Diets rich in starch improve the efficiency of amino acids use by the mammary gland in lactating Jersey cows

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ABSTRACT

The objective of this study was to test whether the greater milk N yield usually observed when feeding diets based on starch versus fiber was the consequence of a higher efficiency of AA use across the mammary gland and whether this effect depended on dietary crude protein (CP) content. Five midlactation multicatheterized Jersey cows were fed 4 isoenergetic diets to provide 2 different carbohydrate compositions (CHO; rich in starch vs. rich in fiber) crossed by 2 different protein levels (12.0 vs. 16.5% CP) and according to a 4 × 4 Latin square design. Blood samples were collected at the end of each treatment period from the mesenteric artery and mammary vein to determine mammary net nutrient fluxes. The nature of nutrients taken up by the mammary gland differed between starch and fiber diets: mammary net uptake of acetate increased with fiber versus starch diets, whereas mammary net uptake and clearance rate of glucose increased with starch versus fiber diets but only at a normal CP level. In addition, the mammary net uptake of total, essential, and branched-chain AA (BCAA) was significantly enhanced (12, 11, and 26% on average, respectively) when feeding starch versus fiber diets, in line with a greater milk protein yield (7% on average) and regardless of the CP level. The conversion efficiency of plasma essential AA into milk protein was improved with starch diets (33.7% on average) compared with fiber diets (27.5% on average). This higher mammary efficiency use of AA with starch diets was accompanied by a greater fractional extraction and clearance rate of AA belonging to group 2 (BCAA, Lys, Thr) by the mammary gland in absence of effects of CHO on either the mammary blood flow or the mammary AA metabolism. The positive effect of starch diets on mammary clearance

rate and uptake of BCAA observed in this study was further improved when increasing dietary CP from 12.0 to 16.5%. Concerning the individual AA, Leu was the only whose mammary uptake accounted for a higher proportion of total essential AA in diets based on starch versus fiber and whose mammary uptake to milk output ratio was modified (together with Pro). Diets rich in starch versus fiber improved the mammary AA utilization; however, some CHO × CP interactions on mammary metabolism support the concept of different metabolic pathways by which starch diets improve milk protein yield at the 2 studied CP levels. Results from this study suggest that mammary Leu and glucose metabolism can be modulated by the supply of glucogenic nutrients to the mammary gland.

Key words: mammary gland, glucogenic, amino acid metabolism, efficiency

INTRODUCTION

Improving milk N efficiency (MNE), the proportion of feed N recovered in milk, is an important issue for dairy farms because it may enhance dairy incomes (Godden et al., 2001) and contribute to decrease the N pollution (Castillo et al., 2001). One of the most effective ways in dairy cattle to increase MNE (Huhtanen and Hristov, 2009), and then to reduce the manure N output (Yan et al., 2006), is to reduce the dietary CP content. However, low-protein diets may dramatically affect milk protein yield unless other dietary components, such as dietary energy content (Hanigan et al., 1998a) or AA profile (Haque et al., 2015), are concurrently modified.

Results from large data sets (North European data set in Huhtanen and Hristov, 2009) have shown that at similar energy and N intakes, diets rich in starch promoted higher MNE compared with diets rich in fiber. In this sense, we have recently found that the proportion of feed N absorbed into the portal vein as total amino acids (TAA), further released to the peripheral tissues and finally used for milk protein yield, was im-

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proved when the main dietary carbohydrate composition (**CHO**) changed from fiber to starch in iso-NE_L diets (Cantalapiedra-Hijar et al., 2014b). However, no direct AA-sparing effect of starch diets was found in that study (Cantalapiedra-Hijar et al., 2014b), in line with others (Larsen and Kristensen, 2012). One of the hypotheses arising was that starch diets increased milk N yield by improving the mammary anabolic use of AA compared with fiber diets, thus reducing the catabolic use of AA by the portal-drained viscera (Hanigan et al., 1998a, 2004) and liver (Reynolds, 2006) and indirectly improving their posthepatic availability.

Glucose availability for mammary metabolism is often considered limiting for lactose and, hence, milk production (Danfaer, 1994). However, increased glucose availability is not always associated with greater lactose synthesis (Lemosquet et al., 2009), as it is also able to increase milk protein yield at isoenergetic and isonitrogenous levels (Hurtaud et al., 2000; Rulquin et al., 2004). Indeed, although AA availability is the main limiting factor for milk protein synthesis (Bequette et al., 1998), the production of milk protein has also been shown to dramatically increase through endocrine signals only (McGuire et al., 1995; Griinari et al., 1997; Mackle et al., 2000). However, AA supply together with endocrine signals prompted by glucogenic nutrients seem to be highly interconnected in pathways involved in milk protein synthesis (Burgos et al., 2010; Bionaz and Looor, 2011), and some interactions between these stimuli have been previously documented at the molecular level (Vinod and Venkatesh, 2009; Burgos et al., 2010; Rius et al., 2010). Hence, a need to assess the effect of CHO on mammary metabolism at different protein intakes levels exists.

We hypothesized that the profile of nutrients arriving to the mammary gland when changing the main CHO from fiber to starch would increase the AA utilization by the mammary gland and that this effect would be different according to the protein supply. Thus, the aim of our study was to evaluate the effects of the dietary carbohydrate composition (starch vs. fiber diets) on mammary nutrient flux and milk performances in dairy cows fed isoenergetic diets at low (12.0% CP) versus normal (16.5% CP) protein levels.

MATERIALS AND METHODS

Details of this study have been described previously (Cantalapiedra-Hijar et al., 2014b). 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 and Experimental Design

Five multiparous Jersey cows in midlactation, averaging 365 ± 33 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 in the Latin square. Chronic indwelling catheters were surgically implanted into major splanchnic vessels (including mesenteric artery) before the lactation peak and at least 5 wk before the beginning of the experiment (Cantalapiedra-Hijar et al., 2014b).

Diet composition, chemical composition, and feed values of diets were described in detail in Cantalapiedra-Hijar et al. (2014a). Briefly, 4 iso-NE_L diets were formulated according to a 2×2 factorial arrangement of treatments to test 2 different CP contents [12.0 (low CP) and 16.5% (normal CP), meeting 80 and 100% of MP requirements (INRA, 2007), respectively] and 2 different carbohydrate compositions (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), leading to large variations in the profile of absorbed nutrients as outlined in Cantalapiedra-Hijar et al. (2014a).

Each experimental period lasted 28 d. To minimize postprandial variations, feed were offered from automated feeders in equal quantities every 3 h from d 1 through 24 and hourly after that point. Cows were housed in tiestalls lighted 24 h/d and water was freely available to all cows.

Measurements, Sampling, and Laboratory Analysis

Net fluxes of nutrients, blood gases, and hormones across the mammary gland were measured on d 27 of each 4-wk period, whereas milk yield was measured and sampled on d 27 and 28. Milk sampling was conducted twice a day at 0600 and 1700 h for milk composition analysis ($n = 4$ per treatment and cow), except for milk AA concentration where only the milk sampled at 1700 h on d 27 was analyzed. The AA composition of the milk protein was analyzed using a HPLC (Alliance System, Waters, Guyancourt, France) following protein hydrolysis with 6 N HCl (24 h). The sulfur AA (i.e., Met and Cys) were separately liberated by oxidation using performic acid before the protein hydrolysis.

Blood was collected hourly from the mesenteric artery [from 1130 to 1630 h ($n = 6$); 30 min before and after each meal] and from the mammary vein by venipuncture [right udder at 1130 and 1530 h; left udder at 1230 and 1630 h ($n = 4$)] into 9-mL tubes containing lithium heparin (5 IU/mL of blood) to determine glucose, lactate, BHBA, individual AA, VFA, O₂, and CO₂ concentrations and EDTA-K (10 μ L/mL of blood) to deter-

mine urea-N, ammonia-N, triglycerides, NEFA, insulin, and IGF-1 concentrations. Sampling procedures and laboratory analysis are detailed in Cantalapiedra-Hijar et al. (2014b). In addition, plasma IGF-1 concentration was determined by RIA (IGF-I RIA-CT; Mediagnost GmbH, Reutlingen, Germany) validated for the bovine.

Calculations and Statistical Analysis

Whole-blood O_2 concentration as well as heat production were calculated as previously detailed (Cantalapiedra-Hijar et al., 2014b), whereas whole-blood CO_2 concentration was calculated according to Siggaard-Andersen et al. (1988). The arteriovenous concentration (AV) difference of a nutrient, hormone, or blood gas was calculated as its mean arterial concentration ($n = 6$) minus its mean venous concentration ($n = 4$). Mammary plasma flow was calculated by the Fick principle based on uptake and output of Phe and Tyr and using a correction factor of 3.5% for blood-borne proteins secreted directly in milk (Cant et al., 1993); therefore, mammary plasma flow = (measured milk output of Phe + Tyr $\times 0.965$) / (measured arteriovenous difference of Phe + Tyr). Mammary blood flow was then calculated as mammary plasma flow divided by $(1 - \text{hematocrit})$.

Mammary net uptake of a nutrient, hormone, or blood gas was calculated as its AV difference multiplied by the mammary plasma (or blood) flow. Mammary fractional extraction of a nutrient, hormone or blood gas was calculated as 100 times its AV difference divided by its arterial concentration. Alternatively, to obtain information on the capacity of mammary net transport system, the mammary clearance rate of nutrients was calculated using the model of Hanigan et al. (1998b) as mammary plasma (or blood flow) $\times$ (AV difference / venous concentration). The uptake to milk output ratio of AA, as an indicator of mammary AA metabolism, was calculated as the net uptake of AA by the mammary gland, divided by the measured milk output of AA. Glutamic acid and Gln, as well as Asp and Asn milk output were individually calculated by using the proportion of Glu or Gln and Asp or Asn in milk true protein according to mean literature values reported by Lapierre et al. (2012).

A power test analysis (longpower package; Diggle et al., 2002) from a feeding trial using similar animal and diets as in the present study (pilot study; Cantalapiedra-Hijar et al., 2014a) revealed that to detect an effect of CHO on MNE (pilot estimate of residual variance), with an effect size of 8% between starch versus fiber diets (compatible with equations reported in the meta-analysis by Huhtanen and Hristov, 2009), a minimum sample size of 10 individuals per level of CHO (starch

vs. fiber) was required to reach a statistical power of 0.70 at $\alpha = 0.05$. However, when the power analysis was carried out for the 2 most important metabolic measurements to be analyzed in the present study (AA and glucose mammary net fluxes of AA and glucose), taking into account residual variances from published data (Lemosquet et al., 2009; Haque et al., 2015), reasonable effect size (20%; Rulquin et al., 2004; Rius et al., 2010), and sample size equal to 10 individuals per CHO level, the resulting statistical power ranged from 0.5 to 0.6.

A linear mixed model using the LME procedure within the nlme package (Pinheiro et al., 2008) in R software (R Development Core Team, 2009) and including a random intercept term for each cow was used. Statistical analyses were carried out as a 4×4 Latin square, following the model

$$Y_{ijkl} = \mu + CHO_i + CP_k + CHO \times CP_{kl} + P_i + C_j + \varepsilon_{ijkl},$$

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_i is the fixed effect of dietary carbohydrate composition (starch vs. fiber), CP_k is the fixed effect of dietary CP level (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. Residual normality was verified and confirmed for each variable by applying Shapiro-Wilk test. Mean values are reported as least squares means with pooled standard error of the mean values ($n = 4$) because of 1 cow $\times$ treatment missing observations (normal CP-starch diet in last period). To assess whether CHO affected the relationship between the AV difference of EAA and arterial concentration of EAA, an analysis of covariance (ANCOVA) was conducted and slopes and intercepts tested for differences across CHO (starch vs. fiber). Statistical significance was declared as $P \leq 0.05$ and trends at $P \leq 0.10$. When the $CHO \times CP$ was significant, the dietary treatment means were compared using Tukey HSD multiple comparison.

RESULTS

Milk Yield and Composition

Milk and ECM yield (Table 1) were not affected ($P \geq 0.65$) by CHO, although a tendency for starch diets to promote lower ECM compared with fiber diets was found ($CHO \times CP$; $P = 0.07$) at normal (-1.3 kg) but not at low CP levels (0.8 kg). Milk NPN, CP, and true

Table 1. Milk yield and composition in lactating Jersey cows fed diets based on either starch or fiber at 2 different dietary CP levels¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	P-value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Milk yield, kg/d	16.4	15.4	18.6	18.9	0.41	0.65	<0.001	0.12
ECM ³ , kg/d	20.0	19.2	22.2	23.5	0.52	0.67	<0.001	0.07
CP, g/kg	35.9	35.3	41.0	38.4	0.64	0.007	<0.001	0.14
True protein, g/kg	34.7	34.2	39.3	36.9	0.60	0.006	<0.001	0.15
Casein, %	81.7	82.0	79.8	82.3	0.72	0.11	0.28	0.14
NPN, mg/kg	178	178	264	237	8.34	0.08	<0.001	0.13
Urea-N, mg/kg	59.2	57.1	189	187	17.3	0.85	<0.001	0.99
Fat, g/kg	55.9	58.5	50.8	55.4	1.81	0.12	0.05	0.57
Lactose, g/kg	51.7	53.4	50.2	52.0	1.48	0.22	0.24	0.95
CP, g/d	588	536	757	725	22.3	0.05	<0.001	0.65
True protein, g/d	569	519	726	696	21.4	0.05	<0.001	0.61
NPN, g/d	2.93	2.75	4.89	4.43	0.20	0.09	<0.001	0.49
Fat, g/d	901 ^a	879 ^a	939 ^a	1,039 ^b	27.8	0.20	0.009	0.05
Lactose, g/d	847	825	931	992	26.5	0.28	0.002	0.14
AA, g/kg of true protein								
Ala	36.5	36.1	36.0	35.5	0.32	0.29	0.11	0.88
Arg	38.1	36.7	37.1	36.5	0.55	0.10	0.40	0.43
Asp + Asn	79.8	80.4	79.6	78.4	1.01	0.83	0.27	0.37
Glu + Gln	218	216	215	212	2.33	0.63	0.17	0.87
Cys	8.44	8.42	8.32	8.04	0.080	0.24	0.009	0.12
Gly	22.4	21.7	21.4	21.4	0.28	0.25	0.06	0.22
His	26.8	27.0	26.5	26.6	0.37	0.32	0.35	0.87
Ile	55.4	55.8	55.5	54.9	0.72	0.79	0.46	0.46
Leu	101	101	98.1	99.1	1.00	0.98	0.02	0.66
Lys	87.8	86.8	86.4	86.8	1.05	0.79	0.45	0.77
Met	30.6	29.3	29.8	29.8	0.57	0.21	0.93	0.29
Phe	52.7	51.6	52.3	50.8	1.29	0.50	0.72	0.88
Pro	102	105	101	100	1.70	0.27	0.09	0.18
Ser	60.4	59.2	59.4	58.3	0.82	0.32	0.26	0.93
Thr	47.7	46.6	47.5	46.6	0.74	0.28	0.88	0.82
Tyr	64.1	61.2	58.5	62.6	0.87	0.36	0.04	0.003
Val	70.5	70.7	70.5	70.2	0.77	0.68	0.68	0.74

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.

¹Least squares means presented with pooled SEM (given for $n = 4$); $n = 5$ for low CP-starch, low CP-fiber, and normal CP-starch and $n = 4$ for normal CP-fiber.

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

³ECM = $[(0.038 \times \text{g of crude fat} + 0.024 \times \text{g of CP} + 0.017 \times \text{g of lactose}) \times \text{kg of milk}]/3.14$.

protein concentrations and yield were higher ($P \leq 0.09$) when feeding starch versus fiber diets, but no effect of CHO on MUN was observed ($P = 0.85$). Milk fat and lactose concentrations and yields were unaffected ($P \geq 0.12$) by CHO, although at normal CP levels milk fat yield was higher when CHO changed from starch to fiber (11%; $\text{CHO} \times \text{CP}$; $P = 0.05$).

Diets formulated at 16.5% CP promoted higher ($P < 0.001$) milk and ECM yield as well as greater ($P < 0.001$) concentration and yield of all analyzed milk N fractions. Although milk fat and lactose concentration decreased ($P = 0.05$) and remained similar ($P = 0.24$), respectively, milk yield increased ($P \leq 0.009$) when changing from 12.0 to 16.5% CP. The casein proportion in milk true protein remained constant ($P = 0.11$) across dietary treatments.

Milk AA composition (g/kg of true protein) was not affected ($P > 0.05$) by CHO, although a tendency for Arg to be higher ($P = 0.10$) for starch versus fiber

diets was observed. Conversely, low-CP diets promoted higher ($P \leq 0.10$) milk concentrations of Cys, Gly, Leu, and Pro compared with normal CP diets. Milk Tyr concentration was higher at low-CP diets, but only with diets rich in starch ($\text{CHO} \times \text{CP}$; $P = 0.003$).

Mammary Flow and Arteriovenous Differences

Mammary plasma and blood flows (Table 2; L/h) were not affected either by CHO ($P \geq 0.38$) or CP ($P \geq 0.58$). However, as mammary flow rates were expressed per kilogram of milk yield, higher values were obtained ($P \leq 0.05$) for low- compared with normal CP diets.

Starch diets promoted lower AV difference for acetate ($P = 0.02$), but higher for BHBA ($P = 0.04$) and glucose ($P = 0.07$) compared with fiber diets (Table 3). Conversely, AV difference for these 3 nutrients was unaffected ($P \geq 0.20$) by CP level. Lactate AV difference tended ($P = 0.08$) to be lower with low-CP diets

Table 2. Mammary plasma and blood flow rates in lactating Jersey cows fed diets based on either starch or fiber at 2 different dietary CP levels¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × ES
Mammary plasma flow								
L/h	447	520	448	459	58	0.38	0.58	0.58
L/kg of milk	677	884	583	599	77	0.14	0.04	0.22
Mammary blood flow								
L/h	583	678	597	609	76	0.41	0.69	0.57
L/kg of milk	880	1,151	775	801	102	0.14	0.05	0.24

¹Least squares means presented with pooled SEM (given for $n = 4$); $n = 5$ for low CP-starch, low CP-fiber, and normal CP-starch and $n = 4$ for normal CP-fiber.

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

compared with normal CP diets, but unaffected ($P = 0.24$) by CHO. On the contrary, the AV difference of IGF-1 tended ($P = 0.06$) to be lower with normal CP diets (negative values) compared with low-CP diets (positive values). Higher ($P \leq 0.04$) AV difference of urea-N, TAA, AA belonging to group 1 and 2, EAA, branched-chain AA (BCAA), and NEAA, but not of ammonia-N ($P = 0.76$), were observed with normal versus low-CP diets, all being similar across CHO ($P \geq 0.20$).

When the AV difference of EAA across the mammary gland was regressed against their arterial concentration, a significant and positive relationship was found ($R^2 = 0.81$; $P < 0.001$, data not shown). The analysis of covariance (Figure 1) showed that starch versus fiber diets promoted higher ($P < 0.05$) AV difference of EAA across the mammary gland when compared at similar EAA arterial concentrations. No effect of either CHO ($P \geq 0.11$) or CP ($P \geq 0.16$) was found on AV difference of propionate, butyrate, NEFA, triglycerides, O_2 , CO_2 , and insulin, although a tendency ($P = 0.09$) for starch diets to have higher respiratory quotient was noted compared with fiber diets. Similar AV difference between starch and fiber diets was observed ($P > 0.10$) for all individual AA, except for Pro which was higher ($P = 0.05$) with starch versus fiber diets. Conversely, AV difference of all individual AA was greater ($P \leq 0.10$) at normal versus low CP levels, except ($P \geq 0.11$) for Ile, Lys, Asn, Glu, Gln, and Gly.

Mammary Net Uptake

Net fluxes of nutrients and hormones across the mammary gland as well as the calculated mammary heat production are presented in Table 4. Mammary net uptake of acetate was higher ($P = 0.04$) for fiber versus starch diets, whereas that of glucose was lower but only at normal CP levels (CHO × CP; $P = 0.02$). Likewise, when the mammary glucose uptake

was expressed per milk lactose output (on a carbon basis) values were higher for starch versus fiber diets, but only at normal CP levels (CHO × CP; $P = 0.001$). Mammary net uptake of TAA, EAA, AA belonging to group 2, and BCAA were higher ($P \leq 0.04$) with starch versus fiber diets, whereas that of ammonia-N, urea-N, AA belonging to group 1, and NEAA were similar ($P \geq 0.28$).

Net uptake of lactate across the mammary gland was different ($P = 0.03$) between low- and normal CP diets, a release of lactate at low CP and an uptake at normal CP was observed. Normal CP diets improved ($P \leq 0.08$) the net mammary uptake of TAA, EAA, AA belonging to group 1 and group 2, BCAA, and NEAA and lowered ($P = 0.04$) that of urea-N compared with low-CP diets. No changes across treatments were observed ($P > 0.10$) when mammary uptake of TAA was expressed per either net portal appearance or splanchnic release of TAA. No differences in mammary net uptake of propionate, butyrate, BHBA, NEFA, triglycerides, insulin, and ammonia-N were observed across either CHO ($P \geq 0.15$) or CP ($P \geq 0.17$) levels. Although heat production by the mammary gland numerically increased with starch versus fiber diets (17%), values were not significantly different ($P > 0.10$) across dietary treatments. Shifting from fiber to starch diets, the mammary net uptake increased ($P \leq 0.10$) for Tyr, Leu, Pro, and Gly, as well as for Thr and Met, but only at low CP (CHO × CP; $P \leq 0.08$). The mammary net uptake of individual AA was greater ($P \leq 0.10$) at normal versus low CP, except ($P \geq 0.13$) for His, Asn, Glu, Gln, and Gly. Finally, the profile of AA taken up by the mammary gland (regarded as the relative mammary uptake of a particular AA or group of AA compared with TAA or EAA) was similar ($P > 0.05$) across dietary treatments (data not shown), except for Leu, for which relative mammary uptake increased with starch versus fiber diets ($P = 0.001$) and with normal versus low-CP diets ($P = 0.008$).

Table 3. Arteriovenous difference across the mammary gland for nutrients, blood gases and hormones in lactating Jersey cows fed diets based on either starch or fiber at two different dietary CP levels¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Arteriovenous difference								
Glucose, μM	0.62	0.55	0.73	0.60	0.059	0.11	0.16	0.58
Acetate, μM	1.26	1.52	1.17	1.62	0.13	0.02	0.98	0.45
Propionate, μM	0.017	0.016	0.020	0.017	0.0059	0.70	0.70	0.85
Butyrate, μM	0.017	0.015	0.018	0.009	0.0038	0.14	0.66	0.30
BHBA, μM	0.43	0.32	0.37	0.34	0.035	0.04	0.50	0.25
Lactate, μM	−0.046	−0.014*	0.0048*	0.011*	0.019	0.24	0.08	0.49
NEFA, μM	−0.028	−0.030	−0.025	−0.023	0.0088	0.96	0.58	0.74
Triglycerides, μM	26.7*	10.5*	7.43*	22.0*	6.90	0.83	0.97	0.06
Ammonia-N, μM	0.015*	0.014*	0.022*	0.017*	0.013	0.76	0.76	0.84
Urea-N, μM	8.95*	14.6*	−37.0	−32.6	19.3	0.98	0.04	0.97
AA, ³ μmol of N/L								
TAA	455	319	555	577	58	0.20	0.01	0.19
EAA	290	204	340	361	41.1	0.30	0.03	0.20
Group 1	64.9	46.4	73.6	82.8	7.9	0.35	0.02	0.10
Group 2	226	157	266	279	33.4	0.29	0.03	0.24
BCAA	144	100	177	178	21.8	0.25	0.03	0.31
NEAA	163	114	215	217	25.4	0.21	0.02	0.32
Individual AA								
His	13.9	10.4	14.2	17.4	1.91	0.73	0.10	0.10
Met	12.0	8.49	13.9	16.1	1.83	0.47	0.03	0.13
Phe	18.2	13.5	20.2	24.2	2.12	0.55	0.01	0.06
Tyr	20.9	14.0	25.3	25.1	2.98	0.17	0.03	0.27
Ile	46.0	50.6	45.6	64.1	6.90	0.11	0.34	0.31
Leu	58.4	36.5	73.5	69.6	8.74	0.11	0.02	0.30
Lys	55.5	39.5	58.3	63.8	7.76	0.36	0.11	0.17
Thr	28.2	19.6	31.1	36.8	4.43	0.52	0.05	0.13
Val	41.8	30.7	53.2	53.1	7.36	0.40	0.05	0.45
Ala	−7.65	9.78	39.1	44.2	8.77	0.25	0.001	0.48
Pro	15.4	7.50	18.1	16.7	2.56	0.05	0.04	0.21
Ser	5.18*	−9.39*	9.86*	16.8	6.30	0.35	0.03	0.10
Asp	1.71	0.44	2.27	2.62	0.55	0.20	0.02	0.15
Asn	22.5	15.7	22.3	20.4	4.70	0.38	0.69	0.60
Glu	87.1	73.4	84.1	77.8	8.13	0.15	0.96	0.64
Gln	35.0	25.4	32.4	35.0	7.49	0.51	0.63	0.41
Gly	4.05*	−3.66*	6.20*	3.50*	5.23	0.22	0.39	0.62
Blood gases, μM								
O ₂	1.45	1.58	1.53	1.75	0.094	0.11	0.17	0.61
CO ₂	−2.59	−2.44	−2.88	−2.40	0.24	0.32	0.64	0.51
RQ ⁴	1.79	1.55	1.88	1.30	0.20	0.09	0.70	0.40
Hormones, nIU/L								
Insulin	−0.25*	0.12*	3.58*	3.74*	2.65	0.90	0.16	0.97
IGF-1	4.08*	1.49*	−8.85*	−13.1	6.08	0.61	0.06	0.89

¹Least squares means presented with pooled SEM (given for $n = 4$); $n = 5$ for low CP-starch, low CP-fiber, and normal CP-starch and $n = 4$ for normal CP-fiber.

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

³TAA = total 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); Group 1 = His, Met, Phe and Tyr (Mephram, 1982); Group 2 = BCAA, Lys and Thr (Mephram, 1982).

⁴Respiratory quotient.

* $P < 0.05$.

Fractional Extraction and Clearance Rates of Nutrients by the Mammary Gland

Mammary fractional extraction and clearance rates of nutrients are presented in Table 5. Fractional extraction by the mammary gland was improved ($P \leq 0.05$) for BHBA, EAA, AA belonging to group 2, and BCAA, but not ($P \geq 0.16$) for lactate, TAA, AA belonging to

group 1, and NEAA when CHO changed from fiber to starch. The fractional extraction by the mammary gland was higher ($P \leq 0.03$) at normal versus low CP for lactate, TAA, and NEAA, but remained unchanged ($P \geq 0.11$) for that of BHBA, AA belonging to group 1 and group 2, EAA, and BCAA. No changes in the mammary fractional extraction were observed for other nutrients across either CHO ($P \geq 0.18$) or CP levels (P

Figure 1. Relationship between mammary arteriovenous difference of EAA and arterial concentration of EAA for starch (Δ) and fiber ($\bullet$) diets. Individual values are adjusted for animal and period effects. The analysis of covariance showed that both equations had similar slopes (0.485 vs. 0.576 for starch and fiber diets, respectively; $P = 0.33$), but different intercept (-41.8 vs. -174 for starch and fiber diets, respectively; $P = 0.03$).

≥ 0.18). The mammary fractional extraction increased ($P \leq 0.09$) for Met, Phe, Tyr, Ile, and Pro and decreased ($P = 0.06$) for His when feeding starch versus fiber and was greater ($P \leq 0.10$) for normal versus low-CP diets for most individual AA, except ($P \geq 0.11$) for Met, Ile, Lys, Val, Asn, Glu, Gln, and Gly.

Similar effects of CHO ($P \leq 0.10$) on mammary fractional extraction and clearance rates were found for most nutrients. However, as CHO shifted from fiber to starch, the mammary clearance rate, unlike the fractional extraction, was improved for glucose (at normal but not at low CP; $P = 0.02$), TAA ($P = 0.02$), AA belonging to group 1 ($P = 0.002$), Lys, and Gly ($P = 0.08$), and remained unchanged for BHBA ($P = 0.35$) and Glu ($P = 0.16$). Similarly, when shifting from 12.0 to 16.5% CP diets the mammary clearance rate, but not the fractional extraction rate, was improved ($P \leq 0.10$) for AA belonging to group 2, BCAA, Ile, and Asn and remained unchanged for Thr ($P = 0.18$).

AA Inflow to the Mammary Gland and Mammary AA Metabolism

Amino acid inflow to the mammary gland was not modified by CHO ($P > 0.05$), although a tendency ($P = 0.09$) for diets rich in starch to promote greater His

inflow to the mammary gland and lower of Val and Ile compared with diets rich in fiber was found (Table 6). Increasing the dietary CP level only improved ($P < 0.10$) the EAA, AA belonging to group 1, and His inflow to the mammary gland and decreased ($P = 0.06$) that of Gly.

The uptake to milk output ratio (Table 6) of all AA was similar ($P > 0.10$) across CHO [except for Leu and Pro, with higher ($P \leq 0.04$) values for starch vs. fiber diet] and across CP levels [except for Leu, Ala, and Ser, with higher ($P \leq 0.07$) values at normal vs. low CP, and Glu with lower ($P = 0.01$) values].

The efficiency of AA use by the mammary gland, defined as the milk output relative to the total mammary inflow, increased ($P \leq 0.10$) when feeding starch versus fiber diets for EAA, AA belonging to group 2, BCAA, and for Met, Phe, Tyr, Ile, Thr, Val, and Ala. When changing the CP level of diets from 12.0 to 16.5% this efficiency was also improved ($P \leq 0.10$) for TAA, AA belonging to group 2, NEAA, and most individual AA, except ($P \geq 0.13$) for Met, Leu, Lys, Val, and Asp.

DISCUSSION

The effect of protein supply on mammary metabolism has been previously discussed in other studies (Guinard

Table 4. Mammary net uptake of nutrients, blood gases, and hormones in lactating Jersey cows fed diets based on either starch or fiber at 2 different dietary CP levels¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	P-value ³		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Mammary net uptake, ² mmol/h								
Glucose	352 ^a	370 ^a	425 ^b	335 ^a	18.7	0.11	0.16	0.02
Milk lactose output ⁴	1.68 ^{ab}	1.96 ^b	1.92 ^b	1.42 ^a	0.090	0.27	0.35	0.001
Acetate	727	959	695	956	114	0.04	0.82	0.90
Propionate	9.57	10.2	12.3	9.17	3.48	0.81	0.79	0.89
Butyrate	9.56	10.8	11.0	5.88	2.63	0.42	0.59	0.23
BHBA	247	216	224	200	28.7	0.35	0.47	0.88
Lactate	-29.7	-9.74*	12.1*	17.0*	13.6	0.30	0.03	0.57
NEFA	-11.6	-15.7	-12.0	-9.48	4.92	0.91	0.56	0.49
Triglycerides	11.8*	5.72*	2.98*	8.71*	3.40	0.72	0.71	0.11
Ammonia-N	5.23*	6.08*	10.8*	6.03*	6.30	0.71	0.65	0.65
Urea-N	4.62*	8.62*	-16.4*	-17.3*	8.20	0.92	0.02	0.76
AA, ⁵ μmol of N/h								
TAA	253	211	309	289	14.3	0.04	0.001	0.45
Mammary/Portal ⁶	0.55	0.61	0.55	0.54	0.046	0.50	0.48	0.44
Mammary/Splanchnic ⁷	0.75	0.92	1.03	0.96	0.090	0.43	0.21	0.28
EAA	158	131	189	181	4.64	0.005	<0.001	0.08
EAA/TAA	0.63	0.61	0.62	0.63	0.031	0.97	0.98	0.62
Group 1	39.6	33.4	45.3	46.7	2.13	0.21	0.002	0.09
Group 1/EAA	0.25	0.26	0.24	0.26	0.010	0.30	0.48	0.52
Group 2	119	97.6	144	134	4.62	0.007	<0.001	0.22
Group 2/EAA	0.75	0.76	0.76	0.74	0.010	0.30	0.48	0.52
BCAA	61.4	50.2	78.6	69.1	2.96	0.006	0.002	0.76
BCAA/EAA	0.39	0.39	0.41	0.39	0.013	0.43	0.38	0.32
NEAA	94.0	80.0	120	108	13.7	0.28	0.08	0.92
NEAA/TAA	0.37	0.39	0.38	0.37	0.031	0.97	0.99	0.62
Individual AA								
His	17.7	15.4	18.9	21.0	1.95	0.98	0.13	0.26
Met	5.16	4.22	6.15	6.26	0.279	0.08	<0.001	0.08
Phe	7.98	6.75	8.99	9.69	0.609	0.51	0.01	0.13
Tyr	8.84	7.02	11.3	9.83	0.37	0.002	0.001	0.60
Ile	43.2	32.7	50.5	55.1	6.55	0.50	0.04	0.25
Leu	25.0	18.3	32.7	27.3	0.88	0.001	<0.001	0.45
Leu/EAA	0.16	0.14	0.17	0.15	0.0040	0.001	0.008	0.53
Lys	46.7	39.1	51.5	50.6	2.82	0.15	0.02	0.24
Thr	11.9	9.82	13.9	14.2	0.38	0.02	<0.001	0.01
Val	17.9	15.4	23.5	20.6	1.48	0.13	0.006	0.89
Ala	-3.22	4.72	17.7	17.2	3.06	0.22	<0.001	0.18
Pro	6.46	3.72	8.09	6.33	0.50	<0.001	0.002	0.33
Ser	1.35*	-5.00	4.18*	6.09	2.68	0.27	0.03	0.14
Asp	0.73	0.17	1.00	1.09	0.26	0.20	0.03	0.22
Asn	18.7	15.3	19.3	16.3	3.12	0.40	0.84	0.94
Glu	38.5	37.5	37.1	34.4	4.63	0.61	0.60	0.84
Gln	30.1	25.7	29.2	27.1	6.24	0.54	0.94	0.85
Gly	1.58*	-1.96*	3.20*	-0.40*	2.39	0.10	0.49	0.99
Hormones, nIU/L								
Insulin	-121*	-381*	1,463*	1,814*	1,283	0.95	0.17	0.81
IGF-1	2,023*	773*	-3,620*	-3,569*	2,173	0.81	0.05	0.76
Heat production, ⁸ MJ/d	8.86	11.4	10.0	10.7	1.16	0.15	0.77	0.43

¹Least squares means presented with pooled SEM (given for n = 4); n = 5 for low CP-starch, low CP-fiber, and normal CP-starch and n = 4 for normal CP-fiber.

²Mammary net uptake = mammary blood or plasma flow × arteriovenous difference; a negative value indicates nutrient release rather than uptake.

³CHO = effect of dietary carbohydrate (starch or fiber); CP = effect of CP.

⁴Calculated on a carbon basis.

⁵TAA = total 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); Group 1 = His, Met, Phe, and Tyr (Mepharm, 1982); Group 2 = BCAA, Lys, and Thr.

⁶Mammary/Portal = mammary net uptake of total AA divided by net portal appearance of total AA (as reported in Cantalapiedra-Hijar et al., 2014b).

⁷Mammary/Splanchnic = mammary net uptake of total AA divided by net splanchnic release of total AA (as reported in Cantalapiedra-Hijar et al., 2014b).

⁸Heat production calculated as 4.89 kcal/L of O₂ consumed (McLean, 1972).

*P < 0.05 (probability that the variable is not different from zero within each treatment, tested using Student's *t*-value).

and Rulquin, 1994; Raggio et al., 2006; Doepel and Lapierre, 2010); hence, the discussion will mainly focus on the effect of dietary carbohydrate composition on mammary metabolism and its possible interaction with the dietary CP level.

Mammary AA Use with Starch Versus Fiber Diets

Our results show that mammary net uptake of TAA, EAA, and BCAA was significantly enhanced (12, 11, and 26% on average, respectively) when feeding starch versus fiber diets, in line with a greater milk protein yield (7% on average) and regardless of the dietary protein content (i.e., lack of significant CHO $\times$ CP interaction). An additive effect of glucogenic substrates and AA supply on milk protein yield has been observed in several experiments carried out in dairy cows (Raggio et al., 2006; Lemosquet et al., 2009; Rius et al., 2010).

In our study, we considered 2 interpretations. First, the positive effect of starch on mammary AA uptake might be interpreted as a consequence of the greater availability of absorbed AA for peripheral tissues (15% net splanchnic release of total AA; Cantalapiedra-Hijar et al., 2014b). Indeed, no differences were found when mammary TAA uptake was expressed with respect to either net portal appearance or net splanchnic release of TAA. This might indicate that mammary uptake of AA is driven by mass action and that the higher availability of AA for peripheral tissues promoted by starch diets could be the consequence of a higher supply of digestible protein to the small intestine. However, the present experiment also showed that the conversion efficiency of plasma EAA into milk protein (milk EAA output/mammary EAA inflow; the latter remaining constant across CHO) was improved with starch diets (33.7% on average) compared with fiber diets (27.5% on average), in line with a greater clearance rate. Consequently, our results may support the concept that the improvement in mammary AA use could have contributed per se to decrease the splanchnic AA catabolism (Hanigan et al., 1998a, 2004; Reynolds, 2006) and thus to enhance the posthepatic AA availability on a net basis (22% net splanchnic release of EAA; Cantalapiedra-Hijar et al., 2014b). Likewise, when the AV difference of EAA across the mammary gland was compared at similar arterial concentrations, starch diets promoted a higher fractional extraction compared with fiber diets (Figure 1). These results agree, considering the splanchnic tissues are a responder (to peripheral AA use) rather than a driver of the AA use by peripheral tissues (Reynolds, 2002; Hanigan et al., 2004).

The AA utilization by the mammary gland for milk protein synthesis is under a finely tuned control system (Clark et al., 1978). Based on the balance be-

tween supply and requirements, the mammary gland is capable of responding to changes by modulating the fractional extraction (Mackle et al., 2000) or clearance rate (Bequette et al., 2000), the blood flow (Prosser et al., 1996), and the intracellular metabolism of AA (Mephram, 1982). Present results show that the higher mammary efficiency of use of AA with starch versus fiber diets was mainly the consequence of a clearance rate of EAA by the mammary gland in absence of effects of CHO on either the mammary blood (plasma) flow or the mammary net uptake to milk output ratio of AA. Our results, thus, may suggest that the mammary gland became more sensitive to the supply of EAA when feeding starch versus fiber diets and increased its ability to extract AA from plasma.

The greater fractional extraction and clearance rates of EAA with starch versus fiber diets was mostly accounted for by AA belonging to group 2 (Mephram, 1982; BCAA, Lys, and Thr), whose mammary uptake is usually greater than their milk output (1.4 on average in the present experiment). The metabolism of these particular EAA is crucial for the formation of the intracellular pool of NEAA (Davis and Mephram, 1976), whose mammary uptake (0.63 on average in the present experiment) is not adequate to account for the needs of milk protein synthesis. It was noted in our study that the clearance rates of all NEAA (except of Pro), unlike that of most EAA, remained similar between starch and fiber diets. This could imply upregulation for the activity of some AA transport systems [Baumrucker, 1985; L-system (Phe, Tyr, Met, and BCAA) and y+L system (Lys), whose clearance rate in the present experiment was improved with starch vs. fiber], but not of others [Baumrucker, 1985; A-system (Pro, Ala, and Gly), N-system (His), ASC-system (Thr and Ser), X_{AG}-system (Asp and Glu), with no changes in the clearance rate across CHO, except for Pro]. This selective upregulation of AA transporters may be mediated in part by changes in endocrine signaling (Bionaz and Looor, 2011). In this regard, a role of insulin in the cellular metabolism of EAA to meet the requirements of NEAA for milk protein synthesis has been hypothesized (Menzies et al., 2009).

The positive effect of starch diets on mammary gland affinity (regarded as clearance rate) and uptake of BCAA observed in our study was further improved when increasing dietary CP level (CHO $\times$ CP, $P = 0.03$), thus explaining the CHO $\times$ CP interaction also observed for BCAA arterial concentrations [lower (−23%) BCAA arterial concentration with starch vs. fiber only at normal CP levels; Cantalapiedra-Hijar et al., 2014b] as well as in other studies infusing glucogenic substrates and casein in dairy cows (Raggio et al., 2006; Rius et al., 2010). In this regard, the expression

Table 5. Mammary fractional extraction and clearance of nutrients in lactating Jersey cows fed diets based on either starch or fiber at 2 different dietary CP levels¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Glucose								
Fractional extraction, ³ %	20.1	19.0	23.8	20.7	2.14	0.27	0.18	0.63
Clearance rate, ⁴ L/h	145 ^a	158 ^a	184 ^b	149 ^a	9.41	0.25	0.07	0.02
Acetate								
Fractional extraction, %	73.6	72.2	73.0	72.9	3.65	0.79	0.91	0.85
Clearance rate, L/h	1,644	1,424	1,694	1,758	260	0.74	0.46	0.57
Propionate								
Fractional extraction, %	23.8	19.6	23.5	24.8	6.27	0.80	0.69	0.65
Clearance rate, L/h	187	177	205	181	64	0.80	0.85	0.90
Butyrate								
Fractional extraction, %	45.6	51.8	56.5	49.7	4.89	0.83	0.26	0.19
Clearance rate, L/h	531	809	627	559	133	0.46	0.71	0.20
BHBA								
Fractional extraction, %	49.7	45.3	50.6	44.8	1.75	0.02	0.95	0.68
Clearance rate, L/h	580	566	643	489	78.0	0.35	0.93	0.36
Lactate								
Fractional extraction, %	−14.3	−4.79*	0.49*	6.19*	5.85	0.26	0.03	0.48
Clearance rate, L/h	−68.6*	−35.3*	37.6*	115	59.3	0.28	0.07	0.70
NEFA								
Fractional extraction, %	−23.0	−28.8	−38.2	−26.4	10.3	0.60	0.60	0.39
Clearance rate, L/h	−81.6	−115	−113	−85.3	29.1	0.97	0.95	0.30
Triglycerides								
Fractional extraction, %	−7.59*	8.85*	2.78*	16.6*	13.6	0.37	0.45	0.92
Clearance rate, L/h	23.7*	74.4*	19.1*	84.1*	52.4	0.39	0.81	0.89
Ammonia-N								
Fractional extraction, %	3.33	1.29*	4.11	1.39*	1.83	0.23	0.92	0.85
Clearance rate, L/h	13.3*	5.70*	19.5	5.50*	9.15	0.27	0.80	0.72
Urea-N								
Fractional extraction, %	0.25*	0.23*	−0.35*	−0.34*	0.32	0.86	0.10	0.95
Clearance rate, L/h	1.78*	1.82*	−1.97*	−2.37*	2.03	0.80	0.09	0.91
AA ⁵								
TAA								
Fractional extraction, %	24.8	19.1	29.9	29.5	2.60	0.16	0.02	0.31
Clearance rate, L/h	127	105	179	155	8.69	0.02	<0.001	0.93
EAA								
Fractional extraction, %	43.0	33.9	46.2	39.4	3.36	0.05	0.29	0.72
Clearance rate, L/h	305	226	371	244	22.4	0.002	0.11	0.29
Group 1								
Fractional extraction, %	41.5	33.8	38.0	38.6	3.58	0.52	0.83	0.27
Clearance rate, L/h	306	204	307	264	29.1	0.05	0.49	0.31
Group 2								
Fractional extraction, %	43.8	34.0	50.1	40.0	3.40	0.02	0.12	0.97
Clearance rate, L/h	308	233	399	242	23.7	0.001	0.07	0.11
BCAA								
Fractional extraction, %	37.2	29.8	44.7	33.8	3.24	0.02	0.11	0.58
Clearance rate, L/h	270 ^b	221 ^a	360 ^c	208 ^a	21.3	0.002	0.09	0.03
NEAA								
Fractional extraction, %	14.4	11.2	19.0	20.9	2.21	0.47	0.01	0.25
Clearance rate, L/h	62.3	55.9	97.9	98.1	9.09	0.43	0.002	0.71
Individual AA								
His								
Fractional extraction, %	54.0	60.0	36.4	46.0	5.84	0.06	0.009	0.74
Clearance rate, L/h	711	873	334	509	153	0.09	0.02	0.96
Met								
Fractional extraction, %	36.6	24.0	38.3	34.0	4.92	0.08	0.25	0.39
Clearance rate, L/h	264	166	284	214	35.6	0.03	0.33	0.69
Phe								
Fractional extraction, %	35.2	24.6	40.8	38.8	2.72	0.02	0.006	0.13
Clearance rate, L/h	247	167	327	275	35.6	0.07	0.03	0.68
Tyr								
Fractional extraction, %	39.0	27.7	51.8	42.0	5.15	0.05	0.03	0.88
Clearance rate, L/h	342	200	539	325	85.0	0.05	0.07	0.67

Continued

Table 5 (Continued). Mammary fractional extraction and clearance of nutrients in lactating Jersey cows fed diets based on either starch or fiber at 2 different dietary CP levels¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -value ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Ile								
Fractional extraction, %	41.5	32.6	49.5	37.5	3.70	0.02	0.12	0.68
Clearance rate, L/h	330	250	453	246	33.2	0.003	0.09	0.08
Leu								
Fractional extraction, %	42.9	39.1	53.6	42.1	3.75	0.07	0.08	0.30
Clearance rate, L/h	346	335	518	308	40.6	0.03	0.07	0.03
Lys								
Fractional extraction, %	70.4	58.9	70.4	63.3	7.00	0.30	0.98	0.74
Clearance rate, L/h	1,285	647	1,275	815	242	0.08	0.97	0.71
Thr								
Fractional extraction, %	32.1	24.1	39.0	33.7	4.70	0.12	0.08	0.78
Clearance rate, L/h	211	183	290	211	44.8	0.19	0.18	0.56
Val								
Fractional extraction, %	29.1	21.7	34.0	24.7	3.28	0.04	0.33	0.76
Clearance rate, L/h	186	146	228	134	19.9	0.02	0.63	0.19
Ala								
Fractional extraction, %	−3.73	4.42	21.1	19.3	3.45	0.40	<0.001	0.17
Clearance rate, L/h	−8.31	24.00	120	103	14.1	0.66	<0.001	0.10
Pro								
Fractional extraction, %	22.2	13.1	24.2	23.6	3.14	0.09	0.08	0.19
Clearance rate, L/h	130	77.0	143	124	15.5	0.03	0.09	0.28
Ser								
Fractional extraction, %	3.77	−8.40	12.0	17.3	5.68	0.34	0.01	0.14
Clearance rate, L/h	18.0	−40.7	63.3	83.5	26.6	0.29	0.01	0.15
Asp								
Fractional extraction, %	9.47	6.94	16.8	14.8	2.60	0.19	0.008	0.91
Clearance rate, L/h	49.6	43.5	97.9	80.7	14.1	0.22	0.008	0.69
Asn								
Fractional extraction, %	36.2	30.0	43.7	46.3	7.60	0.78	0.11	0.56
Clearance rate, L/h	286	215	424	382	89.5	0.55	0.09	0.86
Glu								
Fractional extraction, %	35.8	27.2	33.3	30.6	2.44	0.02	0.94	0.24
Clearance rate, L/h	249	198	219	201	26.3	0.16	0.53	0.53
Gln								
Fractional extraction, %	22.7	16.3	19.2	28.4	4.61	0.95	0.37	0.11
Clearance rate, L/h	137	101	113	163	26.9	0.96	0.48	0.13
Gly								
Fractional extraction, %	0.43*	−1.30*	1.80*	0.73*	1.55	0.22	0.27	0.82
Clearance rate, L/h	2.09*	−6.72*	10.5*	−2.65*	6.99	0.08	0.35	0.75

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$

¹Least squares means presented with pooled SEM (given for $n = 4$); $n = 5$ for low CP-starch, low CP-fiber, and normal CP-starch and $n = 4$ for normal CP-fiber.

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

³Fractional extraction = [arteriovenous difference/arterial concentration] × 100.

⁴Clearance rate = mammary blood or plasma flow × (arteriovenous difference/venous concentration).

⁵TAA = total 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); Group 1 = His, Met, Phe, and Tyr (Mepharm, 1982); Group 2 = BCAA, Lys, and Thr.

* $P < 0.05$ (probability that the variable is not different from zero within each treatment, tested using Student's *t*-value).

of mammary tissue proteins involved in the transport of BCAA appears to be under control of the mammalian target of rapamycin (**mTOR**) pathway (Bionaz and Loor, 2011), which is activated via the insulin signaling cascade and seems to be modulated by the level of AA supply (Burgos et al., 2010; Rius et al., 2010). As discussed in next section, the AA supply and insulin concentration seemed to interact on mammary glucose and BCAA (Leu) metabolism in our study.

Mammary Response to Nutrient Supply and Endocrine Signals

As expected, the nature of nutrients taken up by the mammary gland differed between starch and fiber diets was revealed by differences in the calculated respiratory quotient (1.84 vs. 1.43, respectively, on average). In particular, acetate net uptake increased (35% on average) with fiber versus starch diets, likely driven by

Table 6. Mammary inflow, uptake to output ratio and efficiency use of AA in dairy cows fed diets based on either starch or fiber at 2 different dietary CP levels¹

	Low CP (12.0%)		Normal CP (16.5%)			P-values ²		
Item	Starch	Fiber	Starch	Fiber	SEM	CHO	CP	CHO × CP
TAA ³								
Mammary inflow ⁴ , mmol of N/h	1,023	1,110	1,064	1,039	122	0.68	0.96	0.64
Uptake/milk output, ⁵ %	103*	103*	103*	104*	4.59	0.90	0.95	0.94
Efficiency, output/inflow, ⁶ %	23.8	18.7	29.1	28.7	2.36	0.16	0.01	0.32
EAA								
Mammary inflow, mmol of N/h	375	385	421	466	38.7	0.52	0.09	0.64
Uptake/milk output, %	135	133	131	134	5.56	0.82	0.91	0.67
Efficiency, output/inflow, %	32.1	25.4	35.2	29.6	2.12	0.02	0.20	0.80
Group 1								
Mammary inflow, mmol of N/h	101	98.9	128	123	12.8	0.53	0.04	0.90
Uptake/milk output, %	114	116	109*	117	6.05	0.36	0.66	0.56
Efficiency, output/inflow, %	36.5	29.1	34.8	33.3	2.93	0.24	0.98	0.31
Group 2								
Mammary inflow, mmol of N/h	274	286	293	346	27.2	0.23	0.15	0.45
Uptake/milk output, %	142	138	140	139	6.37	0.82	0.79	0.77
Efficiency, output/inflow, %	30.8	24.3	35.7	28.5	1.99	0.006	0.08	0.86
BCAA								
Mammary inflow, mmol of N/h	169	171	176	226	21.6	0.23	0.16	0.27
Uptake/milk output, %	142	135	145	138	7.35	0.48	0.52	0.98
Efficiency, output/inflow, %	26.9	22.0	31.2	24.6	2.15	0.02	0.19	0.69
NEAA								
Mammary inflow, mmol of N/h	647	725	643	576	88.0	0.76	0.40	0.41
Uptake/milk output, %	62.0	63.5	64.7	62.9	6.76	0.92	0.88	0.80
Efficiency, output/inflow, %	23.2	17.8	29.7	33.8	3.46	0.57	0.009	0.18
Individual AA								
His								
Mammary inflow, mmol of N/h	38.3	26.8	66.1	50.9	6.06	0.01	<0.001	0.76
Uptake/milk output, %	140	144	124	144	15.7	0.39	0.56	0.59
Efficiency, output/inflow, %	41.1	42.9	28.0	32.0	4.96	0.24	0.02	0.82
Met								
Mammary inflow, mmol of N/h	15.3	17.8	17.0	21.6	3.00	0.23	0.39	0.70
Uptake/milk output, %	104*	103*	102*	110*	5.26	0.53	0.52	0.39
Efficiency, output/inflow, %	35.5	23.2	38.4	30.7	4.22	0.03	0.24	0.57
Phe								
Mammary inflow, mmol of N/h	23.6	28.5	22.3	26.7	3.05	0.12	0.64	0.90
Uptake/milk output, %	105*	106*	92.7*	110*	5.00	0.12	0.44	0.11
Efficiency, output/inflow, %	34.9	23.4	43.6	35.9	2.77	0.005	0.005	0.49
Tyr								
Mammary inflow, mmol of N/h	24.3	26.0	22.8	25.5	2.79	0.40	0.72	0.85
Uptake/milk output, %	93.3*	92.7*	104*	89.5	3.94	0.11	0.34	0.09
Efficiency, output/inflow, %	40.5	29.7	50.2	46.3	4.54	0.08	0.02	0.45
Ile								
Mammary inflow, mmol of N/h	46.0	50.5	45.6	64.1	6.94	0.09	0.34	0.31
Uptake/milk output, %	181	189	173	181	11.3	0.39	0.72	0.99
Efficiency, output/inflow, %	23.4	17.5	28.6	21.0	1.96	0.006	0.08	0.65
Leu								
Mammary inflow, mmol of N/h	59.8	47.8	60.9	71.2	7.25	0.87	0.11	0.14
Uptake/milk output, %	135	118*	142	130	6.50	0.04	0.07	0.70
Efficiency, output/inflow, %	33.4	33.6	38.4	33.6	3.08	0.49	0.55	0.40
Lys								
Mammary inflow, mmol of N/h	67.4	69.1	76.5	78.4	8.20	0.95	0.18	0.99
Uptake/milk output, %	158	160	144	150	10.6	0.58	0.31	0.81
Efficiency, output/inflow, %	44.5	37.2	49.6	42.3	4.63	0.19	0.41	0.98
Thr								
Mammary inflow, mmol of N/h	37.3	46.5	40.1	45.0	6.09	0.18	0.96	0.72
Uptake/milk output, %	122	121	114	126	6.40	0.37	0.82	0.33
Efficiency, output/inflow, %	26.4	20.2	34.4	26.4	3.70	0.05	0.06	0.79
Val								
Mammary inflow, mmol of N/h	63.6	72.4	70.0	90.1	8.14	0.09	0.14	0.47
Uptake/milk output, %	124	121	128	120	9.84	0.83	0.91	0.79
Efficiency, output/inflow, %	24.0	17.6	27.0	20.5	1.97	0.007	0.23	0.99

Continued

Table 6 (Continued). Mammary inflow, uptake to output ratio and efficiency use of AA in dairy cows fed diets based on either starch or fiber at 2 different dietary CP levels¹

Item	Low CP (12.0%)		Normal CP (16.5%)		SEM	<i>P</i> -values ²		
	Starch	Fiber	Starch	Fiber		CHO	CP	CHO × CP
Ala								
Mammary inflow, mmol of N/h	93.3	103	85.9	94.0	10.1	0.27	0.38	0.96
Uptake/milk output, %	−39.1	50.6*	145*	149*	30.4	0.11	0.002	0.17
Efficiency, output/inflow, %	10.6	8.10	14.9	12.7	1.12	0.03	0.003	0.92
Pro								
Mammary inflow, mmol of N/h	29.4	28.4	34.9	29.1	3.21	0.35	0.29	0.44
Uptake/milk output, %	29.4	19.9	30.4	25.3	2.79	0.02	0.26	0.42
Efficiency, output/inflow, %	73.3	65.9	79.7	90.3	5.87	0.95	0.03	0.14
Ser								
Mammary inflow, mmol of N/h	50.4	54.7	46.6	38.3	7.47	0.96	0.17	0.39
Uptake/milk output, %	4.57	−41.3	26.5	37.6	18.9	0.25	0.02	0.15
Efficiency, output/inflow, %	29.0	21.7	40.0	42.8	4.46	0.37	0.005	0.26
Asp								
Mammary inflow, mmol of N/h	8.01	7.94	8.90	8.90	1.64	0.79	0.70	0.98
Uptake/milk output, %	10.8	4.00	12.2	14.1	3.71	0.32	0.12	0.25
Efficiency, output/inflow, %	91.2	89.1	124	118	20.8	0.72	0.13	0.93
Asn								
Mammary inflow, mmol of N/h	50.4	52.9	52.1	40.7	9.04	0.74	0.53	0.43
Uptake/milk output, %	46.4	44.1	41.1	35.9	6.61	0.81	0.30	0.82
Efficiency, output/inflow, %	81.1	67.9	101	136	21.5	0.81	0.05	0.26
Glu								
Mammary inflow, mmol of N/h	110	138	114	116	18.7	0.35	0.68	0.49
Uptake/milk output, %	266	304	202	202	26.8	0.52	0.01	0.48
Efficiency, output/inflow, %	19.5	12.5	22.9	24.1	3.20	0.27	0.04	0.21
Gln								
Mammary inflow, mmol of N/h	142	170	152	132	28.4	0.81	0.72	0.39
Uptake/milk output, %	98.2*	101*	73.7*	77.4*	16.6	0.90	0.20	0.97
Efficiency, output/inflow, %	22.8	17.2	27.2	36.7	5.44	0.83	0.06	0.18
Gly								
Mammary inflow, mmol of N/h	165	176	149	125	16.7	0.82	0.06	0.29
Uptake/milk output, %	18.7	−38.8	34.7	−8.77	28.3	0.06	0.43	0.80
Efficiency, output/inflow, %	4.53	3.55	6.07	7.75	0.71	0.87	0.003	0.08

¹Least squares means presented with pooled SEM (given for *n* = 4); *n* = 5 for low CP-starch, low CP-fiber, and normal CP-starch and *n* = 4 for normal CP-fiber.

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

³TAA = total 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); Group 1 = His, Met, Phe, and Tyr (Mepharm, 1982); Group 2 = BCAA, Lys, and Thr.

⁴Total inflow = arterial concentration × mammary plasma flow.

⁵Fractional extraction = [arteriovenous difference/arterial concentration] × 100.

⁶Clearance rate = mammary blood or plasma flow × (arteriovenous difference/venous concentration).

**P* < 0.05 (probability that the variable is not different from 100% within each treatment, tested using Student's *t*-value).

mass action (47% higher total acetate inflow with fiber vs. starch; data not shown), as previously suggested (Cant et al., 1993). Conversely, glucose net uptake, in line with its mammary clearance rate, increased with starch versus fiber diets, but only at normal CP levels (34%). Glucose uptake by the mammary gland has been unrelated to its arterial concentration in different studies (Cant et al., 1993; Lemosquet et al., 2009) and has been proposed to be regulated, in part, by factors within the mammary gland (Madsen et al., 1999). The observed CHO × CP interaction in the present study on net uptake and clearance rate of glucose, as well as in the clearance rate of BCAA, may suggest an interconnection between glucose uptake and protein synthesis via the mTOR signaling pathway (Bionaz and

Loor, 2011). It is already known that the activation of mTOR complex increases expression of glucose transporters (GLUT1 expression; Bionaz and Loor, 2011), the former being strongly regulated by the BCAA, in particular Leu (Kimball and Jefferson, 2006) and insulin concentrations (Menzies et al., 2009). In this regard, Leu was the only AA whose net uptake accounted for a higher proportion of total EAA in diets based on starch versus fiber, no other differences in the profile of AA taken up by the mammary gland were found in the present experiment (data not shown). In addition, the intramammary AA metabolism, regarded as the uptake to milk output ratio of AA, was only modified by CHO for Leu (139 vs. 124 on average) and not for any other individual AA, except Pro. Conversely, the mammary

efficiency use of AA (milk output/mammary inflow) increased for most EAA, except for Leu (as well as Lys and His). These results suggest that mammary uptake of Leu could modulate and be modulated by the protein synthesis signaling cascade rather than driven by a real mammary Leu requirement, as suggested by Bionaz and Loor (2011). In this regard, glutamate released by transamination during mammary BCAA catabolism may be used for the synthesis of Gln, Ala, Asp, and Asn (Li et al., 2009). In our conditions, however, only the mammary efficiency use of Ala increased among the NEAA.

Different studies demonstrated that the production of milk protein can be dramatically increased by altering the endocrine signals (Griinari et al., 1997; McGuire et al., 1995; Mackle et al., 2000). In the present experiment, the increased mammary EAA capture (regarded as clearance rate) and milk protein yield with starch versus fiber diets were consistent with the greater arterial insulin concentration (23%, on average). In this regard, a 15% increase in milk protein yield was found when using a hyperinsulinemic-euglycemic clamp in dairy cows (Mackle et al., 2000); more importantly, in our study this was associated to a higher extraction rate of EAA [mainly BCAA (48%)] rather than to a greater uptake to milk output ratio of EAA. Some other studies (Rius et al., 2010; Rulquin et al., 2004) have also suggested that insulin may play a role in milk protein production and efficiency use of AA; this improvement seems to be supported by an increase in the mammary affinity for some EAA.

A potentiating effect of insulin on BCAA effects may exist. In other studies involving infusion of insulin, plasma concentrations of AA are reduced, with the BCAA being most reduced (McGuire et al., 1995; Griinari et al., 1997; Mackle et al., 2000; Bequette et al., 2001). This was observed in our experiment for starch diets, but only at normal CP levels ($\text{CHO} \times \text{CP}$; $P = 0.03$) corresponding to the highest arterial concentration of insulin. Rius et al. (2010) also found a tendency ($P = 0.10$) for dairy cows infused with starch to have lower BCAA arterial concentrations at high versus low AA supply (with or without casein infusion), an interaction which was significant for Leu ($P = 0.01$). These authors stated that cell-signaling molecules involved in the regulation of protein synthesis differentially responded to these nutritional stimuli (glucogenic substrates and AA supplies).

CONCLUSIONS

At similar energy intakes, the mammary gland of cows receiving diets rich in starch compared with diets rich in fiber showed a higher affinity (clearance rate)

for EAA, leading to a higher efficiency of AA utilization (milk output/total inflow) and milk protein yield. This positive effect of starch was evident both at low (12.0% CP) and normal (16.5% CP) protein levels; thus, additive and independent effects of glucogenic nutrients and AA supplies can be proposed. However, in our study, some interactions between the dietary carbohydrate composition and the protein content of diets on mammary uptake and clearance rates of some nutrients (namely glucose and BCAA) support the concept of different metabolic pathways, likely related to insulin and mTOR signaling pathway, by which starch diets improve milk protein yield at the 2 studied dietary protein content levels. A modulation of mammary Leu and glucose metabolism mediated by glucogenic nutrients (via insulinemia) can be proposed from present results.

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