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doi:10.1017/S1751731113002012

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Dietary carbohydrate composition modifies the milk N efficiency in late lactation cows fed low crude protein diets

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(Received 08 February 2013; Accepted 07 October 2013)

Nitrogen emissions from dairy cows can be readily decreased by lowering the dietary CP concentration. The main objective of this work was to test whether the milk protein yield reduction associated with low N intakes could be partially compensated for by modifying the dietary carbohydrate composition (CHO). The effects of CHO on digestion, milk N efficiency (milk N/N intake; MNE) and animal performance were studied in four Jersey cows fed 100% or 80% of the recommended protein requirements using a 4 × 4 Latin square design. Four iso-energetic diets were formulated to two different CHO sources (starch diets with starch content of 34.3% and NDF at 32.5%, and fiber diets with starch content of 5.5% and NDF at 49.1%) and two CP levels (Low = 12.0% and Normal = 16.5%). The apparent digestible organic matter intake (DOMI) and the protein supply (protein digestible in the small intestine; PDIE) were similar between starch and fiber diets. As planned, microbial N flow (MNF) to the duodenum, estimated from the urinary purine derivatives (PD) excretion, was similar between Low and Normal CP diets. However, the MNF and the efficiency of microbial synthesis (g of microbial N/kg apparently DOMI) were higher for starch v. *fiber diets. Milk and milk N fractions (CP, true protein, non-protein N (NPN)) yield were higher for starch compared with fiber diets and for Normal* v. *Low CP diets. Fecal N excretion was similar across dietary treatments. Despite a higher milk N ouput with starch* v. *fiber diets, the CHO modified neither the urinary N excretion nor the milk urea-N (MUN) concentration. The milk protein yield relative to both N and PDIE intakes was improved with starch compared with fiber diets. Concentrations of β-hydroxybutyrate, urea and Glu increased and those of glucose and Ala decreased in plasma of cows fed starch* v. *fiber diets. On the other hand, plasma concentration of albumin, urea, insulin and His increased in cows fed Normal compared with Low CP diets. This study showed that decreasing the dietary CP proportion from 16.5% to 12.0% increases and decreases considerably the MNE and the urinary N excretion, respectively. Moreover, present results show that at similar digestible OM and PDIE intakes, diets rich in starch improves the MNE and could partially compensate for the negative effects of Low CP diets on milk protein yield.*

Keywords: milk N efficiency, dairy cow, carbohydrate composition, dietary CP concentration, N pollution

Implications

The possibility of binding dairy incomes to environmentally friendly practices would add more interest to formulating diets with a high efficiency of nutrient utilization. A reduction of N emissions from dairy cows can be readily achieved by decreasing the CP concentration of diets. In order to minimize the negative impact of low N diets on milk protein yield, the efficiency of N utilization can be improved by replacing a percentage of dietary fiber by starch at iso-energetic concentrations. Lessening the dietary CP concentration and changing the carbohydrate composition can be two complementary strategies to improve the milk N efficiency.

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Introduction

Important measures to achieve the EU Water Framework Directive (European Union, 2000) include reducing N emissions from animal livestock. In ruminants, N intake has been identified as the principal driver of N excretion and so lowering the N intake reduces N excretion in feces and urines (Castillo *et al.*, 2000). This may necessitate feeding ruminants with low N diets, even lower than those recommended by the current feeding systems. However, this strategy is

detrimental to dairy performance. In this sense, one of the most promising ways to decrease N losses from dairy farms, with minimal detrimental effects on animal performance, is by improving the milk N efficiency (MNE; Kohn *et al*., 1997).

Although MNE is mainly driven by the dietary CP concentration (Huhtanen and Hristov, 2009), other covariates may influence the N partitioning, such as the dietary energy supply (Firkins and Reynolds, 2005) or the type of absorbed energy-yielding nutrients (Leiva *et al*., 2000). Results from literature concerning the effect of the dietary carbohydrate composition (CHO; starch *v.* fiber) on milk protein yield are variable and inconsistent (Kebreab *et al*., 2000; Khalili and Sairanen, 2000; Hristov and Ropp, 2003), likely because the effect of the CHO is often confounded with the total amount of energy consumed. However, in a recent meta-analysis study using a large Northern European data set (Huhtanen and Hristov, 2009), the effects of starch on MNE were significantly positive, whereas effects of NDF concentrations were negative at any given energy and protein intake.

Information about the impact of CHO on mitigation of urinary N excretion is scarce, and as far as we know no evidences exist whether the extent of the MNE improvement mediated by the CHO can be different when feeding cows below the recommended N requirements. Thus, the aim of this work was to test whether milk protein yield reduction associated to low N intakes could be partially compensated for by a higher MNE when dairy cows are fed diets rich in starch compared with diets rich in fiber at similar energy intakes.

Material and methods

The experiment was conducted according to the national legislation on animal care (Certificate of Authorization to Experiment on Living Animals, No. 004495, Ministry of Agriculture, France).

Animals, experimental design and diets

Four multiparous Jersey cows in late lactation, averaging 354 ± 23 kg of BW and 211 ± 13 days in milk at the onset of the experiment, were used in a 4 × 4 Latin Square design experiment, with a 2 × 2 factorial arrangement of dietary treatments. Cows were maintained in tie stalls. Each treatment was applied for 21 days, consisting of 15 days of animal adaptation to diets and 6 days of measurements.

Four diets (Table 1) were formulated to be iso-caloric on a net energy for lactation (NE_L) basis and with a constant 50 : 50 forage to concentrate ratio. The chemical composition of major feed and experimental diets are shown in Table 2 and Table 3, respectively. The dietary treatments varied in the dietary CHO (starch diets (35.0% starch and 32.0% NDF, in a dry matter (DM) basis) *v.* fiber diets (5.0% starch and 49.0% NDF, in a DM basis)) and the dietary CP (12.0% (Low) *v.* 16.5% (Normal), in a DM basis). Diets were formulated to meet 80% (Low CP) and 100% (Normal CP) of the protein to energy ratio recommended by the INRA feeding system ($PDIE/NE_L = 246$ g/MJ; INRA, 2007). Care was taken to ensure an optimum supply of rumen degradable N (PDIN)

Table 1 *Ingredient composition of diets*

	Low CP (12.0%)		Normal CP (16.5%)	
	Starch	Fiber	Starch	Fiber
Diet ingredient composition (% DM)				
Grass silage	12	30	5	33
Grass hay	16	4	8	9
Dehydrated corn plant pellets	22	16	28	8
Molassed straw pellets			9	
Concentrate	50	50	50	50
Supplements				
Mineral-vitamin premix (g/day)	200	200	200	200
Rumen protected methionine[1] (mg/kg DM)	308	431	524	769
Concentrate ingredient composition (% DM)				
Corn	56.0		60.0	
Barley	18.6			
Wheat			5.2	
Wheat bran	18.4		5.8	
Soybean hulls		45.6		51.2
Citrus pulp		14.2		
Dehydrated beet pulp		34.4		22.0
Tannin treated soybean meal	0.8		23.4	21.6
Urea	1.2	1.2	2.6	1.0
Vegegold[2]	0.8	2.6		2.2
Cane molasses	4.2	2.0	3.0	2.0

DM = dry matter.
[1]Smartamine M.
[2]Vegegold: hydrogenated palm fatty acids (palmitic acid 45.5%, stearic acid 45.5% and oleic acid 9%).

Table 2 *Chemical composition of major feed ingredients*

	Grass silage	Grass hay	Dehydrated corn plant pellets	Molassed Straw pellets	Low CP concentrate		Normal CP concentrate	
					Starch	Fiber	Starch	Fiber
Ingredient chemical composition (% DM)								
OM	88.8	90.6	97.3	93.6	96.1	92.6	96.2	93.3
CP	14.7	8.14	7.31	5.67	14.6	12.7	24.2	21.4
Effective degradability[1] (%)	71.3	58.0	62.4	73.3	87.6	74.3	76.9	63.2
Intestinal digestibility[2] (%)	70.4	77.6	70.9	70.1	80.5	71.2	83.9	75.6
NDF	59.6	59.9	39.6	71.9	14.1	44.8	11.0	41.9
ADF	35.4	32.3	18.7	41.3	4.58	26.3	4.19	27.1
ADL	4.89	5.41	1.16	6.78	0.388	1.61	0.448	1.59
Starch[3]	–	–	34.5	–	51.2	3.00	5.12	2.26
Ether extract[3]	2.1	1.2	2.3	2.0	4.5	3.0	3.9	3.3

DM = dry matter; OM = organic matter.
[1]Effective degradability analyzed according to the enzymatic procedure by Aufrère *et al.* (1991).
[2]Intestinal digestibility of rumen undegraded dietary CP according to the three step *in vitro* procedure by Calsamaglia and Stern (1995).
[3]From ingredients pooled from period 1 to 4 ($n = 1$).

Table 3 *Chemical composition and feed values of diets*

	Low CP (12.0%)		Normal CP (16.5%)		Main effects			
					CP		CHO	
	Starch	Fiber	Starch	Fiber	Low	Normal	Starch	Fiber
Diet chemical composition (g/kg DM)								
OM	946	921	954	919	933	937	950	920
CP	120	123	160	169	122	165	141	146
Effective degradability[1] (%)	78.8	71.5	71.4	65.2	75.1	68.4	75.2	68.3
Intestinal digestibility[2] (%)	77.4	71.4	80.9	73.8	74.3	77.4	79.2	72.6
NDF	325	490	308	492	409	401	316	491
ADF	158	280	154	296	219	225	156	288
ADL	18.9	26.6	18.2	29.8	22.8	23.9	18.5	28.2
Starch[3]	332	70	353	39	200	196	343	54
Ether extract[3]	32	25	26	26	29	26	29	26
NDF/Starch	0.98	7.0	0.87	12.6	4.00	6.74	0.93	9.8
Calculated feed values								
NE_L (Mcal/kg DM)	1.72	1.68	1.68	1.63	1.69	1.66	1.69	1.66
PDIE[4] (g/kg DM)	78.7	79.8	97.7	96.8	79.3	97.3	88.2	88.3
PDIN[4] (g/kg DM)	72.8	71.5	106	106	72.2	106	89.2	88.7
PDIN-PDIE (g/kg DM)	−5.9	−8.3	7.8	9.0	−7.1	8.4	1.0	0.4

DM = dry matter; OM = organic matter; NE_L = net energy for lactation.
[1]Effective degradability analyzed according to the enzymatic procedure by Aufrère *et al.* (1991).
[2]Intestinal digestibility of rumen undegraded dietary CP according to the three step *in vitro* procedure by Calsamaglia and Stern (1995).
[3]From ingredients pooled from period 1 to 4 ($n = 1$).
[4]PDIE, PDIN: protein digestible in the small intestine when rumen fermentable energy or nitrogen, respectively, are limiting.

with regard to the available rumen fermentable energy (PDIE) for Normal CP diets (INRA, 2007), with an intended small deficit for Low CP diets (within tolerable limits, PDIN–PDIE/NE_L > − 60 g/MJ; INRA, 2007) in order to benefit from the urea-N recycling into the rumen. Differences in CHO and CP concentration as well as in the supply of rumen degradable protein between dietary treatments were achieved by changing both the nature and the proportion of the ingredients in both the forage and in the concentrate fractions. Finally, diets were supplemented with rumen protected methionine (Smartamine M; Adisseo France SAS, Antony, France) in order for all diets to approach the balanced intestinal digestible lysine and methionine ratio of 3.0 (INRA, 2007). A standard commercial mineral–vitamin premix (Galaphos, Centraliment, Aurillac, France) was also included into the diet.

To prevent confounding the effects of CHO and the energy intake, the animals were fed fixed and limited quantities of rations throughout the experiment according to INRA (2007). Dietary allowances were calculated to meet 100% of the cow

NE_L requirements at the middle of the trial. As-fed quantities of grass silage were adjusted twice a week to reflect changes in DM content.

Because digestibility values from this experiment were intended to be used as an aid in interpreting data from a subsequent metabolic study involving multicatheterized cows fed the same diets in steady state conditions, animals were individually fed every 3 h, using automatic feeders, in eight equal portions (from 0800 h onwards), except for the grass silage and grass hay, which were separately distributed three times per day (25% at 0800 h, 25% at 1200 h and 50% at 1700 h). Water was freely available to all cows.

Measurements, sampling and analyses

Animals were weighed at the beginning and the end of each experimental period. Cows were milked twice a day at 0800 and 1600 h. The N balance and digestibility measurements were made on the last 6 days of each 3-week periods. From days 16 through 21 offered feed and refusals were weighed daily to determine feed intake. Total feces and urine output were measured daily. Feces were collected into wooden containers placed over the gutter behind each stall. Urine was collected using an external urine collector attached to the vulva of the cow by a tail support harness, into polyethylene containers previously filled with 1000 ml of 10% H_2SO_4 (vol/vol). Representative samples of individual feeds (200 g/day), refusals (25%), feces (1%) and urine (1%) were taken each day and frozen at −20ºC before analysis. Feed samples were pooled by period, whereas samples of refusals, feces and urines were composited over the 6-day sampling period by cow and treatment as they were obtained. In addition, on the last 3 days of the measurements daily weighed urine samples were diluted 10-fold with distilled water and stored at −20°C for PD analysis. Individual milk yield was recorded twice daily from days 16 through 21 and milk samples were collected at each milking on days 17 and 19 of each period.

Before analyses, feed, refusals and fecal samples were thawed, dried at 60ºC for 48 h and ground at 1 mm before DM, OM, NDF and ADF analyses. The Kjeldahl N content was determined on thawed fecal as well as urine subsamples and on dried and ground feed and refusal subsamples.

The DM, OM and N concentrations of samples were determined according to the Association of Official Analytical Chemists (AOAC, 2005). The ether extract was assessed for feed samples pooled over the entire experiment by extraction with petroleum ether (AOAC, 2005). The NDF and ADF analyses were performed by the sequential procedure of Van Soest *et al.* (1991) using the Ankom 200 Fiber Analyzer (ANKOM Technology Corporation, Macedon, NY, USA). Additionally, starch was analyzed in dried starch rich feeds pooled over the entire experiment as well as in refusals and feces from animals fed starch diets after grinding through a 0.5 mm sieve (Faisant *et al.*, 1995). The starch content was not measured for fiber diets as the theoretical starch concentration in feces was below the quantification limits of the analytical technique. *In vitro* N degradability (Aufrère *et al.*, 1991) as well as intestinal digestibility (Calsamiglia and Stern, 1995) were assayed in feed samples, composited over the entire experiment, as estimates of rumen effective degradability and intestinal digestibilty of rumen undegraded dietary protein, respectively. Urinary PD (allantoin and uric acid) were determined by liquid chromatography with diode array detection. Separation of PD was performed on a hydrophilic interaction liquid chromatography (HILIC) column (125 mm × 2 mm, 3 μm, Macherey Nagel, Hoerd, France), using a gradient solvent system at 0.5 ml/min (solvent A: 20 mM ammonium formate adjusted to pH 4 and solvent B: acetonitrile). The solvent program was as follows: the initial percentage of solvent A (10%) was maintained for 4 min, then raised to 60% in 10 min, lowered to 10% in 0.1 min and kept constant for 10 min to re-equilibrate the column before the next injection. After thawing, urine samples were diluted 1/5 in acetonitrile (Sigma-Aldrich, Taufkirchen, Germany) containing 20 μM of allopurinol (Sigma Chemical Co., St Louis, MO, USA) as internal standard. The samples were vortex-mixed for 1 min, centrifuged (10 000 × *g*, 10 min), an aliquot of the supernatant was transferred into a clean vial and 10 μl were injected into the liquid chromatography system. Allantoin and uric acid were detected at 218 and 254 nm, respectively. Quantification of PD in urine samples was performed by external calibration with artificial urines spiked with different mixed standard solutions to obtain five different concentrations of each metabolite (from 25 to 300 and 100 to 1200 μM for uric acid and allantoin, respectively). The peak area ratio (allantoin or uric acid to allopurinol) in urinary samples was used to calculate the metabolite concentration from the standard curves.

Individual ($n = 4$ per treatment and cow) fresh milk sub-samples, preserved with $K_2Cr_2O_7$ and stored at 4°C, were analyzed for fat, lactose and MUN concentration by IR analysis (CILAL Laboratory, Saint Genès Champanelle, France). Frozen (−20°C) milk sub-samples ($n = 4$) without preservatives were subsequently thawed, pooled ($n = 1$) by treatment and cow based on daily milk production, and thoroughly homogenized before being analyzed for total Kjeldahl-N and non-NPN concentrations as described elsewhere (Lemosquet *et al.*, 2009).

Jugular blood samples were taken on day 19 at 1100 h to determine plasma amino acids, albumin, urea, glucose, lactate, β-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA) and insulin as detailed before (Majdoub *et al.*, 2003; Savary-Auzeloux *et al.*, 2010).

Calculations and statistical analyses

Dietary PDIE and PDIN contents were calculated from the analyzed ingredients chemical composition and the *in vitro* estimates of the rumen effective degradability of CP and the intestinal digestibility of rumen undegraded dietary protein. The rumen outflow rate for effective CP degradability was set as 6%/h (INRA, 2007).

Microbial N flow (MNF) was estimated from the urinary PD according to the equations proposed by Chen and Gomes (1995), and using a value of 0.158 as the mean ratio of purine-N : total-N for mixed rumen bacteria measured in our

laboratory from an experiment using similar diets (Fanchone *et al.*, 2013). Milk component yield was calculated multiplying the average milk yield from days 16 through 21 ($n = 6$) by the average milk component composition measured at each milking on days 17 and 19 ($n = 4$). Energy-corrected milk (ECM) was calculated according to Reist *et al.* (2003).

Statistical analyses were carried out as a 4×4 Latin Square using the PROC MIXED of SAS v.9.2 (2008) according to the following model: $Y_{ijkl} = \mu + CP_k + CHO_l + \text{CP} \times CHO_{kl} + P_i + C_j + \varepsilon_{ijkl}$, where Y_{ijkl} is the dependent variable, μ is the overall mean, P_i is the fixed effect of the experimental period i, C_j is the random effect of cow j, CP_k is the fixed effect of dietary CP concentration (Low *v.* Normal), CHO_l is the fixed effect of dietary carbohydrate composition (starch *v.* fiber), $\text{CP} \times CHO_{kl}$ is the fixed effect of the two-way interaction between CP and CHO, and ε_{ijkl} is the random residual error. Effects were declared significant at $P \leq 0.05$ and a tendency was considered when $0.05 < P < 0.10$. In order to assess whether at similar total N and PDIE intake (general predictors) the milk N and milk CP yield were affected by the dietary CHO residuals from the regression equations were analyzed for the effect of the CHO by ANOVA (SAS v.9.2, 2008).

Results

Diet intake and digestibility and MNF

Animals consumed all feeds offered, except in the two last periods where the hot weather was responsible for partial refusals of grass silage (around 20%). Consequently, the DM and the calculated NE_L intakes were on average 3.6% ($P = 0.03$) and 2.6% ($P = 0.006$), respectively, lower for fiber diets compared with starch diets (Table 4). However, because the DM ($P = 0.07$) and OM ($P = 0.01$) digestibilities were on average 1.8 and 2.5 percentage units higher for the fiber diets compared with the starch diets, the digestible organic matter intake (DOMI) was similar ($P = 0.11$) across dietary CHO. The apparent CP digestibility was similar ($P = 0.87$) between starch and fiber diets, with no effects of the CHO on the apparent digestible CP ($P = 0.37$) or PDIE ($P = 0.13$) intakes. The NDF and ADF digestibilities were greater ($P < 0.01$) for the fiber compared with the starch diets. As a result of a higher fiber content and digestibility, the proportion of DOMI consumed as digestible NDF was double (50.7% *v.* 24.3% on average; $P < 0.01$) for fiber diets compared with starch diets. Starch digestibility was almost complete for starch diets (96.6% on average). The dietary CP

Table 4 *Effect of the dietary CP concentration (CP) and carbohydrate composition (CHO) on nutrient intake, digestibility and urinary purine derivative excretion*

	Low CP (12.0%)		Normal CP (16.5%)			*P*-values[1]						
								Main effects				
								CP		CHO		
	Starch	Fiber	Starch	Fiber	s.e.m.	CP	CHO	Low	Normal	Starch	Fiber	CP × CHO
Intake (kg/day)												
DM	13.9	13.1	13.9	13.7	0.14	0.18	0.03	13.5	13.8	13.9	13.4	0.16
Apparent digestible OM	9.37	9.15	9.73	9.35	0.232	0.12	0.11	9.26	9.54	9.55	9.25	0.61
Apparent digestible CP	1.00	0.87	1.61	1.66	0.068	<0.01	0.37	0.94	1.64	1.31	1.27	0.08
dNDF : dOM	0.247	0.499	0.239	0.515	0.0056	0.90	<0.01	0.373	0.377	0.243	0.507	0.15
NE_L[2] intake (Mcal/day)	21.1	20.0	20.9	20.4	0.13	0.66	<0.01	20.6	20.7	21.0	20.2	0.12
PDIE[2] intake (g/day)	1.092	1.060	1.359	1.336	14.9	<0.01	0.13	1.076	1.348	1.226	1.198	0.66
Apparent digestibility (%)												
DM	69.1	72.6	71.8	71.9	0.54	0.27	0.07	70.9	71.9	70.5	72.3	0.08
OM	71.2	75.2	73.4	74.4	0.69	0.34	0.01	73.9	73.2	70.5	72.3	0.07
CP	60.1	59.2	71.1	71.6	0.88	<0.01	0.87	59.7	71.4	65.6	65.4	0.46
NDF	50.7	71.2	51.4	71.6	2.72	0.61	<0.01	61.0	61.5	51.1	71.4	0.92
ADF	47.8	71.6	50.7	71.1	2.97	0.53	<0.01	59.7	60.9	49.3	71.4	0.38
Starch	96.5	–	96.8	–	0.77	0.50	–	96.5	96.8	96.7	–	–
Urinary PD excretion (mmol/day)												
Allantoine	219	178	223	200	9.8	0.25	0.02	199	212	221	189	0.40
Allantoine + Uric acid	251	207	252	232	11.1	0.28	0.03	229	242	252	220	0.33
Microbial N flow[3] (g/day)	137	110	138	125	5.7	0.35	0.02	124	132	138	112	0.48
MPSE (g N/kg digestible OM)	14.6	12.1	14.2	13.4	0.37	0.46	0.04	13.4	13.8	14.4	12.8	0.20

DM = dry matter; OM = organic matter; dNDF = digestible nutral detergent fibre; dOM = digestible organic matter; NE_L = net energy for lactation; PD = purine derivatives; MPSE = microbial protein synthesis efficiency.
[1]Effects: CP = dietary CP content; CHO = carbohydrate composition; CP × CHO = interaction between CP and CHO.
[2]Calculated feed values; PDIE = protein digestible in the small intestine when rumen fermentable energy is limiting.
[3]Calculated as reported by Chen and Gomes (1995).

Table 5 *Effect of the dietary CP concentration (CP) and carbohydrate composition (CHO) on milk composition and yield*

	Low CP (12.0%)		Normal CP (16.5%)			*P*-values[1]						
								Main effects				
								CP		CHO		
	Starch	Fiber	Starch	Fiber	s.e.m.	CP	CHO	Low	Normal	Starch	Fiber	CP × CHO
Milk yield (kg/day)	14.1	13.0	16.2	14.9	0.32	<0.01	0.01	13.5	15.5	15.1	13.9	0.82
Milk yield (kg/kg DMI)	1.03	1.00	1.15	1.10	0.043	0.04	0.41	1.01	1.13	1.09	1.05	0.78
ECM[2] (kg/day)	18.4	16.4	20.7	19.4	0.42	<0.01	< 0.01	17.4	20.1	20.0	17.9	0.36
ECM (kg/kg DMI)	1.33	1.25	1.48	1.42	0.011	<0.01	0.09	1.29	1.45	1.41	1.34	0.85
Milk composition (g/kg)												
CP (g/kg)	38.8	35.1	41.0	38.7	0.56	< 0.01	< 0.01	37.0	39.8	39.9	36.9	0.25
True protein (g/kg)	37.4	33.8	39.2	37.0	0.59	<0.01	<0.01	35.6	38.1	38.3	35.4	0.27
NPN (g/kg)	0.220	0.199	0.278	0.257	0.0079	<0.01	0.04	0.210	0.268	0.249	0.228	0.99
Urea-N (g/kg)	0.092	0.077	0.155	0.141	0.0178	<0.01	0.13	0.084	0.148	0.124	0.109	0.94
Lactose (g/kg)	52.9	54.0	52.1	52.7	0.39	0.04	0.05	53.5	52.4	52.5	53.4	0.59
Fat (g/kg)	60.3	58.0	56.4	60.8	2.17	0.82	0.66	59.2	58.6	58.4	59.4	0.18
Milk nutrients yield (g/d)												
CP (g/day)	547	456	663	573	15.2	<0.01	<0.01	502	633	605	529	0.33
True protein (g/day)	527	439	633	549	21.8	<0.01	<0.01	483	591	581	494	0.91
True protein (g/kg DMI)	38.0	33.4	45.6	40.1	1.47	<0.01	0.01	35.7	42.8	41.8	36.8	0.79
NPN (g/day)	3.11	2.59	4.49	3.83	0.234	<0.01	0.02	2.85	4.16	3.80	3.21	0.69
Lactose (g/day)	746	700	842	787	23.1	<0.01	0.06	724	814	793	744	0.84
Fat (g/day)	844	753	912	891	25.9	<0.01	0.07	799	902	879	822	0.23
Feed utilization (g/kg)												
DOMI/ECM	510	559	490	481	19.5	0.05	0.35	534	486	500	520	0.19
PDIE/ECM	59.5	64.7	66.0	68.8	1.48	0.01	0.03	62.1	67.4	62.7	66.8	0.45

DMI = dry matter intake; ECM = energy-corrected milk; NPN = non-protein N; DOMI = digestible organic matter intake; PDIE = protein digestible in small intestine.
[1]Effects: CP = dietary CP content; CHO = carbohydrate composition; CP × CHO = interaction between CP and CHO.
[2]ECM calculated = [(0.038 × g of crude fat + 0.024 × g of CP + 0.017 × g of lactose) × kg of milk]/3.14.

modified neither the dry matter intake (DMI; $P = 0.18$) nor the apparent DM and OM digestibilities ($P = 0.27$ and 0.34, respectively). The apparent CP digestibility was 11.8 percentage units higher ($P < 0.01$) for Normal compared with Low CP diets. Low CP diets resulted in similar digestibilities of NDF ($P = 0.61$), ADF ($P = 0.53$) and starch ($P = 0.50$) compared with Normal CP diets. Urinary PD (allantoine + uric acid) excretion was higher ($P = 0.03$) for starch diets *v.* fiber diets, and consequently the estimated MNF was around 17.0% (on an absolute basis, $P = 0.02$) or 13.2% (per unit of DOMI, $P = 0.04$) higher for the former. The dietary CP content had no effect on these parameters ($P = 0.28$ to 0.46).

Milk yield and composition

Milk yield and composition are presented in Table 5. Starch diets increased milk or ECM yield (+8 and +12%, respectively, $P \leqslant 0.01$) as well as milk CP, true protein and NPN yield (about +18%, $P \leqslant 0.02$) and concentrations ($P \leqslant 0.04$) compared with fiber diets. When data were expressed by DMI to account for the limited intake differences, there was still an effect of CHO on milk true protein yield (+14%, $P = 0.01$) but not on milk or ECM yield (+4 and +5%, respectively, $P > 0.05$). The MUN was not modified by the dietary CHO ($P = 0.13$). Finally, lactose and fat yield tended to be higher for starch *v.* fiber diets ($P = 0.06$ and 0.07, respectively) even though their concentrations were lower ($P = 0.05$) or not modified ($P = 0.66$), respectively.

Normal CP diets promoted greater milk yield (+15%, $P < 0.01$) as well as greater milk CP (+23%, $P < 0.01$), true protein (+22%, $P < 0.01$), NPN (46%, $P < 0.01$) yields and concentrations ($P < 0.01$) compared with Low CP diets. Both milk and milk true protein yield expressed by the DMI was still improved ($P \leqslant 0.04$) by increasing the dietary CP concentration. The MUN increased (+74%, $P < 0.01$) in cows fed Normal *v.* Low CP diets. Although milk lactose and milk fat yields were lower ($P < 0.01$, respectively) for cows fed the Low CP compared with Normal CP, the concentration of milk lactose was improved ($P = 0.04$), whereas milk fat percentage was unchanged ($P = 0.82$).

Efficiency of N and feed utilization

The N partitioning is presented in Table 6. As N intake increased ($P < 0.01$), urinary N excretion (+124%; $P < 0.01$) and milk N yield (+ 23%; $P < 0.01$) increased as well as the BW change ($P = 0.06$), but not the fecal N excretion ($P = 0.17$), which remained at an average of around 7.5 g fecal N/kg DMI. As expected, when data were expressed per unit of N intake, fecal N excretion and milk N yield decreased

Table 6 *Effect of the dietary CP concentration (CP) and carbohydrate composition (CHO) on N partitioning*

	Low CP (12.0%)		Normal CP (16.5%)			*P*-values[1]						
								Main effects				
								CP		CHO		
	Starch	Fiber	Starch	Fiber	s.e.m.	CP	CHO	Low	Normal	Starch	Fiber	CP × CHO
BW (kg)	353	360	356	351	8.9	0.73	0.94	356	353	354	355	0.56
BW change[2] (kg)	0.5	−4.2	4.5	12.5	2.5	0.06	0.74	−3.7	8.5	2.5	4.1	0.30
N intake (g/day)	268	235	362	370	7.2	<0.01	0.12	251	366	315	302	0.04
N output (g/day)												
Fecal N	107	98	105	104	3.2	0.17	0.49	102	105	106	101	0.20
Urinary N	62.2	54.9	128	133	3.9	<0.01	0.70	58.6	131	95.3	93.8	0.20
Milk N	85.6	71.3	104	89.7	2.4	<0.01	<0.01	78.5	96.7	94.7	80.5	0.94
N utilization (%)												
Fecal N/N intake	39.9	41.7	28.9	28.4	0.77	<0.01	0.47	40.8	28.6	34.4	35.0	0.18
Urinary N/N intake	23.1	23.3	35.5	35.8	0.97	<0.01	0.87	23.3	35.6	29.4	29.6	0.87
Milk N/N intake	32.3	30.5	28.6	24.3	1.27	0.01	0.07	31.3	26.4	30.3	27.4	0.33
Milk CP/PDIE intake	50.2	42.9	48.7	42.8	1.67	0.66	<0.01	46.5	45.8	49.4	42.9	0.68

BW = body weight; PDIE = protein digestible in small intestine.
[1]Effects: CP = dietary CP content; CHO = carbohydrate composition; CP × CHO = interaction between CP and CHO.
[2]BW change per period.

($P \leqslant 0.01$) with Normal *v.* Low CP diets while urinary N excretion increased ($P < 0.01$).

On the other hand, starch diets promoted similar N intakes than fiber diets ($P = 0.12$), but differences at Low CP level (+33 g of N) were greater and opposite (CP × CHO, $P = 0.04$) with regard to what happened at Normal CP level (−8 g of N). Starch diets increased milk N secretion (+18%; $P < 0.01$) compared with fiber diets, with no differences in fecal ($P = 0.49$) and urinary ($P = 0.70$) N excretion, and BW change ($P = 0.74$). Moreover, starch diets tended to increase ($P = 0.07$) the MNE (milk N/N intake) compared with fiber diets, without, however, any change in the urinary and fecal N excretion as a % of the N intake ($P = 0.47$ and 0.84, respectively). When milk N yield was regressed against the N intake (Figure 1a), the analysis of residuals showed that at a given N intake starch diets increased ($P < 0.01$) the milk N yield compared with fiber diets. Likewise, the milk CP yield (Figure 1b), positively correlated with PDIE intake ($r^2 = 50.1\%$, $P < 0.01$), was also higher ($P < 0.01$) with starch *v.* fiber diets.

Finally, regarding the feed utilization, Low CP diets needed lower ($P = 0.05$) energy (g of DOMI) and higher ($P = 0.01$) protein (g PDIE) intakes to produce 1 kg of ECM compared with Normal CP diets, whereas starch *v.* fiber diets decreased ($P = 0.03$) the amount of protein (g PDIE) needed to produce 1 kg of ECM.

Plasma metabolites

Starch diets promoted lower ($P < 0.01$) plasma glucose concentrations and higher BHBA ($P = 0.02$) and urea ($P = 0.01$) compared with fiber diets (Table 7). On the other hand, Normal CP diets showed higher albumin ($P = 0.04$), urea

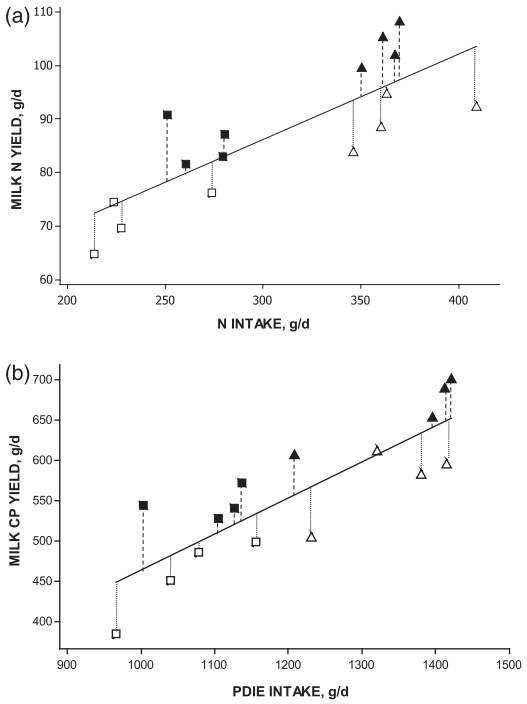

Figure 1 Milk N efficiency in late lactation Jersey cows fed the experimental diets: Low CP – starch diet (■), Low CP – fiber diet (□), Normal CP – starch diet (▲) and Normal CP – fiber diet (△). (a) Milk N yield according to the N intake and adjusted for animal and period effects (fitted equation is: milk N yield = 0.16 (s.e. = 0.043) × N intake + 38.22 (s.e. = 11.7); $r^2 = 47.2\%$; $P < 0.01$), (b) milk CP yield according to the protein digestible in the small intestine (PDIE) and adjusted for animal and period effects (fitted equation is: milk CP yield = 0.448 [s.e. = 0.115] × PDIE intake + 15.5 [s.e. = 140]; $r^2 = 50.1\%$; $P < 0.01$). Analysis of residuals from both equations shows that starch diets increased ($P < 0.01$) the milk N efficiency (milk N yield *v.* N intake and milk CP yield *v.* PDIE intake) compared with fiber diets.

Table 7 *Effect of the dietary CP concentration (CP) and carbohydrate composition (CHO) on plasma metabolites*

	Low CP (12.0%)		Normal CP (16.5%)			*P*-values[1]						
								Main effects				
								CP		CHO		
	Starch	Fiber	Starch	Fiber	s.e.m.	CP	CHO	Low	Normal	Starch	Fiber	CP × CHO
Plasma metabolites												
Glucose (mg/d;l)	48.7	58.2	48.5	60.9	1.77	0.68	< 0.01	53.5	54.7	48.6	59.5	0.63
Lactate (mM)	0.263	0.398	0.288	0.283	0.0271	0.32	0.17	0.331	0.285	0.275	0.341	0.14
BHBA (mM)	1.36	0.74	1.76	0.88	0.172	0.28	0.02	1.05	1.32	1.56	0.811	0.58
NEFA (mM)	60.2	68.6	75.0	60.2	6.66	0.84	0.84	64.4	67.6	67.6	64.4	0.46
Albumin (g/L)	43.9	42.4	46.4	45.7	0.68	0.04	0.33	43.2	47.0	45.2	44.0	0.73
Urea (mg/dl)	8.5	6.6	19.4	14.1	1.39	<0.01	0.01	7.6	16.8	13.9	10.4	0.14
Insulin (μIU/ml)	32.8	29.8	40.3	45.7	3.84	0.02	0.77	31.8	44.0	36.6	37.8	0.32
EAA[2] (μmol/L)	595	546	630	661	38.2	0.76	0.99	570	645	612	604	0.92
NEAA[3] (μmol/l)	1.593	1.478	1.541	1.429	64.4	0.21	0.34	1.535	1.485	1.567	1.455	0.54
Total AA[4] (μmol/l)	2.188	2.023	2.172	2.090	112.7	0.83	0.32	2.106	2.131	2.180	2.057	0.73
Ala[5] (μmol/l)	192	220	166	218	16.1	0.38	0.04	205	192	179	219	0.48
Glu[5] (μmol/l)	300	271	305	263	13.2	0.93	0.04	285	284	303	267	0.61
His[5] (μmol/l)	27.3	17.5	55.9	43.0	6.00	<0.01	0.11	22.4	49.5	41.6	60.5	0.80

BHBA = β-hydroxybutyrate; NEFA = non-essential fatty acids; EAA = essential amino acids; NEAA = non-essential amino acids.
[1]Effects: CP = dietary CP content; CHO = carbohydrate composition; CP × CHO = interaction between CP and CHO.
[2]EAA = His + Ile + Leu + Lys + Met + Phe + Thr + Tyr + Val.
[3]NEAA = total AA − EEA.
[4]Total AA does not include tryptophane.
[5]Only individual AA showing statistical ($P > 0.05$) differences are shown.

($P < 0.01$) and insulin ($P = 0.02$) concentrations than Low CP diets. Total AA plasma concentrations were similar across dietary CHO ($P = 0.32$) and CP concentrations ($P = 0.83$). However, starch diets promoted lower ($P = 0.04$) Ala and higher ($P = 0.04$) Glu concentrations than fiber diets, whereas Normal CP diets showed greater concentrations for His ($P < 0.01$) than Low CP diets. Concentration of individual AA other than Ala, Glu and His did not change across treatments ($P > 0.05$; data not shown).

Discussion

Our data support the general concept that a decrease in the dietary CP content (from 16.5% to 12.0%) improves MNE (Huhtanen and Hristov, 2009), decreasing the urinary N excretion by more than 50%. However, the fall in milk (−2 kg/day) and milk protein yield (−108 g milk true protein/day; −18%) associated with Low CP diets might preclude the use of low protein diets for production purposes. Thus, the question arises: does any improvement in MNE necessarily imply a reduction in milk protein yield? As pointed out by Huhtanen and Hristov (2009) and Calsamiglia *et al.* (2010) who characterized EU and US dairy diets from a large data set, it appears that under certain feeding conditions a higher MNE is compatible with higher milk production. The strategy followed in the present experiment was to modify the dietary CHO as a means to further improve the high MNE usually found at low CP levels. In this situation, our results indicated that diets rich in starch improved milk protein yield compared with diets rich in fiber, with a tendency for starch diets to promote higher MNE than fiber diets.

Although we did not find a significant CP × CHO interaction on milk protein yield and MNE, likely because of the low number of animals, some results support the concept that CHO (starch *v.* fiber) may behave different at low and high dietary CP levels (Sloan *et al.*, 1988). MNF was greater with starch compared with fiber diets in agreement with other studies conducted in dairy cows (Keady *et al.*, 1998; Broderick, 2003; Fanchone *et al.*, 2013) as well as to predictions of the revised Dutch protein evaluation system (DVE/OEB_{2010}; Van Duinkerken *et al.*, 2011). Nevertheless this improvement was more than double at Low *v.* Normal CP diets (24.5% *v.* 10.5%). Preliminary published results from a metabolic study using these same diets (Cantalapiedra-Hijar *et al.*, 2013) showed that the net portal absorption of essential AA was 20% higher for starch *v.* fiber diets at Low CP, whereas similar at Normal CP levels. A significant lower OM digestibility has been reported in the literature (Brandt *et al.*, 1981; Doreau *et al.*, 1990; Peyraud *et al.*, 1997) as the rumen protein balance become negative (higher N flowing to the duodenum compared with N intake). The OM digestibility in our study was significantly depressed with starch compared with fiber diets, in line with a higher microbial protein synthesis, but this reduction was higher at Low when compared to Normal CP level (−4.0 *v.* −1.0 percentage

points, respectively; $P = 0.07$). This reinforces the idea that microbial protein synthesis and consequently the rumen protein sparing effect of starch diets compared with fiber diets is higher at protein deficient diets than with Normal CP levels. Finally, some data on milk performances also support this synergy between Low protein and starch diets. For instance, the difference in feed utilization found in our study between fiber and starch diets was numerically higher at Low *v.* Normal CP levels (+9.6% *v.* −1.8% for DOMI/ECM and +8.7% *v.* +4.2% for PDIE/ECM, respectively).

Results from literature concerning the effect of CHO on milk protein yield are inconsistent (Kebreab *et al.*, 2000; Khalili and Sairanen, 2000; Hristov and Ropp, 2003), likely because: (i) the effect of the CHO is often confounded with the total amount of energy and protein consumed or (ii) variations of CHO composition was not high enough to detect biological or statistical differences. In the present experiment diets were formulated with three objectives: (1) to ensure iso-caloric feeding levels across the four treatments, (2) to ensure similar N intakes within each CP level and (3) to promote an intake of carbohydrates highly different in composition between starch and fiber diets. Although small refusals with fiber diets were found, all four diets were iso-energetic on an ME basis ($ME = 15.58 \times DOMI$; Agricultural Research Council (ARC) 1984) and iso-PDIE within each dietary CP level. As intended, the digestible NDF to DOMI ratio differed widely between fiber and starch diets, suggesting different rumen fermentation profile and energy-yielding nutrient absorption (Nozière *et al.*, 2010). Finally, although the particle size of feed ingredients (not measured in this study) could impact the rumen fermentation through changes in rumen pH, the steady feeding pattern imposed in this experiment (eight meals per day) may have prevented big differences across diets. Our results showed that at similar energy (digestible OM) and protein (PDIE) intakes, diets rich in starch improve milk protein yield, in agreement with others (Sutton *et al.*, 1993; Keady *et al.*, 1998; Kebreab *et al.*, 2000) and increased the MNE (Figure 1a) compared with diets rich in fiber. Conversely, results by Van Knegsel *et al.* (2007) as well as Hristov and Ropp (2003) comparing glucogenic *v.* ketogenic diets in early and late lactation dairy cows, respectively, did not find any differences in milk protein yield ascribed to the CHO. When the limited number of observations from the present study were combined with data from two other INRA-trials (total number of observations = 48) sharing the same iso-NE_L treatment effects (CP level and CHO) the MNE was found to be significantly ($P < 0.01$) improved by 8.6% (Cantalapiedra-Hijar *et al.*, 2012) with diets rich in starch compared with fiber, in agreement with larger data analysis (Northern European data set in Huhtanen and Hristov, 2009). The uniqueness of our study is that this effect was present even when cows were fed diets supplying protein below requirements (INRA, 2007).

Whether the positive effects of the dietary CHO composition on milk protein yield may partially arise from metabolic adaptations has long been a matter of debate. In the present experiment, both digestive and metabolic mechanisms may be involved. At similar DOMI, the conversion of the protein digestible in the small intestine (PDIE) into milk CP is improved by starch compared with fiber diets (Figure 1b), which could indicate either a higher metabolic efficiency of AA utilization or a different true protein supply non predicted by the actual INRA feeding system or a combination of both. Leiva *et al.* (2000) suggested that differences in the type and quantity of absorbed metabolizable nutrients provided by starch *v.* fiber diets may explain differences in MNE in dairy cattle. Plasma metabolite concentrations are indicators of the relative glucogenic *v.* ketogenic potentials of rations, even if some are not fully discriminant (Van Knegsel *et al.*, 2005). Unexpectedly (see review by Van Knegsel *et al.*, 2005), glycemia was reduced for the starch diets, whereas plasma BHBA and insulin levels were increased and not modified, respectively. Recent results from a metabolic trial using identical diets on multi-catheterized Jersey cows (Cantalapiedra-Hijar *et al.*, 2013) showed that net portal absorption of glucose was higher for starch compared with fiber diets. In this situation, the lower glycemia observed in the present study with starch compared with fiber diets could indicate a higher utilization of glucose by both the splanchinc and the peripheral tissues with starch diets. On the other hand, in absence of increased mobilization of adipose tissues, as suggested by measured plasma NEFA concentrations and the calculated energy balance, the higher plasma BHBA concentrations likely arise from increased butyrate absorption and conversion to BHBA in the rumen wall (Kristensen *et al.*, 2000). Because animals were fed every 3 h at restricted intake levels, the possibility of a subacute butyric acidosis associated with starch diets (Eadie *et al.*, 1970) should be considered here. Results from the aforementioned metabolic trial (Cantalapiedra-Hijar *et al.*, 2013) confirmed an almost twofold higher butyrate net portal absorption for starch compared with fiber diets.

Other metabolite data, limited to the systemic circulation where concentrations are the result of supply minus utilization, give some hints of metabolic adaptations without allowing a detailed interpretation. The fact that plasma concentrations of Glu and Ala, both playing important roles in N and carbon shuttles in the body, were affected by the dietary CHO suggest that the different type of absorbed energy-yielding nutrients between starch and fiber diets could interact with the AA utilization at the organ and tissue levels (Cantalapiedra-Hijar *et al.*, 2013). In this sense, both the duodenal infusion of glucose and the ruminal infusion of C3 in dairy cows fed diets based on grass silage were shown (Lemosquet *et al.*, 2009) to decrease Ala and increase Glu plasma concentrations, as in our study.

Despite the higher secretion of N into milk with starch diets (Figure 1a), the urinary N excretion did not decrease compared with fiber diets, in accordance with a similar MUN (Kohn *et al.*, 2002). This unexpected finding disagrees with most published results in the literature, where an increase in milk N output is generally associated with a decrease in urinary N excretion (Kebreab *et al.*, 2000; Broderick, 2003). The fact that in our study animals were in late lactation could have favoured a different pattern of nutrient partitioning

compared with studies using early to mid lactation dairy cows. However, experimental errors and N losses associated with the 24-h urine collection (Spanghero and Kowalski, 1997) should not be rejected.

This study confirms that the manipulation of the dietary CP concentration is an adequate strategy for increasing MNE in dairy cows, and thus to potentially decrease the urinary N excretion. The decline in milk and milk protein yield observed at Low CP content (12.0%) might preclude the general use of this strategy for production purposes. However, present results show that at similar digestible OM and protein (PDIE) intakes, diets with greater amount of starch improve the MNE compared with diets rich in fiber while increasing milk production, and thus, partially compensate the negative effects of low dietary CP content on milk production. Further investigation on the mechanisms actually involved in this improvement is warranted.

Acknowledgments

This study was granted by the Commision of the European Communities; project FP7-KBBE-2007-1 'Rednex'. G. Cantalapiedra-Hijar gratefully acknowledges the receipt of a scholarship from PHASE department (INRA). The authors thank Adisseo (Commentry, France) for financial support to conduct the study. They also thank C. Cossoul, A.S. Bage, J. Fernández, T. Garcia as well as the experimental unit "Les Cèdres" for technical assistance.

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