented.

²S = soluble fraction; WS = washable – soluble fraction; D = nonwashable, potentially degradable fraction; k_d = fractional degradation rate; k_p = fractional passage rate.

³Residual OM [DM minus ash, CP, NDF, starch, sugar, crude fat, and fermentation acids (in silages only)].

⁴NA = not applicable.