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Diets rich in starch increase the posthepatic availability of amino acids in dairy cows fed diets at low and normal protein levels

G. Cantalapiedra-Hijar,^{*1} S. Lemosquet,^{†‡} J. M. Rodriguez-Lopez,^{*} F. Messad,^{*} and I. Ortigues-Marty^{*}

^{*}Institut National de la Recherche Agronomique, UMR 1213 INRA-VetAgroSup, Unité Mixte de Recherches sur les Herbivores, 63122 St Genès Champanelle, France

[†]INRA, UMR 1348 PEGASE, F-35590 Saint-Gilles, France

[‡]Agrocampus Ouest, UMR1348 PEGASE, F-35000 Rennes, France

ABSTRACT

Five mid-lactation multicatheterized Jersey cows were used in a 4 × 4 Latin square design to investigate whether the increase in milk N yield associated with diets rich in starch versus fiber could originate from changes in the splanchnic AA metabolism and if these changes depended upon the dietary crude protein (CP) content. Four isoenergetic diets were formulated to provide 2 different carbohydrate compositions [diets rich in starch (350 g of starch and 310 g of neutral detergent fiber/kg of dry matter) versus rich in fiber (45 g of starch and 460 g of neutral detergent fiber/kg of dry matter)] crossed by 2 different CP contents (12.0 vs. 16.5% CP). At the end of each treatment period, 6 hourly blood samples were collected from the portal and hepatic veins as well as the mesenteric artery to determine net nutrient fluxes across the portal drained viscera (PDV), liver, and total splanchnic tissues. Dry matter and calculated energy intake as well as total absorbed energy were similar across treatments. However, the net portal appearance (NPA) of acetate, total volatile fatty acids, and β-hydroxybutyrate were higher with diets rich in fiber versus starch, whereas that of oxygen, glucose, butyrate, and insulin were lower. Concomitant to these changes, the percentage of N intake recovered as total AA (TAA) in the portal vein was lower for diets rich in fiber versus starch (42.3 vs. 51.4%, respectively), without, however, any difference observed in the NPA of the main AA used as energy fuels by the PDV (Glu, Gln, and Asp). Despite a higher NPA of TAA with starch versus fiber diets, no differences in the net hepatic flux of TAA, essential and nonessential AA were observed, resulting in a higher (+22%) net splanchnic release of AA and, hence, a greater (+7%) milk N yield. The net hepatic flux and hepatic fractional removal of none of the individual AA was affected as the main carbohydrate changed from fiber to starch, except for Gly and

Lys, which were higher for the latter. After correcting for differences in NPA of TAA, the net hepatic uptake of TAA tended to be lower with starch versus fiber diets. The higher transfer of N from feed to milk with diets rich in starch is not the consequence of a direct sparing AA effect of glucogenic diets but rather the result of lower energy requirements by the PDV along with a higher microbial N flow to the duodenum. A better AA use by peripheral tissues with starch versus fiber diets was also hypothesized but more studies are warranted to clarify this issue.

Key words: milk N efficiency, splanchnic metabolism, amino acid, energy source

INTRODUCTION

The efficiency of dietary N conversion into milk N is of particular concern for the dairy industry, because it affects profit margins and has an impact on environmental N pollution (Castillo et al., 2001). At similar energy and N intakes, diets rich in starch have been shown by meta-analysis to slightly improve milk N efficiency in dairy cows compared with diets rich in fiber (North European data set in Huhtanen and Hristov, 2009). Similarly, we have recently shown that high-starch diets improved the milk N efficiency compared with high-fiber diets, especially when they were formulated at low (12.0% CP) compared with normal (16.5% CP) dietary N levels (Cantalapiedra-Hijar et al., 2014). These findings suggest that the dietary carbohydrate (CHO) composition has an effect on the transfer of N from feed into milk beyond the current energy and protein feed values, but little evidence exists in the literature on the mechanism that may be involved.

The splanchnic tissues [i.e., portal drained viscera (PDV) and liver], as a result of their many important functions and their high rate of metabolism, have high requirements for AA, which may limit the systemic availability of absorbed AA to the peripheral tissues (Reynolds, 2006). Anatomically, the splanchnic tissues are positioned between AA intestinal absorption and

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¹Corresponding author: gonzalo.cantalapiedra@clermont.inra.fr

utilization by peripheral tissues. Hence, diet-induced differences in milk N efficiency may originate from splanchnic adaptations.

A mechanism traditionally evoked to explain the higher efficiency of N use with starch has been a sparing effect of glucogenic nutrients on AA utilization by the gastrointestinal tract (Nocek and Tamminga, 1991; Freetly et al., 2010) and liver (Danfær et al., 1995), the spared AA being available for peripheral tissues. Other metabolic adaptations could be involved, such as lower energy expenditure and protein turnover of the gastrointestinal tract as the proportion of dietary fiber decreases (Reynolds et al., 1991a) or lower splanchnic AA catabolism mediated by changes in the hormonal status promoted by glucogenic diets (Brockman, 1990). Recently, no sparing effect of glucogenic nutrients on splanchnic AA removal was demonstrated when using high-protein diets (18–19% CP) in postpartum transition dairy cows (Larsen and Kristensen, 2012). However, data obtained in monogastrics showed a reduction in the splanchnic AA oxidation at the expense of other energy substrates when animals were fed diets at low compared with high N levels (van der Schoor et al., 2001), suggesting that the sparing effect of glucogenic nutrients on AA catabolism could be enhanced when the supply of N is limited.

We hypothesized that increasing the gastrointestinal absorption of glucogenic nutrients in dairy cows by feeding diets rich in starch would (1) decrease the net AA removal by the PDV and liver, (2) increase the net posthepatic availability of absorbed AA, and (3) increase the milk protein secretion compared with the absorption of ketogenic nutrients from fiber-rich diets, and that this effect would be more evident at low than at normal dietary N levels. Thus, the aim of this study was to evaluate the effects of the CHO (starch vs. fiber) on nutrients appearing in the portal circulation, their net hepatic uptake and milk performances in dairy cows fed isoenergetic diets at low (12.0% CP) versus normal (16.5% CP) N levels.

MATERIALS AND METHODS

The experiment was conducted in respect of the national legislation on animal care (Certificate of Authorisation to Experiment on Living Animals, no. 004495; Ministry of Agriculture, Paris, France).

Animals, Experimental Design, and Dietary Treatments

Five multiparous Jersey cows in mid lactation, averaging 365 ± 28 kg of BW and 78 ± 12 DIM at the onset of the experiment, were used in a 4×4 Latin square

design, with the fifth cow used as an extra observation. Chronic indwelling catheters were surgically implanted before the lactation peak and at least 5 wk before the beginning of the experiment. The surgical procedure was adapted from that described by Ortigues et al. (1994) in sheep. Briefly, polyvinyl chloride catheters (Dow Corning Corp., Midland, MI) were implanted into the portal vein (i.d. = 1.5 mm; o.d. = 2.5 mm) by a transhepatic surgical approach, as well as into 2 mesenteric arteries (i.d. = 1.1 mm; o.d. = 1.9 mm), 1 ruminal and 1 mesenteric vein (i.d. = 1.1 mm; o.d. = 1.9 mm) and the hepatic vein (i.d. = 1.5 mm; o.d. = 2.5 mm). The surgery was conducted aseptically under general anesthesia using an oxygen-isoflurane mixture (98% oxygen and 2% isoflurane). Catheter maintenance was carried out twice per week through physiological sterile saline containing 200 IU of heparin. Cows were housed in tie-stalls lighted 24 h daily and milked twice daily in their stall at 0600 and 1700 h. Cows were assessed and treated for pain after the surgical intervention as detailed by Paulmier et al. (2012).

Four iso-NE_L diets were formulated (INRA, 2007) according to a 2×2 factorial arrangement of treatments to test the effects of dietary CP content [low (12.0%) and normal (16.5%), meeting 80 and 100%, respectively, of the Institut national de la recherche agronomique (INRA) recommendations (246 g of protein truly digested in the small intestine/MJ; INRA 2007)] and CHO (350 g of starch and 310 g of NDF/kg of DM for the starch diets, and 45 g of starch and 460 g of NDF/kg of DM for the fiber diets, respectively). Diet composition (Table 1) was the same used in a previous feeding trial (Cantalapiedra-Hijar et al., 2014) and so the chemical composition (Table 2) was almost identical. Diets were supplemented with rumen-protected Met (Smartamine M; Adisseo France SAS, Antony, France) in order for all diets to approach the balanced intestinal digestible Lys and Met ratio of 3.0 (INRA, 2007).

Animals were fed fixed and limited quantities of rations throughout the experiment to achieve isoenergetic and nitrogenous intakes across dietary treatment. Dietary allowances were calculated to meet 100% of the cow average net energy requirements at the middle of the trial as detailed elsewhere (Cantalapiedra-Hijar et al., 2014). Each experimental period lasted 28 d. To minimize postprandial variations, feeds (except grass hay) were hand mixed and offered from automated feeders in equal meals every 3 h from d 1 through 24 and hourly from d 24 to 28. Grass hay was separately distributed 3 times per day (25% at 0800 h, 25% at 1200 h, and 50% at 1700 h). Water was freely available to all cows. Feeds offered were weighed daily and the DM content of feeds was analyzed twice weekly to

Table 1. Ingredient composition of diets

Composition	Low CP (12.0%)		Normal CP (16.5%)	
	Starch	Fiber	Starch	Fiber
Diet ingredient composition, % of DM				
Grass silage ¹	12	30	5	33
Grass hay ¹	16	4	8	9
Dehydrated corn plant pellets ¹	22	16	28	8
Molassed straw pellets ¹			9	
Concentrate	50	50	50	50
Supplement				
Mineral-vitamin premix, g/d	200	200	200	200
Rumen protected methionine, ² mg/kg of DM	308	431	524	769
Concentrate ingredient composition, % of 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, ³ g/kg	0.8	2.6		2.2
Cane molasses, g/kg	4.2	2.0	3.0	2.0

¹Forage chemical composition on a DM basis: grass silage (OM = 88.7%, CP = 15.4%, NDF = 46.7%, and ADF = 29.4%), grass hay (OM = 91.6%, CP = 7.38%, NDF = 64.1%, and ADF = 36.4%), dehydrated corn plant pellets (OM = 97.1%, CP = 7.04%, NDF = 40.8%, ADF = 19.9%, and starch = 36.5%), and molassed straw pellets (OM = 93.3%, CP = 5.75%, NDF = 70.9%, and ADF = 42.0%).

²Smartamine M (Adisseo France SAS, Antony, France).

³Hydrogenated palm FA (45.5% palmitic acid, 45.5% stearic acid, and 9% oleic acid; Sonap, Arzacq, France).

ensure that the DM offered was constant throughout the study.

Measurements, Sampling, and Laboratory Analysis

Net nutrient fluxes were measured on d 27 of each 4-wk period. To account for the time lag between intake, nutrient absorption, and milk secretion, feed intake was measured on d 26 and 27, whereas milk yield and milk N composition were measured on d 27 and 28 of each 4-wk period.

Feed Intake, N Partitioning, and Analysis. Individual feeds were sampled on d 26 and 27, a subsample of each was immediately analyzed for DM content (103°C for 48 h), and the rest was pooled by period and frozen at -20°C before chemical analyses. To determine feed intake around the blood sampling, refusals, if present, were weighed daily, sampled from d 26 through 27, and individually stored at -20° before analyses. Ingredients and refusals samples were treated and analyzed for DM, OM, N, NDF, ADF, starch, and ether extract as detailed by Cantalapiedra-Hijar et al. (2014). The ingredient composition of refusals was also assessed by hand separation to estimate feed values of the intake [energy (NE_L) and protein (MP) contents]. In addition, feed samples were ground through a 0.5-

mm screen and analyzed for their AA content using HPLC (Alliance System; Waters SAS, Guyancourt, France), as described by Haque et al. (2013). Milk was sampled at each milking on d 27 and 28 and stored without preservative for Kjeldahl N analysis (n = 4 per treatment and cow).

Blood Sampling and Analysis. On d 27, a sterile solution of para-aminohippuric acid (pAH; 100 g/L; Sigma Chemical Co., Poole, UK) was infused into the mesenteric and ruminal catheters (Ortigue et al., 1994) for 7 h (1030 to 1630 h) at an identical rate of 3.6 g/h (overall rate of 7.2 g/h). Blood was collected hourly (from 1130 to 1630 h; 30 min before and after each meal) from the mesenteric artery, portal vein, and hepatic vein into 9-mL tubes containing lithium heparin (5 IU/mL of blood) to determine glucose, lactate, BHBA, individual AA, VFA, oxygen, and pAH concentrations, and EDTA-K (10 µL/mL of blood) to determine urea-N, ammonia-N, triglyceride, NEFA, and insulin [previously treated with aprotinin (Trasyol; Boehringer Ingelheim Canada Ltd., Dorval, QC, Canada)] concentrations. Blood samples were kept on ice immediately after collection. Hematocrit was determined by centrifugation at 15,000 × g for 2 min at room temperature. Whole blood was aliquoted into 1.5-mL screw-cap Eppendorf tubes, frozen with liquid N₂, and stored at

Table 2. Chemical composition and feed values of diets

Item	Low CP (12.0%)		Normal CP (16.5%)	
	Starch	Fiber	Starch	Fiber
Diet chemical composition, g/kg of DM				
OM	950	928	951	921
CP	118	121	160	169
NDF	317	467	305	458
ADF	156	287	154	285
Starch ¹	334	59	357	29
Ether extract ¹	32	25	28	26
Feed value, /kg of DM				
NE _L , MJ	7.15	7.02	7.06	6.95
PDIE, ² g	82.5	85.0	96.5	98.2
AADI, ³ % of PDIE				
AlaDI	6.9	6.8	6.7	6.7
ArgDI	4.7	4.6	4.8	4.7
AspDI	11.0	11.1	10.9	11.1
GluDI	13.6	13.1	14.1	13.4
HisDI	2.0	2.0	2.0	2.1
IleDI	5.4	5.4	5.3	5.3
LeuDI	8.6	8.5	8.7	8.5
LysDI	7.1	7.2	6.9	7.1
MetDI ⁴	2.3	2.4	2.5	2.6
PheDI	5.1	5.1	5.1	5.1
ProDI	4.6	4.6	4.6	4.7
SerDI	4.7	4.8	4.8	4.9
ThrDI	5.3	5.3	5.2	5.2
TyrDI	4.4	4.6	4.4	4.4
ValDI	5.8	6.0	5.7	5.8

¹From ingredients pooled from period 1 to 4 (n = 1).

²PDIE = protein digestible in the small intestine when rumen fermentable energy is limiting.

³AADI = AA digestible in the small intestine; 1 g of AADI is equal to 1 g of PDIE.

⁴The percentage MetDI was estimated after Smartamine M (Adisseo France SAS, Antony, France) supplementation. The MetDI from the basal diet was 2.0 and 1.9%, for starch and fiber diets at low CP levels, respectively, and 1.9 and 1.8% for starch and fiber diets at normal CP levels, respectively.

–80°C for later determination of VFA (Reynolds et al., 1988) and hemoglobin using a commercial kit (Biolabo SA, Fismes, France). The blood partial pressure of O₂ and pH were analyzed within 5 min after sampling by an automatic analyzer (ABL700 Blood Gas Analyzer; Radiometer A/S, Copenhagen, Denmark). Heparinized blood samples were deproteinized (1:2, vol/vol) with perchloric acid (0.6 M), centrifuged at 4,500 × g for 15 min at –4°C, and the supernatant fraction stored at –20°C for further analyses of glucose, L-lactate, and BHBA as detailed by Cantalapiedra-Hijar et al. (2014). The remainder of heparinized blood was separated by centrifugation (1,200 × g for 15 min at 4°C) to obtain the plasma and kept at –20°C before analysis. The individual AA plasma concentration was assessed by isotopic dilution as described by Kraft et al. (2011). The EAA analyzed were His, Ile, Leu, Lys, Met, Phe, Thr, Tyr, and Val (n = 9); and the NEAA analyzed were Ala, Asn, Asp, Gln, Glu, Gly, Pro and Ser (n = 8). Total AA (**TAA**) was the sum of EAA and NEAA. Plasma pAH was deacetylated (Rodríguez-López et al., 2014) and analyzed as described by Isserty et al. (1998). Blood from EDTA tubes were kept at –20°C for

urea-N analysis (by a diacetyl monoxime direct assay; Ormsby, 1942) and ammonia-N determination (Ngo et al., 1982) within the first 18 h after sampling. Plasma obtained from the remainder of EDTA-blood was used for insulin (RIA; MP Biomedicals, Illkirch, France), NEFA (C WAKO kit; Unipath SA, Dardilly, France), and triglyceride (kit by Sobioda laboratories, Grenoble, France) determinations.

Calculations and Statistical Analysis

Plasma flows across the PDV were calculated from downstream dilution of pAH according to Katz and Bergman (1969). Plasma flow was divided by 1 – (0.01 × hematocrit) to calculate blood flow. Daily mean blood (or plasma) concentrations, blood (or plasma) flow, and net nutrient fluxes were calculated for each cow × treatment. Net nutrient fluxes were obtained by multiplying the mean (n = 6) venous-arterial difference by the mean (n = 6) blood or plasma flow of the appropriate tissue bed [net portal appearance (**NPA**) for the PDV, net hepatic flux (**NHF**) for the liver, and net splanchnic release (**NSR**) for total splanchnic tis-

sues]. A negative net flux indicates net tissue removal, whereas a positive value denotes net release.

Whole-blood O₂ concentration was calculated using the following equation: O₂ (mL/mL) = (Hb × 1.34 × % sat O₂) + (0.023 × pO₂), where Hb is blood hemoglobin (g/L), 1.34 is mL of O₂/g of hemoglobin, % sat O₂ is the saturation percentage of O₂ calculated according to the equation by Kelman (1966), and pO₂ is the partial pressure of O₂. The volume of oxygen (mL) was transformed into mass (mmol) assuming that 22.4 L of O₂ at standard temperature and pressure equals 1 mol of O₂. Heat production was calculated as 20.44 kJ/L of O₂ consumed. Total absorbed energy was calculated as the addition of net energy fluxes (NPA or NSR) based on the sum of heats of combustion of nutrients whose net flux are positive and PDV or total splanchnic tissue heat production. Thus, any negative net flux, including for individual AA, was regarded as zero for the energy summation. The ME intake was calculated according to the Agricultural Research Council (ARC, 1984; ME = 15.54 × digestible OM intake) and using digestibility data obtained from a previous feeding trial using the same diets as in the current study (Cantalapiedra-Hijar et al., 2014).

Dietary MP [protein truly digestible in the small intestine when the rumen fermentable energy is limiting (PDIE), in the French system] was calculated as detailed by Cantalapiedra-Hijar et al. (2014), whereas the AA composition of the calculated MP was estimated as proposed by Rulquin et al. (2001) from the rumen-undegradable feed protein digestible in the intestine and the analyzed AA composition of feed ingredients.

A linear mixed-effects model that included a random intercept term for each cow was used. The mixed model was fitted using the LME procedure within the nlme package (Pinheiro et al., 2008) in R software (R Development Core Team, 2009). Statistical analyses were carried out as a 4 × 4 Latin square, following the model $Y_{ijkl} = \mu + CHO_1 + CP_k + CHO \times CP_{kl} + P_i + C_j + \varepsilon_{ijkl}$, 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 , CHO_1 is the fixed effect of dietary carbohydrate composition (starch vs. fiber), CP_k is the fixed effect of dietary CP concentration (low vs. normal), $CHO \times CP_{kl}$ is the fixed effect of the 2-way interaction between CP and CHO, and ε_{ijkl} is the random residual error, which was assumed to be $N(0, \sigma^2)$. Mean values are reported as least squares means with pooled standard error of the mean values ($n = 5$ for PDV data and arterial concentrations and $n = 4$ for NHF and NSR data) because of missing observations. To assess whether at similar NPA of TAA (NPA-TAA; general predictor) the NHF of TAA (NHF-TAA) was further affected by CHO, residuals

from the regression equation (NHF-TAA against NPA-TAA) were analyzed for the effect of the CHO by one-way ANOVA. Statistical significance was declared as $P \leq 0.05$ and trends at $P \leq 0.10$.

RESULTS

All catheters remained patent for blood sampling throughout the experiment in 3 out of 5 animals. One cow did not complete the last period due to an acute lameness before the completion of the last treatment (normal CP-fiber diet) and no data for this cow × period was available. In addition, 1 cow did not have a functional hepatic vein catheter in the last 2 periods (low CP-starch diet and normal CP-starch diet), and another cow for the last period (normal CP-starch diet). Therefore, $n = 5$ for arterial concentrations and net flux across PDV (except for the normal CP-fiber diet, where $n = 4$), $n = 4$ for liver and splanchnic net flux (except for the normal CP-starch diet, where $n = 3$, and for the low CP-fiber diet, where $n = 5$).

Feed Intake and N Partitioning

Body weight of animals remained similar across dietary treatments ($P > 0.05$). As planned, the DM and energy (calculated ME and theoretical NE_L) intakes (Table 3) were similar across treatments ($P > 0.05$). The NDF and ADF intakes were higher ($P < 0.001$) and starch intake lower ($P < 0.001$) for fiber compared with starch diets. On the other hand, CP and MP intakes were higher ($P < 0.001$) at normal versus low CP levels.

Milk N and true protein yield was higher with starch versus fiber diets (around +7%; $P \leq 0.046$) and at normal versus low CP levels (around +32%; $P < 0.001$). Although the milk N efficiency (milk N yield/N intake) increased by around 2.4 percentage points, on average (around +8% of increase) with diets rich in starch versus fiber, this effect was not found to be significant ($P = 0.13$). In contrast, low-CP diets tended ($P = 0.069$) to promote a greater milk N efficiency (milk N/N intake) compared with normal-CP diets. The theoretical milk efficiency of digestible AA (milk true protein yield relative to theoretical MP intake above maintenance requirements) did not vary across treatments ($P > 0.05$) and was slightly lower ($59.2 \pm 2.48\%$) than the 64% assumed by the INRA (2007) feeding system.

Blood and Plasma Flow and Arterial Concentrations

Packed cell volume and blood and plasma flow across PDV, liver, and total splanchnic tissues (Table 4) were

Table 3. Feed intake, milk N yield, and urinary N excretion in lactating Jersey cows fed the experimental diets¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
BW, kg	364	368	373	371	7.2	0.33	0.46	0.72
Intake, kg/d								
DM	14.6	14.0	14.9	15.4	0.36	0.94	0.16	0.25
CP	1.72	1.70	2.40	2.55	0.060	0.69	<0.001	0.22
NDF	4.58	6.55	4.48	7.03	0.216	<0.001	0.43	0.24
ADF	2.28	4.03	2.27	4.38	0.164	<0.001	0.34	0.34
Starch	4.84	0.798	5.25	0.550	0.188	<0.001	0.70	0.13
Energy, MJ/d								
ME ³	156	147	160	160	4.3	0.42	0.18	0.32
NE _L	103	96.6	102	105	2.7	0.50	0.22	0.14
MP, g/d	1,226	1,208	1,428	1,474	33	0.95	<0.001	0.35
Milk N yield, g/d	92.1	84.0	119	114	3.1	0.045	<0.001	0.65
Milk true protein yield, g/d	569	519	726	696	19	0.046	<0.001	0.61
Milk N efficiency								
Milk N/N intake, %	33.4	30.8	30.9	28.7	1.16	0.13	0.069	0.86
Milk true protein/available MP ⁴	59.0	56.7	62.6	58.4	3.35	0.33	0.59	0.79

¹Least squares means presented with pooled SEM (n = 5).

²CHO = effect of dietary carbohydrate (starch or fiber); CP = effect of CP.

³Metabolizable energy intake estimated with digestibility data from Cantalapiedra-Hijar et al. (2014) and the equation (ME intake = digestible OM intake × 15.58) proposed by the Agricultural Research Council (ARC, 1984).

⁴Available MP = MP intake – MP for maintenance (INRA, 2007).

similar across dietary treatments ($P > 0.05$). In our experimental conditions (deacetylation of plasma pAH), the average contribution of the hepatic artery to the total hepatic flow was around 8.2% (data not shown) without any effect of CHO or CP on hepatic arterial flow ($P \geq 0.46$).

Diets rich in starch promoted lower arterial concentrations (Table 4) of total VFA ($P = 0.003$) and acetate ($P = 0.002$) and higher concentrations of butyrate ($P = 0.024$) and insulin ($P = 0.003$) compared with diets rich in fiber. Arterial concentrations of glucose, propionate, lactate, BHBA, NEFA, triglycerides, oxygen, ammonia-N, urea-N, and TAA-N were not affected by CHO ($P \geq 0.12$). Concerning the effect of CP content, normal-CP diets only increased the arterial concentration of urea-N ($P < 0.001$) and insulin ($P = 0.002$) and decreased that of NEFA ($P = 0.007$) compared with low-CP diets.

Among the individual AA, the arterial concentrations of His, Pro, and Asn were higher ($P \leq 0.075$) and those of Phe and Met lower ($P \leq 0.074$) with starch versus fiber diets. With normal-CP diets, the arterial concentration was higher for EAA as well as for His, Met, Pro, and Val ($P \leq 0.020$) and lower for Gly ($P = 0.011$) compared with low-CP diets. Finally, a CHO × CP interaction was noted for the arterial concentrations of EAA and BCAA as well as for Ala, Phe, Leu, Ile, and Val, which were all higher in fiber versus starch diets, but only at normal CP levels ($P \leq 0.096$).

Net Fluxes Across PDV, Liver, and Total Splanchnic Tissues

Net portal appearance was lower ($P \leq 0.052$) for total VFA, acetate, propionate, and BHBA and higher ($P \leq 0.055$) for glucose, butyrate, and insulin with starch versus fiber diets (Table 5). Moreover, starch diets promoted higher ($P \leq 0.054$) NPA of TAA-N, NEAA-N, EAA-N, and branched-chain AA-N (BCAA-N) compared with fiber diets, whereas similar values were obtained ($P > 0.523$) for ammonia-N and urea-N (Table 6). Concerning the individual AA (Table 6), starch diets only increased the NPA of Leu ($P = 0.037$), and tended to increase the NPA of Val, Pro, Phe, and His ($P \leq 0.087$).

The dietary carbohydrate composition did not affect the NHF of any metabolite ($P \geq 0.11$) except Gly ($P = 0.038$) and Lys ($P = 0.039$), whose values were higher (lower net hepatic uptake) for starch versus fiber diets. The NHF of TAA-N as a percentage of their NPA increased numerically by 22%, on average, when changing the main CHO from fiber to starch, but this effect was found to be nonsignificant ($P \geq 0.18$). However, a tendency for starch diets ($P = 0.091$) to have higher NHF (lower uptake) of TAA was found (Figure 1) when standardizing data at similar NPA (residuals analysis; Figure 1b). Diets rich in starch did not modify the fractional removal of any individual AA by the liver, except for Gly and Lys, whose hepatic removal was

Table 4. Blood and plasma flow and arterial concentration of metabolites in lactating Jersey cows fed the experimental diets¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Packed cell volume	0.240	0.236	0.235	0.260	0.0087	0.27	0.32	0.15
Blood flow, L/h								
PDV	1,384	1,429	1,186	1,372	70	0.16	0.14	0.37
Liver	1,487	1,558	1,232	1,492	92	0.32	0.25	0.40
Hepatic artery	125	133	132	111	25	0.91	0.52	0.60
Plasma flow, L/h								
PDV	1,058	1,093	892	1,026	51	0.14	0.075	0.38
Liver	1,149	1,191	924	1,113	72	0.40	0.17	0.41
Hepatic artery	98.2	102	102	80.8	19	0.96	0.46	0.58
Total VFA, ³ mM	1.80	2.20	1.66	2.29	0.120	0.003	0.88	0.37
Acetate	1.70	2.09	1.54	2.20	0.112	0.002	0.89	0.29
Propionate	0.067	0.080	0.078	0.069	0.0073	0.61	0.98	0.18
Butyrate	0.036	0.028	0.036	0.020	0.0041	0.024	0.44	0.40
Glucose, mM	3.08	2.94	3.05	2.88	0.065	0.12	0.71	0.83
Lactate, mM	0.351	0.392	0.353	0.383	0.033	0.27	0.87	0.93
BHBA, mM	0.867	0.705	0.742	0.758	0.051	0.15	0.51	0.13
NEFA, mM	0.110	0.104	0.062	0.082	0.0096	0.40	0.007	0.27
Triglycerides, mM	0.131	0.128	0.137	0.127	0.015	0.54	0.85	0.74
Insulin, nIU/L	33.7	27.6	43.1	35.1	1.80	0.003	0.003	0.65
Oxygen, mM	4.74	4.78	4.88	5.02	0.128	0.73	0.20	0.72
Ammonia-N, mM	0.638	0.703	0.570	0.865	0.133	0.46	0.52	0.43
Urea-N, mM	3.37	3.74	10.4	9.52	0.553	0.44	<0.001	0.30
Total AA, ⁴ μM	1,977	1,870	1,951	2,058	84.8	0.93	0.42	0.26
NEAA	1,252	1,209	1,189	1,122	44.6	0.39	0.14	0.80
Ala	215	204	188	224	12.0	0.40	0.67	0.093
Asp	58.6	46.1	55.1	47.7	4.68	0.075	0.79	0.62
Aspartate	18.8	15.8	19.0	20.7	2.12	0.90	0.39	0.31
Glutamate	243	269	251	251	12.5	0.45	0.85	0.34
Glu	160	166	170	135	16.8	0.44	0.68	0.28
Gly	377	345	328	277	18.3	0.20	0.011	0.63
Pro	67.3	56.2	77.2	69.3	3.08	0.014	0.008	0.64
Ser	111	108	100	92.3	9.55	0.64	0.22	0.84
EAA	721	660	752	932	54.4	0.52	0.020	0.056
His	29.9	17.9	48.8	44.9	2.75	0.004	<0.001	0.20
Ile	104	99	102	144	7.32	0.071	0.022	0.018
Leu	138	93.7	142	162	10.6	0.24	0.014	0.020
Lys	77.4	69.0	86.3	101	10.8	0.97	0.10	0.34
Met	33.6	35.8	36.8	47.6	2.46	0.074	0.018	0.13
Phe	52.5	54.8	51.1	61.2	1.98	0.038	0.25	0.096
Thr	86.6	94.5	84.9	110	12.1	0.25	0.64	0.52
Tyr	54.9	53.7	50.6	58.7	3.67	0.53	0.93	0.26
Val	145	142	160	208	11.1	0.13	0.011	0.059
BCAA	385	334	395	513	29.4	0.49	0.014	0.026

¹Least squares means presented with pooled SEM [n = 5 for arterial concentrations and plasma and blood flow for portal drained viscera (PDV), and n = 4 for plasma and blood flow for the liver and hepatic artery].

²CHO = effect of dietary carbohydrate (starch or fiber); CP = effect of CP.

³Total VFA = acetate + propionate + butyrate.

⁴Total amino acids = NEAA + EAA; NEAA = Ala, Pro, Ser, Asp, Glu, Asn, Gly, and Gln; EAA = His, Met, Phe, Tyr, Ile, Leu, Lys, Thr, and Val; BCAA = branched-chain AA (Leu, Ile, and Val).

higher for fiber versus starch diets ($P = 0.010$). The CHO affected the NSR of butyrate, TAA-N, NEAA-N, EAA-N, BCAA-N, Leu, Lys, and Thr, which were all higher with starch versus fiber diets ($P \leq 0.058$). Finally, the effect of CHO was still significant when the NPA ($P = 0.006$) and NSR ($P = 0.038$) of TAA-N were expressed as a proportion of N intake.

As the dietary CP content increased from 12.0 to 16.5%, the net flux across the PDV and total splanchnic tissues increased ($P \leq 0.038$), whereas that across the

liver decreased ($P \leq 0.062$) for urea-N, TAA-N, NEAA-N, EAA-N, and BCAA-N. In the case of ammonia-N, for diets at normal CP content, its NPA increased ($P = 0.004$) and NHF decreased ($P = 0.004$) compared with low-CP diets, whereas its NSR remained similar ($P = 0.64$). In addition, normal- versus low-CP diets decreased the NHF and NSR of BHBA, but only in starch diets (CHO × CP; $P \leq 0.042$). Concerning the individual AA, normal-CP diets increased the NPA of all AA ($P \leq 0.086$) except Ala ($P = 0.28$); the NHF of

Table 5. Net portal appearance (NPA), net hepatic flux (NHF), and net splanchnic release (NSR) of VFA, glucose, lactate, BHBA, NEFA, triglycerides, oxygen, and insulin in lactating Jersey cows fed the experimental diets¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Total VFA, ³ mmol/h								
NPA	1,550	1,802	1,494	1,833	128	0.043	0.89	0.75
NHF	-300	-574	-540	-623	137	0.20	0.41	0.56
NSR	1,406	1,241	1,123	1,247	85.5	0.78	0.26	0.18
Acetate, mmol/h								
NPA	983	1,191	921	1,231	102	0.032	0.90	0.64
NHF	195*	3.55*	33.2*	-50.9*	110	0.21	0.41	0.68
NSR	1,350	1,209	1,086	1,212	85.0	0.67	0.30	0.21
Propionate, mmol/h								
NPA	409	504	424	493	37.6	0.052	0.98	0.76
NHF	-376	-489	-453	-469	45.6	0.19	0.73	0.39
NSR	13.0*	16.3	18.7*	22.4	6.40	0.67	0.59	0.98
Butyrate, mmol/h								
NPA	158	105	149	110	10.4	0.003	0.75	0.56
NHF	-117	-88.9	-117	-94.5	11.5	0.11	0.76	0.84
NSR	41.5	15.7	21.4	15.6	3.16	0.004	0.032	0.037
Glucose, mmol/h								
NPA	-3.51*	-81.6	47.6	-97.4	27.1	0.003	0.42	0.27
NHF	400	492	441	462	38.4	0.26	0.83	0.44
NSR	426	410	446	378	48.5	0.45	0.85	0.65
Lactate, mmol/h								
NPA	108	112	100	105	11.1	0.65	0.67	0.96
NHF	-187	-161	-117	-135	21.5	0.99	0.14	0.39
NSR	-85.0	-49.5	-23.9*	-29.7*	19.9	0.89	0.14	0.38
BHBA, mmol/h								
NPA	163	204	167	229	12.3	0.003	0.15	0.35
NHF	280	194	140	183	20.2	0.12	0.087	0.042
NSR	437	398	303	414	20.4	0.72	0.37	0.035
NEFA, mmol/h								
NPA	18.2	21.3	23.0	35.4	5.69	0.39	0.14	0.46
NHF	-34.7	-39.9	-30.8	-51.3	7.50	0.32	0.65	0.39
NSR	-19.6	-18.6	-5.64*	-15.9	4.57	0.19	0.14	0.31
Triglycerides, mmol/h								
NPA	4.19*	7.21*	4.81*	2.46*	4.44	0.80	0.60	0.58
NHF	-1.12*	-1.92*	-6.08*	-5.21*	3.70	0.56	0.46	0.84
NSR	-3.59*	4.63*	-9.72*	-2.71*	4.99	0.85	0.25	0.91
Oxygen, mmol/h								
NPA	-2,147	-2,391	-2,077	-2,434	102	0.019	0.79	0.61
NHF	-1,361	-1,538	-1,456	-1,552	99.5	0.17	0.74	0.73
NSR	-3,554	-3,927	-3,500	-3,913	222	0.11	0.80	0.92
Heat production, ⁴ MJ/d								
PDV	23.2	25.8	22.4	26.3	1.10	0.019	0.79	0.61
Liver	14.7	16.6	15.7	16.7	1.08	0.17	0.74	0.73
Splanchnic tissues	38.5	42.4	37.8	42.2	2.40	0.11	0.80	0.92
Energy, ⁵ MJ/d								
NPA	83.5	83.7	91.8	92.0	5.44	0.999	0.18	0.997
NHF	10.7	3.55	-3.61	-7.44	5.42	0.20	0.14	0.79
NSR	106	98.3	95.6	98.5	7.52	0.61	0.90	0.56
Total absorbed energy, MJ/d								
From NPA ⁶	111	119	121	128	5.35	0.24	0.15	0.93
From NSR ⁷	145	143	136	146	9.34	0.84	0.96	0.62
Insulin, μ IU/h								
NPA	11.1	6.12	9.04	6.58	1.51	0.055	0.57	0.45

Continued

all AA ($P \leq 0.096$) except for Ser ($P = 0.10$), Glu ($P = 0.17$), Gly ($P = 0.30$), Met ($P = 0.49$), and Val ($P = 0.46$); and the NSR of Gly, His, Phe, Ile, Leu, Thr, and Val ($P \leq 0.081$) compared with low-CP diets. As the dietary CP content increased, the hepatic fractional

removal increased for TAA-N, NEAA-N, and EAA-N ($P \leq 0.071$) as well as for Pro, Ser, Gly, Gln, Phe, Tyr, Leu, Lys, and Thr ($P \leq 0.092$).

The PDV O₂ consumption and heat production decreased (+14%; $P = 0.019$) with starch versus fiber

Table 5 (Continued). Net portal appearance (NPA), net hepatic flux (NHF), and net splanchnic release (NSR) of VFA, glucose, lactate, BHBA, NEFA, triglycerides, oxygen, and insulin in lactating Jersey cows fed the experimental diets¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
NHF	-7.16	-3.94	-7.95	-3.14	1.85	0.13	0.94	0.71
NSR	4.04*	2.22*	2.29*	3.20*	1.59	0.58	0.94	0.48

¹Least squares means presented with pooled SEM [n = 5 for portal drained viscera (PDV) data, and n = 4 for NHF and NSR data].

²CHO = effect of dietary carbohydrate (starch or fiber); CP = effect of CP.

³Total VFA = acetate + propionate + butyrate.

⁴Heat production calculated as 4.89 kcal/L of O₂ consumed (McLean, 1972).

⁵Net fluxes of energy, based on individual heats of combustion (MJ/mol), across the portal drained viscera (NPA) and splanchnic tissues (NSR): acetate = 0.875, propionate = 1.54, butyrate = 2.19, glucose = 2.81, BHBA = 2.06, lactate = 1.37, NEFA = 11.28, urea = 0.64, Ala = 1.58, Pro = 2.74, Ser = 1.45, Asp = 1.60, Glu = 2.24, Asn = 1.93, Gly = 0.97, Gln = 2.57, His = 3.18, Met = 2.92, Phe = 4.65, Tyr = 4.43, Ile = 3.58, Leu = 3.58, Lys = 3.68, Thr = 2.05, and Val = 2.92.

⁶Total absorbed energy from the gastrointestinal tract calculated from PDV fluxes = summation of net energy flux (based on individual heats of combustion) across the PDV, for which net flux is positive + heat production of PDV.

⁷Total absorbed energy from the gastrointestinal tract calculated from NSR fluxes = summation of net energy fluxes (based on individual heats of combustion) across the total splanchnic tissues + heat production of total splanchnic tissues.

*Net fluxes not different from 0 ($P < 0.05$) according to the asymptotic CI within each treatment.

diets and remained similar ($P = 0.79$) between normal- and low-CP diets. However, neither the net flux of O₂ (and thus the energy expenditure) of the liver nor that of total splanchnic tissues was affected by treatments ($P > 0.05$). The total absorbed energy from the gastrointestinal tract was similar ($P > 0.05$) across dietary treatments as well as the gross energy flux across the splanchnic tissues ($P > 0.05$), calculated both from PDV and total splanchnic tissues measures.

DISCUSSION

To improve current feeding systems, nutrient supply and utilization must be represented at a more detailed level than the current aggregated energy and protein feed values (Nocek and Tamminga, 1991; Hanigan et al., 2005). For instance, at similar calculated net energy and protein intakes, results from the current study confirm previous findings showing a small (+7% in the present study) but significant improvement in milk N yield when dairy cows were fed starch versus fiber diets (Huhtanen and Hristov, 2009; Cantalapiedra-Hijar et al., 2014). Moreover, such an improvement was accompanied by a greater availability of absorbed AA for the peripheral tissues (+15% NSR of TAA-N) even though diets were formulated to supply similar MP. This questions the validity of reasoning only on aggregated feed values, raising the issue of whether the profile of absorbed nutrients associated with a particular diet can affect the efficiency of nutrient utilization beyond their energy and protein feed values (Leiva et al., 2000; Hanigan et al., 2005).

The high AA requirements and metabolic rate of splanchnic tissues have a profound effect on the avail-

ability and profile of absorbed AA presented to peripheral tissues (Lapierre et al., 2005). Therefore, differences in milk N yield promoted by CHO could originate from changes in splanchnic AA metabolism, and, as observed in monogastrics, this phenomenon could be modulated by the N supply (van der Schoor et al., 2001). Care was taken in diet formulation to minimize the effect of potential interfering factors on AA metabolism and animal performance, and so energy and N intake as well as the Lys:Met ratio in MP were kept similar between starch and fiber diets within a given CP level. Because the portal absorption of AA is carried predominantly in plasma and no evidence exists for erythrocytes to be involved in liver AA flux (Lobley et al., 1996), the AA analysis was performed from plasma samples. The effect of different N intake levels on ruminant splanchnic metabolism has been extensively discussed in other studies (Lapierre et al., 2000; Raggio et al., 2004); thus, this discussion will focus mainly on the CHO effect and its possible interaction with the dietary CP level.

Net Portal Fluxes

Data from this study showed a greater proportion of feed N intake recovered as portal absorbed AA (NPA of TAA-N) when changing the main CHO from fiber to starch. However, our results confirm a lack of a direct sparing effect of starch diets on AA used by the PDV as energy fuels and suggest that the improvement in the NPA of TAA-N with starch versus fiber diets is rather the consequence of lower energy requirements by PDV along with a higher microbial N flow to the duodenum.

Total absorbed energy from the gastrointestinal tract (calculated from PDV measurements accord-

Table 6. Net portal appearance (NPA), net hepatic flux (NHF), and net splanchnic release (NSR) of urea-N, ammonia-N, and AA in lactating Jersey cows fed the experimental diets¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Net flux, ³ g of N/d								
Urea-N								
NPA	-67.8	-70.8	-100	-110	14.4	0.86	0.038	0.83
NHF	98.4	135	193	200	11.5	0.11	0.002	0.34
NSR	36.9	63.9	92.5	90.0	10.6	0.36	0.026	0.26
Ammonia-N								
NPA	83.9	107	161	159	14.9	0.52	0.004	0.45
NHF	-81.9	-113	-171	-161	12.2	0.12	0.004	0.20
NHF Urea-N, %	81.2	84.4	90.8	79.4	7.35	0.80	0.97	0.42
NSR	-5.68*	-5.85*	-1.61*	-1.73*	9.98	0.99	0.64	0.998
TAA-N								
NPA	147	110	189	182	8.82	0.019	<0.001	0.15
N intake, %	53.0	40.2	49.7	44.4	2.39	0.005	0.97	0.17
NHF	-37.6	-34.0	-78.2	-74.1	10.1	0.90	0.014	0.98
NPA, %	25.1	32.6	37.3	42.1	5.70	0.32	0.12	0.85
Fractional removal, %	2.82	2.64	6.25	5.10	0.865	0.83	0.026	0.63
NSR	113	86.1	129	116	5.26	0.013	0.007	0.29
N intake, %	42.0	32.6	39.5	28.3	3.04	0.038	0.24	0.78
NEAA-N								
NPA	64.0	44.3	81.6	81.4	5.42	0.054	0.001	0.12
NHF	-25.3	-16.8	-40.8	-43.3	6.75	0.84	0.022	0.47
NPA, %	36.4	38.6	44.8	53.4	10.6	0.67	0.24	0.79
Fractional removal, %	3.13	2.31	5.88	5.95	0.955	0.95	0.018	0.68
NSR	39.4	27.6	42.7	39.3	2.30	0.030	0.021	0.15
EAA-N								
NPA	83.3	65.9	106	100	4.74	0.022	<0.001	0.32
NHF	-11.8	-17.0	-33.3	-30.6	5.29	0.60	0.035	0.53
NPA, %	12.9	27.2	27.8	32.3	5.30	0.18	0.20	0.50
Fractional removal, %	2.19	3.18	6.06	4.41	0.980	0.76	0.071	0.34
NSR	65.5	48.9	78.8	69.2	5.00	0.035	0.017	0.57
BCAA-N								
NPA	29.5	23.1	37.7	35.5	1.95	0.046	0.002	0.39
NHF	-0.322*	-3.18*	-6.32*	-6.21*	2.26	0.37	0.062	0.80
NSR	25.2	19.7	31.0	29.2	1.67	0.018	0.008	0.56
Net flux of individual AA, mmol/h								
NEAA								
Alanine								
NPA	63.7	48.1	60.5	63.7	4.73	0.18	0.28	0.093
NHF	-34.5	-31.6	-36.9	-47.8	5.69	0.51	0.096	0.28
Fractional removal, %	9.28	10.2	10.6	11.3	2.28	0.89	0.42	0.96
NSR	27.5	16.8	24.2	17.9	3.42	0.10	0.80	0.62
Proline								
NPA	14.6	10.8	18.9	16.5	1.72	0.076	0.022	0.71
NHF	-5.19	-4.66	-9.85	-8.82	1.44	0.97	0.029	0.88
Fractional removal, %	4.83	4.83	9.04	7.72	1.22	0.60	0.045	0.54
NSR	9.87	6.29	11.0	7.84	1.96	0.15	0.50	0.93
Serine								
NPA	30.7	21.2	32.4	32.5	2.97	0.11	0.078	0.16
NHF	-28.7	-19.0	-31.9	-32.4	4.48	0.45	0.10	0.32
Fractional removal, %	14.2	10.4	19.5	18.3	1.98	0.55	0.026	0.59
NSR	-0.449*	2.09*	-2.14*	2.26*	2.17	0.78	0.82	0.70
Aspartate								
NPA	2.16	2.13	2.45	3.27	0.208	0.24	0.012	0.087
NHF	-0.476	-0.634	-1.13	-1.31	0.266	0.50	0.056	0.97
Fractional removal, %	2.26	3.33	3.52	4.48	0.877	0.14	0.12	0.96
NSR	1.54	1.49	1.39	1.93	0.318	0.85	0.58	0.43
Glutamate								
NPA	1.88*	0.294*	9.44	6.37	2.00	0.34	0.013	0.74
NHF	45.4	52.5	29.9	46.7	5.78	0.098	0.17	0.45
Fractional removal, %	-13.0	-12.8	-8.23	-13.0	1.84	0.42	0.45	0.27
NSR	48.3	52.8	40.2	53.2	7.21	0.24	0.69	0.59
Asparagine								
NPA	29.1	22.4	31.2	32.2	2.82	0.23	0.086	0.23

Continued

Table 6 (Continued). Net portal appearance (NPA), net hepatic flux (NHF), and net splanchnic release (NSR) of urea-N, ammonia-N, and AA in lactating Jersey cows fed the experimental diets¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
NHF	-13.5	-10.2	-17.4	-17.7	2.49	0.71	0.070	0.52
Fractional removal, %	12.4	10.9	18.4	17.5	3.27	0.97	0.16	0.95
NSR	14.5	12.1	12.2	15.0	2.92	0.81	0.84	0.44
Glycine								
NPA	19.3	18.6	34.7	39.2	4.14	0.74	0.003	0.57
NHF	-34.8	-37.1	-33.8	-41.5	2.20	0.038	0.30	0.31
Fractional removal, %	6.06	6.63	6.73	9.54	0.715	0.010	0.025	0.23
NSR	-11.8	-18.3	2.28*	-2.84*	4.72	0.28	0.031	0.89
Glutamine								
NPA	0.068*	-3.53*	5.61	4.60	1.91	0.16	0.008	0.53
NHF	5.50	5.48	1.74*	-3.80*	3.12	0.75	0.080	0.43
Fractional removal, %	-3.19	-2.67*	-0.40	1.86*	1.47	0.65	0.057	0.61
NSR	5.49	1.93	7.02	0.803	2.28	0.22	0.99	0.59
EAA								
Histidine								
NPA	9.07	6.18	11.0	11.3	0.776	0.085	0.003	0.088
NHF	-2.60	-1.57	-4.89	-4.35	0.760	0.43	0.021	0.76
Fractional removal, %	5.74	4.98	7.89	6.25	1.14	0.65	0.40	0.75
NSR	5.52	4.57	5.78	7.20	0.575	0.46	0.036	0.11
Methionine								
NPA	9.96	9.45	11.1	12.9	1.04	0.85	0.067	0.40
NHF	-4.85	-5.31	-6.36	-5.54	1.15	0.43	0.49	0.62
Fractional removal, %	7.44	6.99	10.5	6.37	2.00	0.65	0.66	0.43
NSR	4.32	4.10	4.74	7.30	1.31	0.89	0.22	0.36
Phenylalanine								
NPA	17.2	12.4	20.6	18.8	2.06	0.087	0.048	0.51
NHF	-6.76	-6.32	-13.5	-9.31	1.58	0.30	0.035	0.31
Fractional removal, %	7.10	5.95	15.9	8.09	1.53	0.098	0.033	0.11
NSR	8.26	6.07	8.17	10.1	1.00	0.42	0.081	0.12
Tyrosine								
NPA	17.2	13.9	20.3	20.1	2.07	0.38	0.074	0.50
NHF	-5.60	-6.90	-11.3	-10.7	1.39	0.69	0.025	0.55
Fractional removal, %	5.61	6.82	13.0	9.60	1.06	0.72	0.011	0.11
NSR	8.34	6.83	7.50	9.77	1.38	0.43	0.38	0.26
Isoleucine								
NPA	27.6	24.0	32.5	32.0	2.14	0.28	0.026	0.54
NHF	-0.450*	-4.34*	-7.24*	-4.02*	3.04	0.95	0.58	0.33
Fractional removal, %	0.041	2.18	4.22	1.22	1.52	0.69	0.64	0.18
NSR	25.2	19.7	28.1	27.8	1.85	0.31	0.021	0.27
Leucine								
NPA	34.3	24.6	44.9	40.8	3.26	0.037	0.004	0.43
NHF	0.953*	-1.27*	-6.84*	-9.84	2.39	0.26	0.024	0.88
Fractional removal, %	-0.058	0.718	3.18	3.79	1.23	0.37	0.077	0.95
NSR	29.6	23.0	34.6	31.5	2.56	0.020	0.037	0.55
Lysine								
NPA	32.2	28.9	46.8	39.2	3.17	0.14	0.006	0.53
NHF	-2.67*	-6.34	-8.67	-11.5	1.56	0.039	0.023	0.80
Fractional removal, %	1.21	4.26	6.12	6.56	0.619	0.010	0.007	0.12
NSR	31.3	22.9	42.1	27.1	3.96	0.058	0.12	0.48
Threonine								
NPA	22.7	15.2	27.1	27.5	2.31	0.14	0.010	0.14
NHF	-4.24	-4.89	-10.4	-11.4	2.71	0.75	0.072	0.95
Fractional removal, %	2.89	3.32	7.13	6.34	1.59	0.91	0.092	0.74
NSR	17.2	10.3	19.0	16.4	1.49	0.026	0.036	0.23
Valine								
NPA	25.5	20.1	34.9	31.9	2.08	0.057	0.001	0.59
NHF	-1.53*	-3.99*	-7.34*	-4.01*	2.75	0.80	0.46	0.36
Fractional removal, %	0.358	1.44	2.15	1.24	0.920	0.84	0.45	0.38
NSR	21.7	16.1	29.7	27.3	2.42	0.12	0.009	0.56

¹Least squares means presented with pooled SEM [n = 5 for portal drained viscera (PDV) data, and n = 4 for NHF and NSR data].

²CHO = effect of dietary carbohydrate (starch or fiber); CP = effect of CP.

³TAA-N = nitrogen from total AA (NEAA-N + EAA-N); NEAA-N = nitrogen from NEAA (Ala, Pro, Ser, Asp, Glu, Asn, Gly, and Gln); EAA-N = nitrogen from EAA (His, Met, Phe, Tyr, Ile, Leu, Lys, Thr, and Val); BCAA-N = nitrogen from branched-chain AA (Leu, Ile, and Val).

*Net fluxes not different from 0 ($P < 0.05$) according to the asymptotic CI within each treatment.

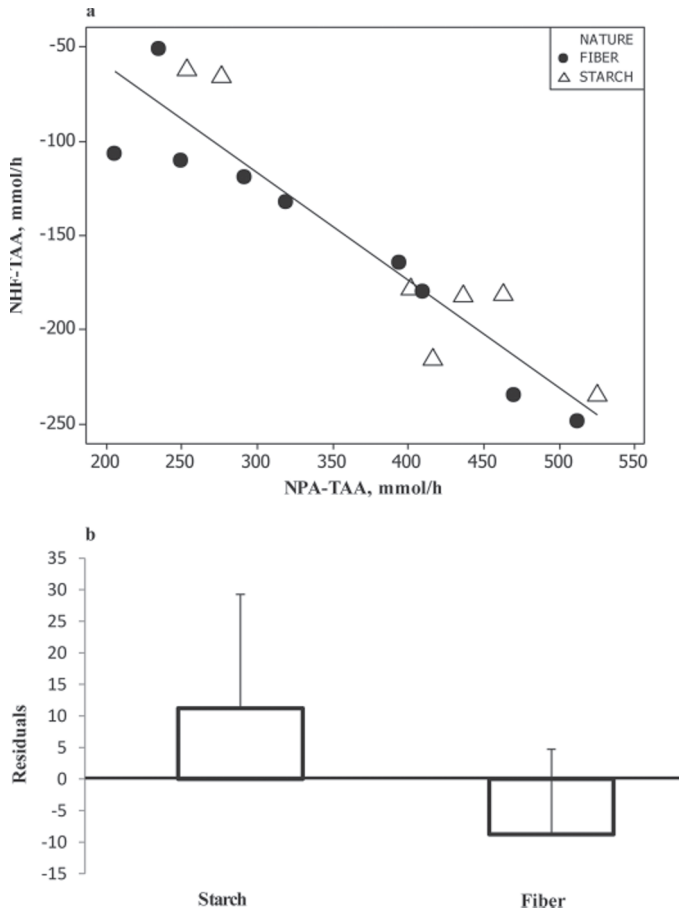


Figure 1. Net hepatic flux (NHF; mmol/h) of total AA (TAA) in Jersey lactating cows fed the experimental diets. (a) Net hepatic flux regressed against the net portal appearance (NPA; mmol/h) of TAA and adjusted for animal and period effects [fitted equation is $\text{NHF-TAA} = -0.569$ (SE = 0.060; $P < 0.001$) \times $\text{NPA-TAA} + 54.2$ (SE = 22.8; $P = 0.032$); $R^2 = 85.5$]; (b) residuals from the regression shown in panel a and according to the dietary carbohydrate composition (starch vs. fiber). Analysis of residuals shows that starch diets tended to increase ($P = 0.091$) the NHF of TAA (lower liver uptake) compared with diets rich in fiber.

ing to Loncke et al., 2011) was, as expected, similar across treatments and accounted for $77.0 \pm 4.76\%$ of the calculated ME intake (using digestibility data from Cantalapiedra-Hijar et al., 2014) and for $118 \pm 7.22\%$ of the theoretical NE_L intake. Lower absorbed energy compared with ME intake was in agreement with other studies [68–74% (Lindsay, 1993); $86.0 \pm 9.5\%$ (Loncke et al., 2011)] and may originate from biases in the calculation of ME intake from digestible OM intake (Loncke et al., 2011) and methodological limits of the arteriovenous differences technique (Lindsay, 1993).

Despite similar absorbed energy across diets, fiber diets increased the energy expenditure of PDV by 14%, on average, compared with starch diets. Reynolds et al. (1991a) and McLeod and Baldwin (2000) found that dietary NDF content increased the energy expenditure

of PDV independently of the DMI. A higher gastrointestinal workload (Reynolds et al., 1991a) as well as a greater size of PDV (Bailey, 1986; McLeod and Baldwin, 2000) is expected as the dietary bulk and fiber content of diets increase. Moreover, although non-significant, the blood flow across the PDV was higher (+9%) with fiber diets, compatible with a higher PDV size and energy expenditure (Reynolds et al., 1991a; Han et al., 2002).

The profile of energy-yielding nutrients absorbed into the portal vein changed according to CHO as empirically predicted by meta-analysis (Loncke et al., 2009). In particular, the higher NPA of glucose associated with starch diets was compensated by a higher NPA of acetate promoted by fiber diets. The glucogenic nature of starch diets was reflected by the higher insulin secretion (NPA of insulin) and concentration (van Knegsel et al., 2005) compared with fiber diets; however, the lower NPA of propionate found with starch versus fiber diets as well as the marked difference in NPA of butyrate across CHO was unexpected. Sucrose from molasses increased ruminal butyrate proportions (Kellogg and Owen, 1969) and thus a slightly higher NPA of butyrate could be expected with starch versus fiber diets. In addition, feeding diets hourly and at a restricted intake level may promote high rumen protozoa proliferation and thus a shift in the rumen VFA pattern toward butyrate at the expense of propionate (Brossard et al., 2004). Interestingly, the NPA of butyrate plus BHBA were similar among treatments. Because butyrate is an efficient energy source for rumen (Kristensen, 2005) and gut (Topping and Clifton, 2001) epithelia, as well as the major precursor for BHBA (Kristensen, 2005), a higher NPA of butyrate and lower NPA-BHBA with starch versus fiber diets agrees with the lower energy expenditure of PDV found with starch diets.

The observed differences in the profile of absorbed energy-yielding nutrients with CHO was accompanied by a significant increase in the proportion of dietary N recovered as TAA-N in the portal vein with starch versus fiber diets (51.4 vs. 42.3%). This higher NPA of TAA-N was compatible with a greater milk N yield and whole-body Leu turnover (Cantalapiedra-Hijar et al., 2012) and could be the consequence of lower energy expenditure of PDV, and thus AA requirements, as the size of viscera decreases with lower dietary NDF content (Reynolds et al., 1991a; McLeod and Baldwin, 2000).

The loss of AA between intestinal absorption and their NPA is a result of AA oxidation and non-reabsorbed endogenous protein secretion (Lobley et al., 2003). The NEAA are used by the gut tissues mainly as an energy source, as opposed to the EAA, which are mostly used for protein synthesis (Lobley et al.,

2003). Therefore, specific responses of EAA versus NEAA may help explain the changes in PDV AA use for protein synthesis or oxidation. In the present study, although a tendency existed for NPA-NEAA to be higher in animals fed diets rich in starch versus fiber, none of the AA considered as the main energy fuels for PDV tissues (Glu, Gln, and Asp; Berthiaume et al., 2001) were significantly affected. This indicates that the effect of CHO on the NPA-TAA is not likely the consequence of a direct sparing effect of starch diets on AA catabolism by PDV.

Attempts to improve glucose supply to spare AA from PDV metabolism have not always been successful. No improvement in the NPA-TAA was measured when glucose was infused in the abomasum (Larsen and Kristensen, 2009; Freetly et al., 2010), duodenum (Krehbiel et al., 1996), or intravenously (Balcells et al., 1995). Conversely, encouraging results have been obtained when nutrient supply was altered by diet (starch vs. fiber) formulation [+68% higher NPA of TAA, nonsignificant (Seal et al., 1992); +22% higher NPA of EAA, significant (Larsen and Kristensen, 2012)]. In this sense, starch diets has been shown to improve the efficiency of microbial N synthesis (Ouellet et al., 2002; Fanchone et al., 2013), as assumed by some feeding systems [CNCPS (Sniffen et al., 1992); DVE/OEB₂₀₁₀ (Van Duinkerken et al., 2011); NorFor (Volden, 2011)] but not by others (NRC, 2001; INRA, 2007). A higher microbial N flow to the duodenum and, thus, NPA of TAA-N could be then expected with starch versus fiber diets above theoretical predictions (INRA, 2007). Interestingly, in the feeding trial of Cantalapiedra-Hijar et al. (2014), which used the same diets as in the current study, the microbial N flow to the duodenum was improved when changing from fiber to starch diets; this improvement was more than 2-fold higher at low (24.5%) versus normal (10.5%) CP contents, which is compatible with data obtained in the present study. In contrast, changes in CHO did not promote differences in the NPA of urea-N, in agreement with others (Larsen and Kristensen, 2012).

According to Lobley et al. (2003), the loss of EAA across PDV metabolism is more probably associated with incomplete reabsorption of endogenous protein (secretions and cellular desquamations) rather than with high rates of PDV protein turnover. However, no differences in endogenous fecal N secretion were observed between low and high-fiber diets in direct comparisons (Ouellet et al., 2002) or by meta-analysis (Marini et al., 2008). One hypothesis, based on the model by Hanigan et al. (2004), is that the increased anabolic use of AA by extrasplanchnic tissues would reduce the catabolic use of AA by the PDV, resulting in a higher NPA. Indeed, for some AA, such as the BCAA,

the PDV may contribute to the catabolic regulation of AA balance (Lobley et al., 2003; El-Kadi et al., 2006). Interestingly, the arterial concentration of BCAA was lower (−23%) with diets rich in starch versus fiber, but only at normal CP levels, which raises the hypothesis of a different mechanism by which starch diets improve the NPA-TAA based on the dietary CP levels.

Net Hepatic Fluxes

Our results show a tendency for diets rich in starch to decrease the NHF-TAA when standardizing data at similar NPA-TAA. This tendency did not seem to be driven by a direct sparing effect of glucogenic nutrients on hepatic AA uptake but more likely as a consequence of better utilization by extrahepatic tissues associated with high insulin concentrations.

Data in the literature show that the contribution of AA to hepatic gluconeogenesis can vary considerably in the fed dairy cow (6.1 to 20%; Larsen and Kristensen, 2012) and this could logically have a non-negligible effect on the efficiency of N utilization and, thus, on protein requirements (Danfær et al., 1995). Because the contribution of AA to gluconeogenesis decreases as the insulin concentration (Brockman, 1990) and NPA of propionate increases (Danfær et al., 1995), a supply of glucogenic nutrients has been thought to decrease the uptake of AA by the liver (Danfær et al., 1995; Larsen and Kristensen, 2012). Moreover, it is believed that an increased NPA of glucose with diets rich in starch could be partly compensated for by a decrease in hepatic gluconeogenesis (Freetly et al., 2010), further sparing AA (Larsen and Kristensen, 2009).

No difference in the NHF of either total, NEAA-N or EAA-N was found between diets differing in CHO. However, because one of the main factors driving the AA liver uptake is their NPA (Reynolds, 2006), a residuals analysis from the regression of NHF-TAA on NPA-TAA was carried out to evaluate whether the CHO itself could affect the AA hepatic uptake, regardless of its positive effect on NPA-TAA (Figure 1b). Thus, at similar NPA-TAA, diets rich in starch versus fiber tended to promote a higher NHF-TAA (lower uptake). Likewise, Larsen and Kristensen (2012) found a similar NHF-TAA in ruminants fed a glucogenic compared with a ketogenic diet, despite a significantly higher NPA of EAA with the glucogenic diets. When no differences in NPA were observed (Huntington, 1989), diets rich in starch promoted a significantly lower (−68%) α -amino N hepatic uptake compared with diets rich in fiber. This would suggest an effect of CHO on liver AA metabolism in our study, hidden by differences in NPA.

The fractional removal of AA by the liver does not remain constant across different dietary and physiologi-

cal conditions and can vary with changes in supply and requirements (Blouin et al., 2002). This was the case for most individual AA when dietary CP content increased. In contrast, fractional removal of AA by the liver was only modified by CHO for 2 AA: Gly and Lys. Because Lys (together with Leu) is the only non-glucogenic AA and the potential contribution of Gly to gluconeogenesis does not seem to be of quantitative importance (Larsen and Kristensen, 2012), our results do not indicate a lower use of AA for hepatic gluconeogenesis when feeding starch versus fiber diets. In practice, the major gluconeogenic AA substrates are the NEAA, alanine, glutamine (converted to glutamate), glycine, and serine (Wolff et al., 1972). Alanine has been proposed as the most important AA contributing to gluconeogenesis (Wolff et al., 1972; Larsen and Kristensen, 2013) but its net liver uptake was not modified by CHO in our study. Glycine was the only AA whose liver uptake increased when shifting from starch to fiber diets in one study (Larsen and Kristensen, 2012) and this could be related to the hepatic synthesis of hippuric acid (glycine conjugate of benzoic acid) as the dietary fiber increases (Martin, 1969). In contrast, no clear explanation was found in our study for changes in NHF of Lys across CHO.

Finally, as stated before for PDV, a lower net hepatic uptake of AA with starch diets can also be the consequence of better use by non-splanchnic tissues, as liver metabolism seems to be regulated not only by the feed supply (NPA) but also by the nutrients demand of peripheral tissues (Reynolds, 2006). Because the uptake of AA by muscle is stimulated by insulin concentrations in ruminants (see review by Brockman and Laarveld, 1986), lower hepatic AA uptake with starch versus fiber diets could be the consequence of better use of AA for protein synthesis.

Net Splanchnic Flux

The amount of energy released by the splanchnic tissues remained similar across dietary treatments and accounted for $91.4 \pm 4.83\%$ of the theoretical NE intake. In addition, the only change in the profile of energy-yielding nutrients released by the splanchnic tissues was observed for butyrate and BHBA, whose splanchnic fluxes respond to CHO, depending on the dietary CP level. In contrast, the improvement in milk N secretion (+7%) found in our experiment when starch replaced fiber as the main CHO was accompanied by a greater NSR of TAA-N (+22%); that is, a higher systemic availability of absorbed AA for the peripheral tissues at similar calculated MP and NE_L intakes. In this sense, Larsen and Kristensen (2012) recently found a greater NSR-TAA (calculated values of 7 and 29%,

respectively) associated with higher milk N yield when postpartum cows (between 4 and 29 DIM) were fed a glucogenic diet versus a ketogenic diet, but the reduced number of observations ($n = 2$ for the glucogenic diet) did not result in a significant effect. Likewise, Reynolds et al. (1991b) and Huntington et al. (1996) observed a higher (although nonsignificant) NSR of α -amino N (+17 and +24%, respectively) when beef cattle were fed a glucogenic diet compared with a ketogenic diet at similar energy intakes. As far as we know, no more data exist in the literature comparing the posthepatic net flux of AA when animals are fed glucogenic versus ketogenic diets at similar energy and N intakes, but the few results found in the literature along with ours indicate that diets rich in starch can modify the net flux of AA presented to the peripheral (productive) tissues without remarkable changes in both energy and type of energy-yielding nutrients.

CONCLUSIONS

At similar N intake and total absorbed energy, the dietary carbohydrate composition affected the profile of portal absorbed energy-yielding nutrients as well as the net posthepatic availability of AA. In particular, this study confirmed previous results observing a higher milk N yield with starch versus fiber diets and demonstrated that the net splanchnic release of AA was significantly improved by formulating diets high in starch, regardless the dietary N content. The higher transfer of N from feed to milk with diets rich in starch compared with fiber seems not to be the consequence of a direct AA sparing effect of starch diets but rather the result of lower PDV energy requirements and higher microbial N flow to the duodenum. More studies are needed to clarify whether the AA use by peripheral tissues could have been improved by starch versus fiber diets, as suggested in this experiment. Results from this study may justify the need to evolve toward a nutrient-based system.

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