Feed and nitrogen efficiency are affected differently but milk lactose production is stimulated equally when isoenergetic protein and fat is supplemented in lactating dairy cow diets

K. Nichols,*†¹ A. Bannink,† S. Pacheco,‡ H. J. van Valenberg,‡ J. Dijkstra,* and H. van Laar§

*Animal Nutrition Group, Wageningen University and Research, PO Box 338, 6700 AH Wageningen, the Netherlands

†Wageningen Livestock Research, Wageningen University and Research, PO Box 338, 6700 AH Wageningen, the Netherlands

‡Dairy Science and Technology Group of Food Quality and Design, Wageningen University and Research, PO Box 17, 6700 AH Wageningen, the Netherlands

§Trouw Nutrition R&D, PO Box 220, 5830 AE Boxmeer, the Netherlands

ABSTRACT

Fifty-six Holstein-Friesian cows were used in a randomized complete block design to test the effects of supplemental energy from protein (PT) and fat (FT) on lactation performance and nutrient digestibility in a 2 × 2 factorial arrangement. During the control period, cows were adapted for 28 d to a basal total mixed ration consisting of 34% grass silage, 33% corn silage, 5% grass hay, and 28% concentrate on a dry matter (DM) basis. Experimental rations were fed for 28 d immediately following the control period and consisted of (1) low protein, low fat (LP/LF), (2) high protein, low fat (HP/LF), (3) low protein, high fat (LP/HF), or (4) high protein and high fat (HP/HF). To obtain the HP and HF diets, intake of the basal ration was restricted and supplemented isoenergetically (net energy basis) with 2.0 kg/d of rumen-protected protein (soybean + rapeseed, 50:50 mixture on DM basis) and 0.68 kg/d of hydrogenated palm fatty acids (FA) on a DM basis. Milk production and composition, nutrient intake, and apparent digestibility were measured during the final 7 d of the control and experimental periods. No interaction was found between PT and FT on milk production and composition. Yields of milk, fat- and protein-corrected milk, and lactose increased in response to PT and FT and lactose concentration was unaffected by treatment. Milk protein concentration and yield increased in response to PT, and protein yield tended to increase in response to FT. Milk fat concentration and yield increased in response to FT and were unaffected by PT. Milk urea concentration increased and nitrogen efficiency decreased in response to PT. Feed and nitrogen efficiency were highest on the LP/HF diet and both parameters increased in response

to FT, whereas milk urea concentration was not affected by FT. Energy from fat increased the concentration and yield of ≥ 16 -carbon FA in milk and decreased the concentration of FA synthesized de novo, but had no effect on their yield. Concentration and yield of de novo-synthesized FA increased in response to PT. Concentration and yield of polyunsaturated FA increased and decreased in response to PT and FT, respectively. Apparent total-tract digestibility of crude fat decreased in response to PT, and FT increased crude protein digestibility. Energy supplementation through rumen-inert hydrogenated palm FA appears to be an efficient feeding strategy to stimulate milk production with regard to feed and nitrogen efficiency compared with supplementing an isoenergetic level of rumen-protected protein.

Key words: rumen-protected protein, hydrogenated palm fatty acid, milk lactose, digestibility

INTRODUCTION

Rising demand for sustainably produced milk protein products (OECD/FAO, 2015) emphasizes the importance of understanding how energy-yielding feedstuffs affect efficient synthesis of milk and its components. Several studies compare the effects of glucogenic substrates with lipogenic substrates (Grum et al., 1996; van Knegsel et al., 2007; Boerman et al., 2015), but comparisons between isoenergetic supplements of aminogenic and lipogenic nutrients are scarce. It is well established that protein degradation in the rumen increases with dietary CP content. If available protein in the rumen exceeds microbial needs, or if availability of AA exceeds postabsorptive requirements, excess NH_3 is produced and excreted as urea (Colmenero and Broderick, 2006; Dijkstra et al., 2013). Energy is required to process and excrete a surplus of N, which increases heat production and decreases retained energy and milk energy (Reed et al., 2017). As an alternative to

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¹Corresponding author: kelly.nichols@wur.nl

feeding high-CP diets, balancing rations to supply sufficient levels of EAA in support of milk protein synthesis has been shown to improve postabsorptive N efficiency and increase milk protein yield (Haque et al., 2012; Lee et al., 2012; Arriola Apelo et al., 2014). Thus, great focus has been placed on maximizing dietary protein utilization by increasing post-ruminal supply of EAA and energy precursors through rumen-protected (**RP**) products.

Lactose is the main osmotic driver of total milk yield; therefore, the glucogenic capacity of a dietary ingredient can have a profound effect on lactation performance. Glucose supply to the mammary gland can be increased through absorption of glucogenic nutrients and flux through hepatic gluconeogenesis. Ideally, the majority of dietary AA would be used by the mammary gland for milk protein synthesis at first pass, but AA will be used for gluconeogenesis when concomitant supply of glucogenic energy is limiting for productive purposes. The corollary is that, by supplying rapidly available energy precursors, a larger proportion of dietary AA can be used for milk protein synthesis (Rius et al., 2010a,b). However, lactation responses to supplemental glucose availability are not always positive and depend on the nutritional status and production potential of the animal (Cant et al., 2002; Nichols et al., 2016). Saturated fat is energy dense and included in lactating cow diets as a source of nonfermentable energy. Saturated long-chain fatty acids (**LCFA**) are supplemented into dairy rations with the goal of increasing milk production while minimizing inhibitory effects on functional digestibility (Jenkins, 1993). In contrast, supplementation with UFA has been associated with perturbed DMI, altered ruminal biohydrogenation and microbial activity, and depressed milk fat synthesis (Allen, 2000; Baumgard et al., 2001). Saturated LCFA have the potential to provide high amounts of gross energy to the animal, but on a net basis do not directly contribute to glucose precursors necessary for lactose synthesis by the mammary gland. The apparent effect of supplemental fat on DMI and digestibility leads to variation in cow performance across studies (Rabiee et al., 2012). If DMI is not severely decreased and digestibility remains unaffected, the high energy density of rumen-inert fat supplements increases ME consumption and may improve energetic efficiency through the direct transfer of FA into milk (Hammon et al., 2008; Boerman et al., 2015).

The interaction between AA and glucose or glucose precursors on milk production has been examined in several studies (Raggio et al., 2006; Lemosquet et al., 2009; Nichols et al., 2016). Furthermore, many studies compare the effects of glucogenic or lipogenic nutrients through forage substitution in the diet (Cantalapiedra-

Hijar et al., 2014; Boerman et al., 2015; Piantoni et al., 2015) or abomasal infusion (Oldick et al., 1997), but the interaction between protein and fat supplementation has not been extensively investigated. We expected that, at isoenergetic levels, the inherent properties of aminogenic versus lipogenic energy would differently affect whole-body metabolism, which may be reflected in milk production responses. Supplemental protein may yield AA and stimulate protein synthesis in both the mammary gland and extramammary tissues, but as a glucogenic substrate, AA may yield glucose potentially in support of lactose synthesis. In contrast, fat supplementation provides FA that may contribute to milk fat yield but do not directly yield substrates to increase milk protein or lactose synthesis. However, this may be achieved if intramammary glucose is spared through a reduction in de novo FA synthesis. Thus, our objective was to characterize the independent and interactive effects of isoenergetic protein and fat supplementation on milk production and composition and nutrient digestibility, where changes to the energy content of treatment diets were accomplished by supplementation with RP protein and rumen-inert fat.

MATERIALS AND METHODS

Experimental Design and Treatment Diets

All experimental procedures were approved by the Animal Care and Use Committee for Nutreco Nederland B.V. (Amersfoort, the Netherlands) and conducted under the Dutch Law on Animal Experiments. Fifty-six Holstein-Friesian dairy cows (167 ± 87 DIM; 2.8 ± 1.9 lactations; 20 primiparous, 36 multiparous) were used in a randomized complete block design where two 28-d feeding periods (control and experimental) consisted of 21 d of diet adaptation and 7 d of data collection. Supplemental energy from protein (**PT**) or fat (**FT**) was tested in a factorial arrangement. During the control period, cows were fed a basal diet as a TMR meeting NE_L and MP requirements consisting of 34% grass silage, 33% corn silage, 5% grass hay, and 28% concentrate on a DM basis. Cows were blocked by parity, DIM, and DMI of the final 7 d of the control period. Within blocks, cows were randomly assigned to 1 of 4 diets for the experimental period: (1) low protein, low fat (**LP/LF**; 95% MP, 95% NE_L), (2) high protein, low fat (**HP/LF**; 131% MP, 107% NE_L), (3) low protein, high fat (**LP/HF**; 95% MP, 107% NE_L), or (4) high protein and high fat (**HP/HF**; 131% MP, 119% NE_L), where MP and NE_L are expressed relative to animal requirements in the control period. For all treatments, basal diet intake for individual cows was restricted to 95% of their ad libitum intake recorded

during the control period. A 2.0-kg 50:50 mixture (DM basis) of RP soybean meal and rapeseed meal (SoyPass + RaPass; both rumen-protected by xylose-treatment; Borregaard LignoTech, Sarpsborg, Norway) and 0.68 kg of rumen-inert hydrogenated LCFA (mainly C16:0 and C18:0; Hidropalm; Norel, Madrid, Spain) on a DM basis were supplemented into the concentrate portion of each basal TMR to obtain the HP and HF diets. Therefore, feed intake restriction was accomplished by limiting only the basal portion of the diet (to minimize variation in basal diet intake across treatments), and additional daily intake on HP and HF diets arose from the addition of the respective supplement. Therefore, differences in DMI between each diet were created by design (Table 1) to allow isoenergetic intake of protein and fat on diets with HP and HF.

Ingredient and chemical composition of the control and experimental diets are presented in Tables 1 and 2. During the final 10 d of each period, concentrates contained 0.25% titanium dioxide as an inert marker for estimation of apparent total-tract digestibility (ATTD). Cows were housed in a freestall barn with ad libitum access to water. The TMR were mixed and distributed once daily at 1000 h via electronic intake

control boxes (Insentec, Marknesse, the Netherlands). Refused feed was removed 3 times per week and intake control boxes were checked daily for functionality. Feed intake measurements of individual animals were facilitated via electronic ear transponders, which allowed cows ($n = 14$) access to a set of 9 intake control boxes supplying their respective diet. When individual cows reached their set daily feed intake according to their treatment, access to the intake control boxes was denied until the next day at 0400 h, when the boxes were reset for the next 24-h period. Cows were milked twice daily at 530 and 1630 h and milk production was recorded electronically at each milking.

Sample Collection and Analysis

Samples of TMR, concentrates, grass silage, corn silage, and grass hay were collected once weekly during each period and stored at -20°C until analysis. Feces was collected by rectal grab sampling on d 24, 25, and 26 of each period at 730 and 1330, 930 and 1530, and 1230 and 1830 h, respectively, to account for potential diurnal variation in digestibility over 3 d. At each collection time point, 75-g aliquots of fresh feces

Table 1. Formulated TMR component intake and composition of concentrate fed during the control period (CNTL) and during the experimental period for the basal diet (LP/LF) or diets supplemented with energy from protein (HP/LF), fat (LP/HF), or from protein and fat (HP/HF)

Item	CNTL	LP/LF	HP/LF	LP/HF	HP/HF
Ingredient, kg of DM/d					
Grass silage	7.1	6.7	6.7	6.7	6.7
Corn silage	6.9	6.5	6.5	6.5	6.5
Grass hay	1.00	0.98	0.98	0.98	0.98
Concentrate ¹	5.8	5.5	5.5	5.5	5.5
SoyPass + RaPass ²	—	—	2.0	—	2.0
Hidropalm ³	—	—	—	0.68	0.68
Total predicted DMI, kg/d	20.8	19.7	21.7	20.5	22.5
Concentrate composition, ⁴ g/kg of DM					
Soy hulls	328	328	239	292	219
Wheat	264	264	193	235	177
Soybean meal	228	228	166	203	152
Rapeseed meal	100	100	73	89	67
Limestone, 36% Ca	10	10	7	9	7
Magnesium oxide	7	7	5	6	5
Monocalcium phosphate	7	7	5	6	5
Mineral and vitamin premix	5	5	6	5	6
Sodium chloride	5	5	4	5	3
Urea	3	3	—	3	—
SoyPass	43	43	167	38	154
RaPass	—	—	136	—	125
Hidropalm	—	—	—	109	82

¹Portion of concentrate excluding rumen-protected protein and hydrogenated palm fatty acid supplements. The basal concentrate composition (excluding the addition of SoyPass + RaPass or Hidropalm) was identical for all treatments.

²A 50:50 mix (DM basis) of SoyPass (xylose-treated rumen-protected soybean meal) and RaPass (xylose-treated rumen-protected rapeseed meal) manufactured by Borregaard LignoTech, Sarpsborg, Norway.

³Hidropalm (hydrogenated palm fatty acids; 85% free fatty acids and 15% triglycerides; 50% C16:0 and 47% C18:0) manufactured by Norel Animal Nutrition (Madrid, Spain).

⁴Titanium dioxide was included at 0.25% of DM to the concentrate fed in the final 10 d of each period.

Table 2. Analyzed and calculated chemical composition of ingredients (grass silage, corn silage, grass hay, and concentrates) and complete TMR fed during the control period (CNTL) and during the experimental period for the basal diet (LP/LF) or diets supplemented with energy from protein (HP/LF), fat (LP/HF), or from protein and fat (HP/HF)

Item	Roughage ¹			Concentrate						TMR ²			
	Grass silage	Corn silage	Grass hay	CNTL	LP/LF	HP/LF	LP/HF	HP/HF	CNTL	LP/LF	HP/LF	LP/HF	HP/HF
Analyzed ³													
DM, g/kg	539	359	867	889	893	860	907	902	519	522	539	534	553
Gross energy, MJ/kg of DM	18.5	18.6	18.0	17.9	17.9	17.7	20.8	20.0	18.3	18.3	18.2	19.2	19.1
Crude ash	119	35	63	70	80	75	73	75	75	78	76	76	76
CP	187	61	61	239	251	285	221	273	153	157	177	151	176
Crude fat	45	44	25	32	34	24	156	126	40	41	37	77	73
NDF	445	369	607	336	308	338	272	285	398	390	393	376	371
ADF	258	203	338	216	206	201	179	169	232	229	225	220	213
ADL	14	14	34	13	13	26	11	21	15	15	19	14	17
Starch	NA ⁴	363	NA	208	182	144	155	119	178	171	159	163	149
Sugar	16	NA	124	60	60	49	52	64	28	28	27	27	34
Calculated ⁵													
DVE ⁶	80	45	58	152	152	205	131	178	87	87	111	83	105
OEB ⁷	32	-40	8	56	56	47	49	40	14	14	14	13	13
NE _L ⁸ , MJ/kg DM	6.25	6.87	5.45	7.22	7.19	7.57	8.95	8.63	6.68	6.68	6.85	7.23	7.26

¹Identical roughages were used in the control and experimental periods.
²Values for TMR were calculated based on ration composition and analyzed and calculated values obtained for roughages and concentrate.
³Values for all components were analyzed by wet chemistry (Wageningen University, Wageningen, the Netherlands) and are presented in g/kg of DM unless otherwise stated.
⁴NA = not analyzed.
⁵Calculated values for grass silage and corn silage were obtained from near-infrared spectroscopy analysis (Eurofins Agro, Wageningen, the Netherlands) and values for grass hay were obtained from the CVB Table Ruminants (CVB, 2008). Concentrate values were estimated from raw material composition from the CVB Table Ruminants (CVB, 2008).
⁶Intestinal digestible protein (CVB, 2008).
⁷Rumen-degradable protein balance (CVB, 2008).
⁸NE_L calculated with the VEM system (CVB, 2008).

were immediately pooled into a composite sample by cow and stored at -20°C until analysis. Milk samples were obtained from each cow 4 times per week at 2 subsequent morning and afternoon milkings via automatic samplers in the milking parlor collecting a fixed volume of milk per kilogram produced. Samples were pooled into a weekly morning and afternoon sample by individual cow, stored at 4°C , and analyzed within 3 d. An additional aliquot of each milk sample was stored separately at -20°C until analysis of milk FA.

Samples of grass silage, corn silage, grass hay, concentrates and feces were analyzed for DM, ash, N, crude fat, starch (except grass silage and grass hay), sugars (except corn silage), NDF, ADF (except feces), ADL (except feces), gross energy, and titanium (concentrates and feces only). After thawing at room temperature, samples were dried at 60°C until a constant weight was reached and ground to pass a 1-mm screen using a Wiley mill (Peppink 100AN, Olst, the Netherlands). Fresh samples of silages and feces were used to determine N concentration. For the determination of NH_3 content, fresh silage samples were deproteinized by the addition of 10% (wt/vol) trichloroacetic acid solution followed by centrifugation (10 min at $14,000 \times g$ at room temperature). Subsequently, indophenol blue was formed using the Berthelot reaction with phenol and hypochlorite in an alkaline solution, which was determined spectroscopically at 623 nm. The DM content of air-dried samples was gravimetrically determined by drying at 103°C until a constant weight was reached (ISO 6496; ISO, 1999b). Ash was determined after combustion at 550°C (ISO 5984; ISO, 2002a). Crude protein content was calculated as $\text{N} \times 6.25$, where N was determined using the Kjeldahl method with CuSO_4 as a catalyst (ISO 5983; ISO, 2005). Hydrolysis with HCl and extraction with light petroleum was used to determine crude fat content of samples (ISO 6492; ISO, 1999a). Starch was determined enzymatically (ISO 15914; ISO, 2004). Grass silage, grass hay, and concentrates were analyzed for sugars according to Abrahamse et al. (2008). The NDF content of samples was analyzed according to Van Soest et al. (1991) after pretreatment with α -amylase but without sodium sulfite. Acid detergent fiber and ADL were analyzed in feed samples using methods described by Van Soest et al. (1991) and Robertson and Van Soest (1981) using sulfuric acid, respectively. An adiabatic bomb calorimeter (IKA-C700, Janke and Kunkel, Heitersheim, Germany) was used for determination of gross energy content (ISO 9831; ISO, 1998). Titanium content was analyzed using sulfuric acid digestion in the presence of Cu at 420°C . The subsequent Cu complex formed by addition of peroxide was determined spectroscopically at 408 nm. Reported values for nutrient content

of the TMR were calculated from ration composition and analyzed values obtained for roughage and concentrates. Reported intestinal digestible protein (DVE; see Table 2), RDP balance (OEB; see Table 2), and NE_L were obtained by near-infrared spectroscopy analysis for corn and grass silage (Eurofins Agro, Wageningen, the Netherlands) and were estimated from CVB tables for grass hay (CVB, 2008). For concentrates, intestinal digestible protein (DVE; see Table 2), RDP balance (OEB; see Table 2), and NE_L were calculated based on table values and the composition of raw materials (CVB, 2008), and for each TMR these parameters were calculated from ration composition of all roughage and concentrate ingredients.

Pooled morning and afternoon milk samples were analyzed weekly for fat, protein, lactose, and milk urea by mid-infrared spectroscopy (ISO 9622; ISO, 2013; Qlip NV, Zutphen, the Netherlands). Milk FA composition was analyzed by GC. Frozen milk samples were thawed and 50 mL from each sample was pooled into a composite sample from which milk fat was extracted. Fatty acid methyl esters were prepared from fat fractions (ISO 15884; ISO, 2002b) and analyzed (ISO 16958; ISO, 2015) on a GC (Thermo Focus GC, Thermo Fisher, Milan, Italy) with a split/splitless injector operated in split injection mode (split ratio 10:1; split flow 25.5 mL/min) at a temperature of 250°C , using a WCOT fused silica capillary column (Agilent CP-Sil 88, Agilent, Santa Clara, CA) with CP-select CB for FAME as stationary phase (100 m $\times$ 0.25 mm i.d.) and helium as carrier gas, and fitted to a flame ionization detector (275°C). The initial temperature was held at 60°C for 5 min, then raised to 165°C at a rate of $15^{\circ}\text{C}/\text{min}$ and held for 1 min, then raised to 225°C at a rate of $2^{\circ}\text{C}/\text{min}$ and held for 20 min. A volume of 1 μL was injected. Peaks were identified and quantified using pure methyl esters (Larodan, Malmö, Sweden; Lipidox, Stockholm, Sweden; Nu-Chek Prep, Elysian, MN). Results of FA were corrected for flame ionization detector response and expressed as grams per 100 g of total FA. Yields of FA (g/d) were calculated using milk fat yield and individual FA weight proportions (g/100 g of FA) to determine yield on a mass basis using the average proportion of FA in milk fat (93.7 g/100 g) derived from individual FA molecular weight and corrected for the glycerol portion of triglycerides and other milk lipid classes according to Glasser et al. (2007).

Statistical Analysis

Milk yield, milk composition, and DMI were averaged over the final 7 d of each period. Apparent total-tract digestibility was calculated by the marker ratio technique using titanium dioxide measured in feed and feces

samples collected in the final week of each period. Variances in lactation performance, milk FA profile, and digestibility were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), with level of protein and fat and their interaction as fixed effects and parameters measured during the control period used as covariates according to the following model:

$$Y_{ijkl} = \mu + \beta \times \mu_l + \text{block}_i + \text{PT}_j + \text{FT}_k \\ + (\text{PT} \times \text{FT})_{jk} + \varepsilon_{ijkl},$$

where Y_{ijkl} = observed trait, μ = overall mean, β = control covariate parameter, μ_l = observed trait in the control period of cow l , block_i = random block effect ($i = 1$ to 14), PT_j = fixed protein effect ($j = 1$ to 2), FT_k = fixed fat effect ($k = 1$ to 2), $(\text{PT} \times \text{FT})_{jk}$ = interaction between fixed PT and FT effects, and ε_{ijkl} = residual random error term. Differences were considered significant at $P \leq 0.050$ and tendencies at $0.050 < P \leq 0.100$. Multiple comparisons between treatment means were made using the Tukey-Kramer method when a PT $\times$ FT interaction was detected at $P \leq 0.1$.

RESULTS AND DISCUSSION

Dietary treatments in this study were designed such that manipulation of supplemental energy from PT and from FT contributed most significantly to the quantity of aminogenic or lipogenic nutrients reaching the small intestine. The proportion of all basal diet ingredients was maintained, individual intake was restricted to 95% of ad libitum level, and RP protein and rumen-inert fat were supplemented in isoenergetic amounts to the restricted basal diet. Therefore, additional daily intake of MP and NE_L on HP and HF diets arose only from the addition of the respective supplement. Wright et al. (1998) employed a similar design, where allocation of their basal diet was restricted by 10 or 20% and additional intake was made up of varying amounts of a low-RDP, EAA-balanced concentrate. Our design was unique compared with studies where manipulation of forage or concentrate type changes the profile of glucogenic and lipogenic nutrients available from the diet (Cantalapiedra-Hijar et al., 2014; Boerman et al., 2015; van Hoeij et al., 2017), or where fat-rich concentrates and RUP sources are added as a portion of the total ration DM without feed restriction (Canale et al., 1990; Hoffman et al., 1991; Chan et al., 1997). No significant effects of PT or FT ($P > 0.13$) were found when DMI, milk production, or milk composition from the final 7 d of the control period were tested considering block and treatment of the cows in the experimental period,

indicating that cows were distributed evenly across the experimental diets after the control period.

Dry matter intake was designed to differ between treatments due to the addition of PT and FT supplements to the basal TMR through the concentrate (Table 1). The LP/HF TMR contained more gross energy and calculated NE_L (on a DM basis) compared with HP/LF (Table 2). However, as intended, calculated NE_L intake was similar between HP/LF and LP/HF, 147 MJ/d and 141 MJ/d, respectively, and higher (155 MJ/d) when HP and HF were combined, and this pattern is reflected in the significant increase in digestible energy intake in response to PT and FT (Table 3). Therefore, our objective to increase energy intake through protein and fat supplementation was achieved despite a lower observed DMI than designed for LP/HF and HP/HF diets (Table 3). Feeding supplemental fat is associated with variable responses in DMI, but commonly results in no change or a hypophagic effect (Harvatine and Allen, 2006a), where DMI is affected less as the degree of FA saturation increases (Pantoja et al., 1996). The significant effect of PT on DMI (Table 3) is due to our experimental design.

Lactation Performance

The main objective of this work was to study the effects on lactation performance when extra energy comes from aminogenic or lipogenic supplements. The response to PT and FT was independent and additive, as the interaction between these factors had no effect on milk yield or composition (Table 3). Cows on HP diets produced 1.9 kg/d more milk compared with LP diets, and cows on HF diets produced 1.6 kg/d more milk compared with LF diets. Milk protein concentration and yield were increased by PT whereas fat concentration and yield were increased by FT. This production response is in agreement with previous studies feeding aminogenic or lipogenic nutrients. Schor and Gagliostro (2001) increased milk and protein yield when pasture-based forage was supplemented with blood meal of low rumen degradability compared with high rumen-degradable soybean meal. Lock et al. (2013) increased milk fat yield and concentration in dairy cattle by replacing soyhulls with a C16:0-rich FA supplement at 2% of DM, and Relling and Reynolds (2007) stimulated 187 g/d of milk fat synthesis when rumen-inert SFA were fed compared with a nonsupplemented diet. However, few studies exist that compare the effect of aminogenic and lipogenic nutrients supplemented at an isoenergetic level. Chan et al. (1997) supplemented Met and Lys from a mix of fish, blood, and soybean meal compared with a low-quality protein source from

corn gluten meal, with and without supplementation of rumen-inert LCFA at 2.5% of DM, and observed similar lactation responses to our study with regards to milk protein and lactose yield. Canale et al. (1990) supplemented RP Met and Lys, calcium salts of FA, or both, and reported increased milk yield and decreased protein content in response to FA, increased protein content in response to RP AA, and found an interaction for milk fat yield where it increased with FA but only when RP AA were supplemented.

Lactose content was unaffected by PT or FT, but both factors independently increased lactose yield, suggesting postruminal supplementation with AA and saturated LCFA affected mammary glucose utilization through alterations in whole-body glucose availability, local mammary gland glucose sequestration, or intramammary glucose partitioning. The effect of PT on lactose yield is not surprising, as infusions of casein or AA mixtures increase milk protein but also lactose yield (Galindo et al., 2011; Nichols et al., 2016), suggesting AA supplementation affects whole-body glucose partitioning and mammary gland glucose metabolism

(Lemosquet et al., 2009; Haque et al., 2012). Contrary to AA, LCFA are not conventionally gluconeogenic, yet the same level of lactose synthesis was maintained on the LP/HF diet compared with the HP/LF diet. Compared with glucogenic diets, lactose yield may be equal to or higher from cows fed lipogenic diets (van Knegsel et al., 2007; Hammon et al., 2008; Lohrenz et al., 2010), but is lower in other studies (Cantalapiedra-Hijar et al., 2014; Boerman et al., 2015; van Hoeij et al., 2017). Differences in response to glucogenic and lipogenic diets, with regard to lactose yield, could be due to energy partitioning and whole-body glucose availability in different stages of lactation. On a net basis, FA do not directly contribute to glucose precursors necessary for mammary lactose synthesis. Increased lactose yield in response to dietary supplementation with SFA has been attributed to greater mammary supply of LCFA and their direct incorporation into milk fat reducing mammary glucose requirement for de novo FA synthesis, thus sparing glucose for lactose synthesis (Grummer and Carroll, 1991; Chilliard, 1993; Hammon et al., 2008). In agreement, we observed increased incorporation of

Table 3. Performance of lactating dairy cows fed the basal diet (LP/LF) or diets supplemented with energy from protein (HP/LF), fat (LP/HF), or from protein and fat (HP/HF)¹

Item	Treatment ²				SEM	P-value ³		
	LP/LF	HP/LF	LP/HF	HP/HF		PT	FT	PT × FT
DMI, kg/d	19.7	21.4	19.5	21.4	0.28	<0.001	0.758	0.668
DE intake, ⁴ MJ/d	252	276	268	291	4.8	<0.001	0.001	0.967
Yield								
Milk, kg/d	26.6	28.7	28.4	30.0	0.70	0.012	0.031	0.707
Fat, g/d	1,199	1,254	1,325	1,375	33.0	0.102	<0.001	0.931
Protein, g/d	915	1,013	974	1,041	24.1	<0.001	0.054	0.495
Lactose, g/d	1,205	1,307	1,301	1,375	30.8	0.007	0.012	0.656
Composition, %								
Fat	4.52	4.51	4.75	4.64	0.078	0.435	0.022	0.520
Protein	3.48	3.60	3.47	3.51	0.029	0.009	0.111	0.185
Lactose	4.54	4.57	4.58	4.57	0.018	0.453	0.352	0.278
FPCM, ⁵ kg/d	28.5	30.6	31.0	32.4	0.73	0.019	0.005	0.655
Milk urea, mg/dL	21 ^a	28 ^b	22 ^a	26 ^b	0.7	<0.001	0.430	0.059
Feed efficiency ⁶	1.44	1.43	1.58	1.51	0.040	0.298	0.004	0.471
Feed energy efficiency, ⁷ %	34.7	34.2	35.8	34.7	0.98	0.405	0.409	0.791
N efficiency, ⁸ %	28.9 ^a	26.1 ^b	32.2 ^c	27.1 ^{ab}	0.69	<0.001	0.002	0.071

^{a-c}Means within a row with no common superscripts differ ($P < 0.05$).

¹Data are LSM from the final week of the experimental period using data from the final week of the control period (CNTL) as covariates for each parameter.

²LP/LF = basal TMR fed at 95% of CNTL ad libitum intake with no protein or fat supplement; HP/LF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass (Borregaard LignoTech, Sarpsborg, Norway) on a DM basis; LP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 0.68 kg of Hidropalm (Norel Animal Nutrition, Madrid, Spain) on a DM basis; HP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass and 0.68 kg of Hidropalm on a DM basis. For all treatments $n = 14$.

³PT = effect of energy from protein; FT = effect of energy from fat.

⁴Digestible energy intake = gross energy (GE) intake (MJ/d) × apparent total-tract GE digestibility.

⁵Fat- and protein-corrected milk (FPCM) = $(0.337 + 0.116 \times \text{fat \%} + 0.06 \times \text{protein \%}) \times \text{milk yield (kg/d)}$ (CVB, 2008).

⁶FPCM (kg/d)/DMI (kg/d).

⁷[Milk energy (MJ/d)/DE intake (MJ/d)] × 100. Milk energy content is calculated according to the equation of Tyrrell and Reid (1965) based on observed milk fat, protein, and lactose content.

⁸[Milk N yield (g/d)/N intake (g/d)] × 100.

preformed LCFA into milk when energy was supplemented from fat. Concentration of total preformed (>16 carbons) and mixed (16 carbons) FA increased 4 and 7%, respectively, in response to FT (Table 4), and the yield of total preformed and mixed FA increased 15 and 17%, respectively, in response to FT (Table 5). The concentration of total de novo-synthesized FA (<16 carbons) decreased 13% in response to FT. Total yield of de novo-synthesized FA was not affected by FT, as the decline in concentration was offset by the increase in total milk FA yield. If triglycerides compose 98% of milk fat (Jensen and Newberg, 1995), total milk triglyceride production increased 121 g/d and de novo FA synthesis decreased only 12 g/d in response to FT. Although this shift in milk FA profile from de novo-synthesized FA toward LCFA in response to FT may have facilitated some intramammary repartitioning of glucose toward lactose synthesis, we suspect mammary glucose sequestration was stimulated by gluconeogenesis from AA or other endogenous energy precursors increasing arterial glucose supply and by responses in local mammary gland extraction mechanisms in support of lactose synthesis on HF diets.

Individually, concentration of all de novo FA, with the exception of C4:0, decreased in response to FT, whereas yield of C8:0, C10:0, C11:0, C12:0, *iso* C14:0, C15:0, and *iso* C15:0 decreased, yield of C4:0 increased, and yield of the remaining de novo FA were unaffected (Table 4 and 5). In response to PT, concentration and yield of C4:0 (yield only), C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, and *iso* C14:0 increased as well and concentration and yield of total de novo FA. Other studies have shown higher proportions of de novo FA when glucogenic diets are compared with lipogenic diets (Grum et al., 1996; van Kneysel et al., 2007; Boerman et al., 2015). In our study, supplementation of RP protein may have allowed greater production of short-chain FA through metabolism of AA into α -ketoacids to produce acetyl-CoA and other tricarboxylic acid cycle intermediates. In support, we observed a numerical increase in milk fat yield of 53 g/d on HP compared with LP diets, which agrees with previous reports of stimulated milk fat production when postruminal EAA are administered (Vanhatalo et al., 2003; Nichols et al., 2016). The pattern in concentration and yield of C4:0 across treatments differed from other de novo FA identified in milk fat. The C4:0 FA is produced directly by reduction of BHB or by condensation of acetyl units in a malonyl-CoA-independent pathway, whereas other de novo FA are synthesized via the malonyl-CoA pathway mainly from acetyl-CoA (Palmquist et al., 1993a). Similar to results presented by others (Cant et al., 1993; van Kneysel et al., 2007; Lock et al., 2013), C4:0 concentra-

tion did not respond to PT or FT, but yield increased in response to FT alongside total milk fat yield.

The increase in concentration and yield of C16:0 is largely responsible for the increase in concentration and yield of total mixed FA in response to FT, as concentrations and yield of *iso* C16:0 and C16:1 *trans*-9 decreased or did not change, respectively, in response to FT, and concentration of C16:1 *cis*-9 was unchanged but the yield of this FA increased. Similarly, the response in concentration and yield of total preformed FA in response to FT is driven by individual increases in concentration and yield of both C18:0 and C18:1 *cis*-9, which together make up the largest proportion of reported preformed FA. Concentration and yield of all other preformed FA were unchanged or decreased in response to FT. The rumen-inert fat supplement used in our study supplies almost exclusively C16:0 and C18:0 and contributes very little as substrate for rumen biohydrogenation reactions, which suggests increases in the profile of these SFA in milk was due to their extraction by the mammary gland and incorporation into milk fat. The concentration of total SFA was unaffected by FT because, despite the increase in C16:0 and C18:0 stimulated by FT, all saturated de novo FA (except C4:0) actually decreased in response to FT. Subsequently, SFA yield increased alongside total milk fat yield in response to FT.

Fat supplementation tended to increase milk protein yield by 45 g/d. Depending on the concomitant supply of AA available to the glands, non-AA energy precursors can allow the use of available EAA for milk protein synthesis by sparing them from gluconeogenesis or energy-yielding oxidative processes (Raggio et al., 2006; Rius et al., 2010a). Milk urea concentration was highest and N efficiency was lowest on HP diets, indicating greater AA catabolism when energy was supplemented from an aminogenic source compared with supplementation from a lipogenic source. Milk urea concentration was not affected by FT, but a tendency for a PT $\times$ FT interaction on this parameter suggests FT may have depressed AA catabolism when in the presence of PT. Interestingly, although adding fat to the basal diet tended to improve milk N efficiency to a greater extent at the low than at the high PT level, milk urea content was not affected. Milk urea concentration in general may serve as an on-farm indicator to guide nutritional strategies in efforts to reduce emissions of N to the environment, but the relationship between milk urea and N excretion is variable (reviewed by Spek et al., 2013). Our current results with respect to supplementation of PT and FT indicated that milk urea concentration was not necessarily a sound indicator of N efficiency. Increased AA catabolism and lowered N

Table 4. Milk fatty acid (FA) composition of lactating dairy cows fed the basal diet (LP/LF) or diets supplemented with energy from protein (HP/LF), fat (LP/HF), or from protein and fat (HP/HF)

Item	Treatment ¹				SEM	P-value ²		
	LP/LF	HP/LF	LP/HF	HP/HF		PT	FT	PT × FT
Concentration, g/100 g of FA								
C4:0	2.93	2.94	2.96	2.95	0.034	0.999	0.629	0.771
C6:0	2.08	2.19	1.94	2.02	0.026	<0.001	<0.001	0.596
C8:0	1.30	1.40	1.11	1.21	0.022	<0.001	<0.001	0.949
C10:0	2.95	3.27	2.38	2.63	0.066	<0.001	<0.001	0.561
C11:0	0.41	0.45	0.33	0.34	0.013	0.040	<0.001	0.367
C12:0	3.49	3.87	2.73	3.06	0.084	<0.001	<0.001	0.753
C14:0	11.8	12.0	10.2	10.7	0.17	0.020	<0.001	0.446
iso C14:0	0.21	0.24	0.15	0.18	0.011	0.008	<0.001	0.813
C14:1 <i>cis</i> -9	1.19	1.23	1.03	1.04	0.035	0.452	<0.001	0.796
C15:0	1.15	1.13	0.91	0.96	0.023	0.514	<0.001	0.154
iso C15:0	0.29	0.27	0.24	0.24	0.009	0.265	<0.001	0.331
anteiso C15:0	0.47	0.45	0.40	0.40	0.010	0.459	<0.001	0.296
C16:0	32.4 ^a	31.3 ^b	34.4 ^c	34.2 ^c	0.34	0.025	<0.001	0.076
iso C16:0	0.28	0.28	0.24	0.23	0.010	0.771	<0.001	0.766
C16:1 <i>cis</i> -9	1.56	1.65	1.65	1.83	0.079	0.105	0.104	0.565
C16:1 <i>trans</i> -9	0.44	0.43	0.38	0.39	0.013	0.961	<0.001	0.418
C17:0	0.54	0.54	0.44	0.44	0.010	0.802	<0.001	0.801
iso C17:0	0.68	0.62	0.63	0.62	0.025	0.163	0.335	0.301
C17:1 <i>cis</i> -9	0.22	0.24	0.19	0.19	0.008	0.551	<0.001	0.200
C18:0	9.3	9.0	10.9	10.4	0.23	0.082	<0.001	0.713
C18:1 <i>cis</i> -9 ³	19.2	18.9	21.0	20.0	0.42	0.081	<0.001	0.389
C18:1 <i>cis</i> -12	0.31	0.31	0.27	0.26	0.010	0.569	<0.001	0.403
C18:1 <i>cis</i> -13	0.43 ^{ab}	0.44 ^a	0.38 ^{bc}	0.33 ^c	0.015	0.203	<0.001	0.089
C18:1 <i>trans</i> -9	0.02	0.04	0.03	0.03	0.010	0.337	0.591	0.253
C18:1 <i>trans</i> -10 + <i>trans</i> -11	1.53	1.67	0.98	0.99	0.061	0.213	<0.001	0.273
C18:1 <i>trans</i> -15 + C18:1 <i>cis</i> -11	0.61	0.66	0.57	0.59	0.024	0.055	0.003	0.310
Total CLA ⁴	0.90	0.96	0.69	0.67	0.038	0.675	<0.001	0.289
C18:2n-6	2.11 ^a	2.57 ^b	1.88 ^a	2.00 ^a	0.074	<0.001	<0.001	0.024
C18:3n-3	0.49	0.53	0.39	0.44	0.011	<0.001	<0.001	0.924
C18:3n-6	0.23	0.25	0.22	0.22	0.012	0.551	0.135	0.367
C20:0	0.04	0.04	0.03	0.03	0.003	0.739	0.004	0.721
C20:2n-6	0.019	0.022	0.015	0.017	0.0011	0.013	<0.001	0.627
C20:3n-6	0.07	0.09	0.07	0.08	0.005	0.034	0.408	0.376
C20:4n-3	0.03	0.04	0.03	0.04	0.004	0.427	0.607	0.726
C20:4n-6	0.12	0.14	0.11	0.12	0.005	0.003	<0.001	0.470
C20:5n-3	0.050	0.049	0.045	0.044	0.0024	0.540	0.035	0.986
C22:0	0.08	0.08	0.07	0.07	0.002	0.901	<0.001	0.882
C22:5n-3	0.06	0.06	0.05	0.05	0.002	0.105	<0.001	0.200
C24:0	0.033	0.033	0.027	0.027	0.0014	0.917	<0.001	0.993
n-6 to n-3 ratio ⁵	4.09	4.56	4.45	4.25	0.158	0.392	0.889	0.035
Summation ⁶								
De novo	28.3	29.5	24.4	25.6	0.41	0.003	<0.001	0.953
Preformed	37.1	37.0	39.0	37.7	0.61	0.176	0.020	0.240
Mixed	34.7 ^a	33.6 ^b	36.6 ^c	36.7 ^c	0.36	0.079	<0.001	0.042
SFA	70.4	70.1	70.0	70.7	0.48	0.713	0.862	0.290
MUFA	25.5	25.3	26.5	25.7	0.45	0.245	0.124	0.502
PUFA	4.08 ^a	4.66 ^b	3.49 ^c	3.65 ^c	0.107	0.001	<0.001	0.058

^{a-c}Means within a row with no common superscripts differ ($P < 0.05$).

¹LP/LF = basal TMR fed at 95% of control period (CNTL) ad libitum intake with no protein or fat supplement; HP/LF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass (Borregaard LignoTech, Sarpsborg, Norway) on a DM basis; LP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 0.68 kg of Hidropalm (Norel Animal Nutrition, Madrid, Spain) on a DM basis; HP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass and 0.68 kg of Hidropalm on a DM basis. For all treatments $n = 14$.

²PT = effect of energy from protein; FT = effect of energy from fat.

³C18:1 *cis*-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12, as these 2 FA could not be separated in the analysis. The proportion of C18:1 *trans*-12 is considered negligible.

⁴Total CLA consists of mainly C18:2 *cis*-9, *trans*-11.

⁵Ratio between the sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, and C20:4n-6 and the sum of C18:3n-3, C20:4n-3, C20:5n-3, and C22:5n-3.

⁶De novo-synthesized FA (<16 carbons) originate from mammary de novo synthesis, preformed FA (>16 carbons) originate from mammary plasma extraction, mixed FA (16 carbons) originate from both de novo-synthesized and preformed FA. Sum of the respective SFA, MUFA, and PUFA reported in this table.

Table 5. Milk fatty acid (FA) yield (g/d) of lactating dairy cows fed the basal diet (LP/LF) or diets supplemented with energy from protein (HP/LF), fat (LP/HF), or from protein and fat (HP/HF)

Item	Treatment ¹				SEM	P-value ²		
	LP/LF	HP/LF	LP/HF	HP/HF		PT	FT	PT × FT
C4:0	32.9	34.8	36.8	38.3	0.98	0.077	<0.001	0.838
C6:0	23.4	25.8	24.2	26.2	0.62	<0.001	0.287	0.796
C8:0	14.6	16.4	13.9	15.7	0.40	<0.001	0.062	0.955
C10:0	33.0	38.1	29.8	34.2	1.01	<0.001	<0.001	0.718
C11:0	4.64	5.30	4.12	4.40	0.193	0.014	<0.001	0.282
C12:0	39.0	44.8	34.1	39.6	1.28	<0.001	<0.001	0.910
C14:0	131	140	127	139	3.6	0.004	0.408	0.781
iso C14:0	2.26	2.80	1.90	2.26	0.172	0.004	0.004	0.558
C14:1 <i>cis</i> -9	13.4	14.4	12.8	13.4	0.52	0.107	0.115	0.765
C15:0	12.8	13.3	11.4	12.3	0.44	0.108	0.006	0.663
iso C15:0	3.29	3.36	2.90	3.11	0.184	0.436	0.077	0.696
anteiso C15:0	5.17	5.20	4.99	5.09	0.166	0.671	0.385	0.826
C16:0	369	374	427	440	11.5	0.344	<0.001	0.679
iso C16:0	3.05	3.26	2.99	3.01	0.160	0.425	0.294	0.514
C16:1 <i>cis</i> -9	17.5	19.6	20.4	23.9	1.40	0.034	0.007	0.581
C16:1 <i>trans</i> -9	4.90	5.03	4.73	5.04	0.222	0.337	0.715	0.684
C17:0	5.88	6.36	5.51	5.77	0.245	0.144	0.057	0.652
iso C17:0	7.55	7.25	7.82	8.00	0.340	0.858	0.146	0.489
C17:1 <i>cis</i> -9	2.49	2.78	2.40	2.43	0.137	0.233	0.119	0.345
C18:0	103	105	136	135	4.2	0.922	<0.001	0.743
C18:1 <i>cis</i> -9 ³	211	221	259	257	8.3	0.606	<0.001	0.436
C18:1 <i>cis</i> -12	3.38	3.60	3.38	3.29	0.166	0.704	0.350	0.356
C18:1 <i>cis</i> -13	4.74	5.09	4.64	4.34	0.271	0.927	0.122	0.240
C18:1 <i>trans</i> -9	0.26	0.56	0.38	0.37	0.142	0.295	0.806	0.268
C18:1 <i>trans</i> -10 + <i>trans</i> -11	16.6	19.5	12.0	12.3	0.85	0.068	<0.001	0.163
C18:1 <i>trans</i> -15 + C18:1 <i>cis</i> -11	6.69	7.76	7.07	7.58	0.354	0.013	0.739	0.370
Total CLA ⁴	9.9	11.3	8.6	8.4	0.61	0.277	0.002	0.173
C18:2n-6	23.7 ^a	30.1 ^b	23.9 ^a	25.5 ^a	1.20	0.002	0.072	0.056
C18:3n-3	5.37	6.25	4.82	5.66	0.168	<0.001	0.002	0.903
C18:3n-6	2.45	2.87	2.70	2.81	0.211	0.148	0.593	0.411
C20:0	0.46	0.49	0.40	0.44	0.034	0.351	0.120	0.908
C20:2n-6	0.20	0.26	0.19	0.22	0.015	0.001	0.081	0.441
C20:3n-6	0.81	1.02	0.93	1.02	0.078	0.035	0.432	0.403
C20:4n-3	0.37	0.44	0.41	0.47	0.062	0.265	0.603	0.941
C20:4n-6	1.34	1.65	1.31	1.49	0.066	<0.001	0.126	0.270
C20:5n-3	0.54	0.58	0.57	0.58	0.040	0.549	0.601	0.570
C22:0	0.86	0.94	0.83	0.89	0.039	0.079	0.289	0.725
C22:5n-3	0.64	0.67	0.56	0.68	0.038	0.040	0.274	0.253
C24:0	0.35	0.38	0.33	0.35	0.022	0.193	0.162	0.672
Summation ⁵								
De novo	316	344	305	332	8.5	0.001	0.144	0.995
Preformed	410	432	483	485	14.0	0.379	<0.001	0.477
Mixed	395	401	455	472	12.4	0.268	<0.001	0.600
SFA	794	827	871	912	21.6	0.070	<0.001	0.835
MUFA	282	296	327	331	10.3	0.375	<0.001	0.619
PUFA	45.8	54.6	43.4	46.6	1.96	0.004	0.012	0.159

^{a,b}Means within a row with no common superscripts differ ($P < 0.05$).

¹LP/LF = basal TMR fed at 95% of control period (CNTL) ad libitum intake with no protein or fat supplement; HP/LF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass (Borregaard LignoTech, Sarpsborg, Norway) on a DM basis; LP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 0.68 kg of Hidropalm (Norel Animal Nutrition, Madrid, Spain) on a DM basis; HP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass and 0.68 kg of Hidropalm on a DM basis. For all treatments $n = 14$.

²PT = effect of energy from protein; FT = effect of energy from fat.

³C18:1 *cis*-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12, as these 2 FA could not be separated in the analysis. The proportion of C18:1 *trans*-12 is considered negligible.

⁴Total CLA consists of mainly C18:2 *cis*-9, *trans*-11.

⁵De novo-synthesized FA (<16 carbons) originate from mammary de novo synthesis, preformed FA (>16 carbons) originate from mammary plasma extraction, mixed FA (16 carbons) originate from both de novo-synthesized and preformed FA. Sum of the respective SFA, MUFA, and PUFA reported in this table.

efficiency associated with feeding increased CP levels has been well characterized (Broderick, 2003; Colmenero and Broderick, 2006). However, when low-quality dietary protein is replaced with highly digestible and EAA-balanced RUP (Wright et al., 1998; Noftsker and St-Pierre, 2003), or when energy is supplemented (Rius et al., 2010b), this response is altered in support of lowered milk urea and improved efficiency of N use by the mammary gland. Cantalapiedra-Hijar et al. (2014) found improved N efficiency on a starch-based, 12.0% CP diet when it stimulated the same level of milk protein yield as a fiber-based, 16.5% CP diet, but resulted in lower urinary N excretion. In the present study, N efficiency was improved on LP/LF compared with HP/LF, or on LP/HF compared with HP/HF. Importantly, our results support the addition of fat as an energy source to improve N utilization by lactating cows at high and low MP levels, although the effect was more pronounced at the low protein level.

Apparent Total-Tract Digestibility

Efficacy of energy supplementation from protein or fat for milk production is related to the digestibility of the added component and its effect on availability of other nutrients. Therefore, a second objective was to assess the effects of RP protein and rumen-inert fat on digestibility. Improved energy utilization is one of the primary purposes for supplementing saturated fat to dairy cow diets. Fat- and protein-corrected milk yield was stimulated to the same extent in response to isoenergetic supplementation of extra NE_L from RP protein and rumen-inert fat, and feed energy efficiency, calculated as milk energy as a proportion of digestible energy intake, did not differ. Therefore, efficiency of energy use for milk production was similar between aminogenic and lipogenic diets. Feed efficiency in the present study increased in response to FT as a result of increased milk production stimulated by the fat-containing TMR, which supplied more energy at a similar DMI (Table 3). Many others have reported increased feed efficiency when supplemental fat is fed (Lock et al., 2013; Rico et al., 2014; Boerman et al., 2015) or infused postruminally (Oldick et al., 1997).

Energy from protein and fat had little effect on nutrient ATTD. The HP diets had lower dietary crude fat contents than the LP diets (Table 2). Therefore, the decrease in crude fat digestibility in response to PT may reflect the relatively higher contribution of endogenous fat losses to fecal fat excretion at low dietary fat contents (Kil et al., 2010). Fat supplementation has been associated with a reduction in nutrient digestibility (Harvatiné and Allen, 2006b), but the effect depends highly on the particular nutrient, the location in

the digestive tract where digestibility is measured, the amount of fat added, and the degree of saturation of the FA fed (Palmquist and Jenkins, 1980; Jenkins and Jenny, 1989; Pantoja et al., 1995). Adding a high level of fat to a diet may increase apparent fat digestibility if endogenous fat becomes a smaller part of total fecal fat. In contrast, apparent digestibility may decrease if the true digestibility of the fat supplement is lower than that of the basal diet. Recently, Weld and Armentano (2017) showed that the inclusion of saturated fats to dairy rations in 38 studies did not affect total-tract NDF digestibility. The level of fat supplementation in the present experiment (i.e., 36 g/kg of DM) was similar to that in the meta-analysis of Weld and Armentano (2017; on average, 33 g of FA/kg of DM, ranging from 11 to 66 g of FA/kg of DM). With our fat supplement, we observed no effect of FT or of a PT $\times$ FT interaction on ATTD of DM, OM, NDF, crude fat, starch, or gross energy (Table 6), which agrees with other studies where saturated fats were fed or infused postruminally (Chan et al., 1997; Oldick et al., 1997; Bremmer et al., 1998). However, in contrast to these studies, we observed a small positive effect of FT on CP digestibility. Palmquist et al. (1993b) suggested that increased N digestibility with fat supplementation might be associated with the lower starch contents generally found in fat-supplemented diets. Although starch content was similar between the HP/LF and LP/HF diets, lower DMI on LP/HF could have slightly reduced starch entering the rumen. Under these conditions, microbial protein synthesis in the rumen was expected to be lower, and N loss through NH_3 -N production was expected to be higher. If intestinal absorption of NH_3 -N is higher compared with that of N originating from synthesized microbial protein, apparent N digestibility will increase.

CONCLUSIONS

This study demonstrated independent and additive stimulation of milk yield when protein and fat were supplemented at isoenergetic levels. Energy from RP protein stimulated the greatest response in milk protein yield. Energy from rumen-inert hydrogenated palm FA increased feed efficiency and stimulated milk fat yield by incorporation of LCFA into milk fat and reduction of mammary de novo FA synthesis. Energy from fat improved N utilization at high and low MP levels. In addition, milk urea concentration was identified as a poor indicator of actual N efficiency when fat supplementation was used to increase energy intake. Energy from fat produced the same level of lactose yield as energy from protein. During fat supplementation, repartitioning of intramammary glucose in support

Table 6. Apparent total-tract digestibility (%) of nutrients in lactating dairy cows fed the basal diet (LP/LF) or diets supplemented with energy from protein (HP/LF), fat (LP/HF), or from protein and fat (HP/HF)¹

Item	Treatment ²				SEM	P-value ³		
	LP/LF	HP/LF	LP/HF	HP/HF		PT	FT	PT × FT
DM	71.0	72.8	72.0	72.3	1.08	0.348	0.810	0.530
OM	73.0	73.7	73.9	73.9	0.75	0.598	0.460	0.638
CP	67.1	67.5	68.5	69.2	0.65	0.389	0.017	0.747
NDF	58.7	62.0	61.5	62.0	1.41	0.178	0.332	0.332
Crude fat	66.1	61.9	67.7	64.0	1.33	0.006	0.159	0.858
Starch	98.2	98.0	98.2	98.0	0.09	0.085	0.759	0.528
Gross energy	70.3	70.8	71.4	71.0	0.80	0.915	0.414	0.606

¹Data are LSM calculated from feed and feces sampled during the final week of each period using data from the final week of the control period (CNTL) as covariates for each parameter.

²LP/LF = basal TMR fed at 95% of CNTL ad libitum intake with no protein or fat supplement; HP/LF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass (Borregaard LignoTech, Sarpsborg, Norway) on a DM basis; LP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 0.68 kg of Hidropalm (Norel Animal Nutrition, Madrid, Spain) on a DM basis; HP/HF = TMR fed at 95% of CNTL ad libitum intake supplemented daily with 2.0 kg of SoyPass + RaPass and 0.68 kg of Hidropalm on a DM basis. For all treatments n = 14.

³PT = effect of energy from protein; FT = effect of energy from fat.

of lactose synthesis may have been allowed in part through decreased synthesis of de novo FA, but major glucose sequestration by the gland was likely stimulated by arterial glucose concentrations and local mammary gland kinetic mechanisms. These mechanisms should be investigated to elucidate the dynamics of mammary glucose partitioning when lipogenic substrates change milk precursor availability.

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REFERENCES

- Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. *J. Dairy Sci.* 91:2033–2045.
- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- Arriola Apelo, S. I., A. L. Bell, K. Estes, J. Ropelewski, M. J. de Veth, and M. D. Hanigan. 2014. Effects of reduced dietary protein and supplemental rumen-protected essential amino acids on the nitrogen efficiency of dairy cows. *J. Dairy Sci.* 97:5688–5699.
- Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of *trans*-10,*cis*-12 conjugated linoleic acid. *J. Nutr.* 131:1764–1769.
- Boerman, J. P., S. B. Potts, M. J. VandeHaar, and A. L. Lock. 2015. Effects of partly replacing dietary starch with fiber and fat on milk production and energy partitioning. *J. Dairy Sci.* 98:7264–7276.
- Bremmer, D. R., L. D. Ruppert, J. H. Clark, and J. K. Drackley. 1998. Effects of chain length and unsaturation of fatty acid mixtures infused into the abomasum of lactating dairy cows. *J. Dairy Sci.* 81:176–188.
- Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86:1370–1381.
- Canale, C. J., L. D. Muller, H. A. McCahon, T. J. Whitsel, G. A. Varga, and M. J. Lormore. 1990. Dietary fat and ruminally protected amino acids for high producing dairy cows. *J. Dairy Sci.* 73:135–141.
- Cant, J. P., E. J. DePeters, and R. L. Baldwin. 1993. Mammary uptake of energy metabolites in dairy cows fed fat and its relationship to milk protein depression. *J. Dairy Sci.* 76:2254–2265.
- Cant, J. P., D. R. Trout, F. Qiao, and N. G. Purdie. 2002. Milk synthetic response of the bovine mammary gland to an increase in the local concentration of arterial glucose. *J. Dairy Sci.* 85:494–503.
- Cantalapiedra-Hijar, G., J. L. Peyraud, S. Lemosquet, E. Molina-Alcaide, H. Boudra, P. Nozière, and I. Ortigues-Marty. 2014. Dietary carbohydrate composition modifies the milk N efficiency in late lactation cows fed low crude protein diets. *Animal* 8:275–285.
- Chan, S. C., J. T. Huber, C. B. Theurer, Z. Wu, K. H. Chen, and J. M. Simas. 1997. Effects of supplemental fat and protein source on ruminal fermentation and nutrient flow to the duodenum in dairy cows. *J. Dairy Sci.* 80:152–159.
- Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: a review. *J. Dairy Sci.* 76:3897–3931.
- Colmenero, J. J., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 89:1704–1712.

- CVB (Centraal Veevoederbureau). 2008. CVB Table Ruminants 2008, series nr. 43. CVB, The Hague, the Netherlands.
- Dijkstra, J., O. Oenema, J. W. van Groenigen, J. W. Spek, A. M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N₂O emissions. *Animal* 7:292–302.
- Galindo, C. E., D. R. Ouellet, D. Pellerin, S. Lemosquet, I. Ortigues-Marty, and H. Lapierre. 2011. Effect of amino acid or casein supply on whole-body, splanchnic, and mammary glucose kinetics in lactating dairy cows. *J. Dairy Sci.* 94:5558–5568.
- Glasser, F., M. Doreau, A. Ferlay, and Y. Chilliard. 2007. Technical note: Estimation of milk fatty acid yield from milk fat data. *J. Dairy Sci.* 90:2302–2304.
- Grum, D. E., J. K. Drackley, L. R. Hansen, and J. D. Cremin Jr. 1996. Production, digestion, and hepatic lipid metabolism of dairy cows fed increased energy from fat or concentrate. *J. Dairy Sci.* 79:1836–1849.
- Grummer, R. R., and D. J. Carroll. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. *J. Anim. Sci.* 69:3838–3852.
- Hammon, H. M., C. C. Metges, P. Junghans, F. Becker, O. Bellmann, F. Schnieder, G. Nürnberg, P. Dubreuil, and H. Lapierre. 2008. Metabolic changes and net portal flux in dairy cows fed a ration containing rumen-protected fat as compared to a control diet. *J. Dairy Sci.* 91:208–217.
- Haque, M. N., H. Rulquin, A. Andrade, P. Faverdin, J. L. Peyraud, and S. Lemosquet. 2012. Milk protein synthesis in response to the provision of an “ideal” amino acid profile at 2 levels of metabolizable protein supply in dairy cows. *J. Dairy Sci.* 95:5876–5887.
- Harvatine, K. J., and M. S. Allen. 2006a. Effects of fatty acid supplements on feed intake, and feeding and chewing behavior of lactating dairy cows. *J. Dairy Sci.* 89:1104–1112.
- Harvatine, K. J., and M. S. Allen. 2006b. Effects of fatty acid supplements on ruminal and total tract nutrient digestion in lactating dairy cows. *J. Dairy Sci.* 89:1092–1103.
- Hoffman, P. C., R. R. Grummer, R. D. Shaver, G. A. Broderick, and T. R. Drendel. 1991. Feeding supplemental fat and undegraded intake protein to early lactation dairy cows. *J. Dairy Sci.* 74:3468–3474.
- ISO. 1998. Animal feeding stuffs, animal products, and feces or urine. Determination of gross calorific value—Bomb calorimeter method. International Standards Organization (ISO), Geneva, Switzerland.
- ISO. 1999a. Animal feeding stuffs. Determination of fat content. ISO, Geneva, Switzerland.
- ISO. 1999b. Animal feeding stuffs. Determination of moisture and other volatile matter content. International Standards Organization, Geneva, Switzerland.
- ISO. 2002a. Animal feeding stuffs. Determination of crude ash. ISO, Geneva, Switzerland.
- ISO. 2002b. Milk fat. Preparation of fatty acid methyl esters. ISO, Geneva, Switzerland.
- ISO. 2004. Animal feeding stuffs. Enzymatic determination of total starch content. ISO, Geneva, Switzerland.
- ISO. 2005. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content—Part 1: Kjeldahl method. ISO, Geneva, Switzerland.
- ISO. 2013. Milk and liquid milk products. Guidelines for the application of mid-infrared spectrometry. ISO, Geneva, Switzerland.
- ISO. 2015. Milk, milk products, infant formula and adult nutritional. Determination of fatty acids composition—Capillary gas chromatographic method. ISO, Geneva, Switzerland.
- Jenkins, T. C. 1993. Lipid metabolism in the rumen. *J. Dairy Sci.* 76:3851–3863.
- Jenkins, T. C., and B. F. Jenny. 1989. Effect of hydrogenated fat on feed intake, nutrient digestion, and lactation performance of dairy cows. *J. Dairy Sci.* 72:2316–2324.
- Jensen, R. G., and D. S. Newberg. 1995. Bovine milk lipids. Pages 543–575 in *Handbook of Milk Composition*. R. G. Jensen, ed. Academic Press, San Diego, CA.
- Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. *J. Anim. Sci.* 88:2959–2967.
- Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.* 95:6042–6056.
- Lemosquet, S., G. Raggio, G. E. Lobley, H. Rulquin, J. Guinard-Flament, and H. Lapierre. 2009. Whole-body glucose metabolism and mammary energetic nutrient metabolism in lactating dairy cows receiving digestive infusions of casein and propionic acid. *J. Dairy Sci.* 92:6068–6082.
- Lock, A. L., C. L. Preseault, J. E. Rico, K. E. DeLand, and M. S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. *J. Dairy Sci.* 96:6650–6659.
- Lohrenz, A.-K., K. Duske, F. Schneider, K. Nürnberg, B. Losand, H. M. Seyfert, C. C. Metges, and H. M. Hammon. 2010. Milk performance and glucose metabolism in dairy cows fed rumen-protected fat during mid lactation. *J. Dairy Sci.* 93:5867–5876.
- Nichols, K., J. J. M. Kim, M. Carson, J. A. Metcalf, J. P. Cant, and J. Doelman. 2016. Glucose supplementation stimulates peripheral branched-chain amino acid catabolism in lactating dairy cows during essential amino acid infusions. *J. Dairy Sci.* 99:1145–1160.
- Noftsker, S., and N. R. St-Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. *J. Dairy Sci.* 86:958–969.
- OECD/FAO (Food and Agriculture Organization of the United Nations). 2015. Dairy. Pages 121–138 in *OECD-FAO Agricultural Outlook 2015*. OECD Publishing, Paris, France.
- Oldick, B. S., C. R. Staples, W. W. Thatcher, and P. Gyawu. 1997. Abomasal infusion of glucose and fat—Effects on digestion, production, and ovarian and uterine functions of cows. *J. Dairy Sci.* 80:1315–1328.
- Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993a. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* 76:1753–1771.
- Palmquist, D. L., and T. C. Jenkins. 1980. Fat in lactation rations. *J. Dairy Sci.* 63:1–14. (Review).
- Palmquist, D. L., M. R. Weisbjerg, and T. Hvelplund. 1993b. Ruminal, intestinal, and total digestibilities of nutrients in cows fed diets high in fat and undegradable protein. *J. Dairy Sci.* 76:1353–1364.
- Pantoja, J., J. L. Firkins, and M. L. Eastridge. 1995. Site of digestion and milk production by cows fed fats differing in saturation, esterification, and chain length. *J. Dairy Sci.* 78:2247–2258.
- Pantoja, J., J. L. Firkins, and M. L. Eastridge. 1996. Fatty acid digestibility and lactation performance by dairy cows fed fats varying in degree of saturation. *J. Dairy Sci.* 79:429–437.
- Piantoni, P., A. L. Lock, and M. S. Allen. 2015. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. *J. Dairy Sci.* 98:3309–3322.
- Rabiee, A. R., K. Breinhild, W. Scott, H. M. Golder, E. Block, and I. J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and meta-regression. *J. Dairy Sci.* 95:3225–3247.
- Raggio, G., S. Lemosquet, G. E. Lobley, H. Rulquin, and H. Lapierre. 2006. Effect of casein and propionate supply on mammary protein metabolism in lactating dairy cows. *J. Dairy Sci.* 89:4340–4351.
- Reed, K. F., H. C. Bonfá, J. Dijkstra, D. P. Casper, and E. Kebreab. 2017. Estimating the energetic cost of feeding excess dietary nitrogen to dairy cows. *J. Dairy Sci.* 100:7116–7126.
- Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. *J. Dairy Sci.* 90:1506–1515.
- Rico, D. E., Y. Ying, and K. J. Harvatine. 2014. Effect of a high-palmitic acid fat supplement on milk production and apparent

- total-tract digestibility in high- and low-milk yield dairy cows. *J. Dairy Sci.* 97:3739–3751.
- Rius, A. G., J. A. D. R. N. Appuhamy, J. Cyriac, D. Kirovski, O. Becvar, J. Escobar, M. L. McGilliard, B. J. Bequette, R. M. Akers, and M. D. Hanigan. 2010a. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. *J. Dairy Sci.* 93:3114–3127.
- Rius, A. G., M. L. McGilliard, C. A. Umberger, and M. D. Hanigan. 2010b. Interactions of energy and predicted metabolizable protein in determining nitrogen efficiency in the lactating dairy cow. *J. Dairy Sci.* 93:2034–2043.
- Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis. Pages 123–158 in *The Analysis of Dietary Fibre in Food*. W. P. T. James, and O. Theander, ed. Marcel Dekker, New York, NY.
- Schor, A., and G. A. Gagliostro. 2001. Undegradable protein supplementation to early-lactation dairy cows in grazing conditions. *J. Dairy Sci.* 84:1597–1606.
- Spek, J. W., J. Dijkstra, G. van Duinkerken, and A. Bannink. 2013. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. *J. Agric. Sci.* 151:407–423.
- Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. *J. Dairy Sci.* 48:1215–1223.
- van Hoeij, R. J., J. Dijkstra, R. M. Bruckmaier, J. J. Gross, T. J. G. M. Lam, G. J. Remmelink, B. Kemp, and A. T. M. van Knegsel. 2017. Consequences of dietary energy source and energy level on energy balance, lactogenic hormones, and lactation curve characteristics of cows after a short or omitted dry period. *J. Dairy Sci.* 100:8544–8564.
- van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, M. J. W. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. *J. Dairy Sci.* 90:1467–1476.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Vanhatalo, A., T. Varvikko, and P. Huhtanen. 2003. Effects of various glucogenic sources on production and metabolic responses of dairy cows fed grass silage-based rations. *J. Dairy Sci.* 86:3249–3259.
- Weld, K. A., and L. E. Armentano. 2017. The effects of adding fat to diets of lactating dairy cows on total-tract neutral detergent fiber digestibility: A meta-analysis. *J. Dairy Sci.* 100:1766–1779.
- Wright, T. C., S. Moscardini, P. H. Luimes, P. Susmel, and B. W. McBride. 1998. Effects of rumen-undegradable protein and feed intake on nitrogen balance and milk production in dairy cows. *J. Dairy Sci.* 81:784–793.