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Invited review: Moving from dietary fat to fatty acids—New insights into how fatty acids affect digestibility, metabolism, and performance in dairy cows

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ABSTRACT

In dairy nutrition, "fat" broadly refers to lipid compounds primarily composed of fatty acids (FA), which play diverse roles in digestion, metabolism, and milk production. The main FA present in dairy cow diets are palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. In the rumen, FA are extensively modified, which decreases the toxicity of UFA to rumen bacteria. Rumen bacteria can also synthesize FA from end products of rumen fermentation and AA, primarily producing odd- and branched-chain FA. As FA flow to the intestine, digestibility is influenced by several factors. These include total FA flow, FA profile, the presence of emulsification compounds, and the physical characteristics of fat supplements. The digestibility of FA typically decreases as total FA intake and flow to the intestine increase, especially with 18:0, which exhibits a more pronounced reduction in digestibility than 16:0. Some dietary UFA escape rumen biohydrogenation. Therefore, supplemental blends containing 18:1 can increase its postruminal delivery, improving FA digestibility and absorption. These effects are especially beneficial during early lactation and in high-producing cows. Additionally, the form and purity of supplements influence FA absorption, with highly saturated and pure supplements having lower digestibility. The source of 18-carbon FA in the diet also affects digestibility, with oilseeds being associated with less reduction in digestibility than prilled supplements that provide 18:0. Historically, UFA and medium-chain FA, commonly found in vegetable oils, have been shown to negatively affect NDF digestibility due to various mechanisms, including the coating of fiber particles, microbial toxicity, and reduced cation availability. However, recent studies indicate that FA sources high in 16:0 can enhance NDF digestibility. These improvements are not linked to reduced DMI, suggesting that other mechanisms, such as altered gut peptide activity or microbial community shifts, may be involved. In terms of production responses, 16:0 supplementation consistently improves milk fat yield, ECM, and nutrient utilization across lactation. In contrast, 18:1 enhances BW gain and FA digestibility and is particularly effective in increasing ECM in high-producing cows (over 45 kg/d of milk yield) and cows in early lactation. At the same time, 18:0 often reduces FA digestibility, limiting its production benefits. Across multiple studies, blends containing higher proportions of 16:0, especially in comparison with 18:0, led to linear increases in milk fat yield and ECM, supporting the preferential use of 16:0 for fat synthesis in the mammary gland. Although some earlier work suggested that combining 16:0 and 18:0 in a supplement would optimize FA utilization, this approach has been largely discredited by recent findings. High levels of 18:0 in FA supplements have been shown to reduce FA digestibility and energy intake, limiting their value in most production contexts. Combining blends containing 16:0 and 18:1 resulted in better production responses during early lactation than other supplements containing only 16:0 or 16:0 + 18:0. Overall, our review highlights the complexity of FA digestion and metabolism and underscores the importance of tailoring supplementation strategies to the production stage and physiological status of dairy cows and the outcomes desired. Future research should refine dietary formulations to optimize energy partitioning, enhance productivity, and support metabolic health throughout lactation.

Key words: energy partitioning, milk production, oleic acid, palmitic acid, stearic acid

INTRODUCTION

The addition of supplemental fat to the diet is a common practice in dairy nutrition to improve animal performance (Palmquist and Jenkins, 2017). In general, fat supplementation has been shown to increase the yield

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of milk, milk fat, and reproductive performance, but results have varied greatly between different fat types and even for the same supplement across different diets and studies (Rabiee et al., 2012; Rodney et al., 2015). This variability across experiments could be due to the use of different types of fat supplements, the level of fatty acid (**FA**) supplementation, interactions with other diet ingredients, and the physiological state of cows. In addition, milk fat is highly responsive to changes in nutrition and management (Bauman et al., 2011). At the same time, it is important to avoid excessive weight loss in early lactation and to support recovery of body condition in postpeak cows to improve reproductive performance and farm profitability, while also ensuring that excessive body condition does not occur in later lactation (Piantoni and VandeHaar, 2023). Therefore, the ability to understand and model FA digestion and metabolism is crucial for optimal diet formulation strategies.

Understanding how different sources of fat and their composition influence animal metabolism and energy partitioning is key to advancing FA nutrition. The importance of individual FA in a diet extends beyond their energy contribution and includes potential metabolic and physiological effects. The digestion, metabolism, and utilization of FA are complex processes in ruminants, and recent research highlights the importance of dietary FA in performance outcomes. In this regard, palmitic acid (16:0), stearic acid (18:0), and oleic acid (*cis*-9 18:1; hereafter referred to as 18:1) are the most abundant FA present in milk fat and adipose tissue of dairy cows (de Souza et al., 2018). These FA have distinct metabolic functions and pathways, yet they may interact through competitive or complementary mechanisms depending on the cow's physiological state. Just as nutrition practices have been advanced by focusing on individual AA rather than CP, understanding the specific effects of individual FA is central to furthering both scientific knowledge and practical applications.

A schematic representation of FA digestion, metabolism, and partitioning is shown in Figure 1. The main FA present in dairy cow diets are 16:0, 18:0, 18:1, linoleic (18:2), and linolenic (18:3) acids. These FA are extensively modified in the rumen and influence the digestion of other dietary fractions (e.g., fiber). They also influence the secretion of hormones and peptides in the gut that can affect feed intake and rumen emptying. After rumen metabolism, FA reach the intestine, where they are absorbed. After absorption, FA are partitioned into different tissues and used as building blocks for end products (e.g., milk fat) and as a source of energy. They can also be stored in adipose tissues, influencing transcription, gene expression, and hormone synthesis. The supply of preformed FA to the mammary gland influences de novo synthesis and total FA synthesis. Partitioning of nutrients to adipose tissue is influenced by the physiological state, FA profile of the diet, and other dietary conditions.

Our objective in this review is to summarize and discuss recent advances in the understanding of the role of individual FA in dairy cows, with a focus on 16:0, 18:0, and 18:1 and their effects on nutrient digestibility, energy partitioning, and production responses. Our goal is to highlight major advancements, issues that need to be addressed, and some practical implications.

INTAKE AND RUMEN METABOLISM

Intake of Dietary FA

Dietary FA in lactating dairy cattle primarily come from forages, grains, byproducts, and fat supplements (Daley et al., 2020). Most of these lipids are present as triglycerides (**TG**), free FA, glycolipids, or phospholipids (Lock and Bauman, 2004). The most abundant FA in grass is 18:3, with legume forage sources and fresh grasses providing significant amounts of FA to the diet (Glasser et al., 2013). The main FA in concentrates based on corn and soybeans and in corn silage is 18:2 (Baldin et al., 2018), and in most corn silage-based diets, it is the predominant FA ingested. Oilseeds commonly used in diets contain primarily 18:2 (e.g., cottonseed and soybeans) or 18:3 (e.g., flaxseed), but new varieties of soybeans have been developed with high levels of 18:1 (Bales and Lock, 2024a). Regarding FA supplements, calcium salts and saturated prilled supplements are some of the most common rumen-inert FA supplements in dairy cow diets, and because they are produced from a variety of raw material streams and fat byproducts that yield free FA, the FA profile can vary across different supplements. The main SFA present in dietary ingredients and commercially available FA supplements are 16:0 and 18:0 (dos Santos Neto et al., 2021b). The 16:0-enriched supplements contain high levels of 16:0 (>80%) and small amounts of UFA, whereas saturated mixed prills contain 18:0 and 16:0 as the main FA. Calcium salts of palm FA are usually manufactured from palm FA distillate (**PFAD**) and contain 16:0 and 18:1 as their major FA (dos Santos Neto et al., 2021a). Therefore, in most feeding conditions, 16:0, 18:0, 18:1, 18:2, and 18:3 are the predominant FA ingested by dairy cows.

Rumen Metabolism

Although most dietary FA are UFA, the FA reaching the intestine are mostly saturated owing to lipid metabolism in the rumen (Harfoot and Hazlewood, 1997). Two major modification processes for ingested FA that occur in the rumen are (1) hydrolysis of ester linkages in lipids, which releases free FA, and (2) subsequent

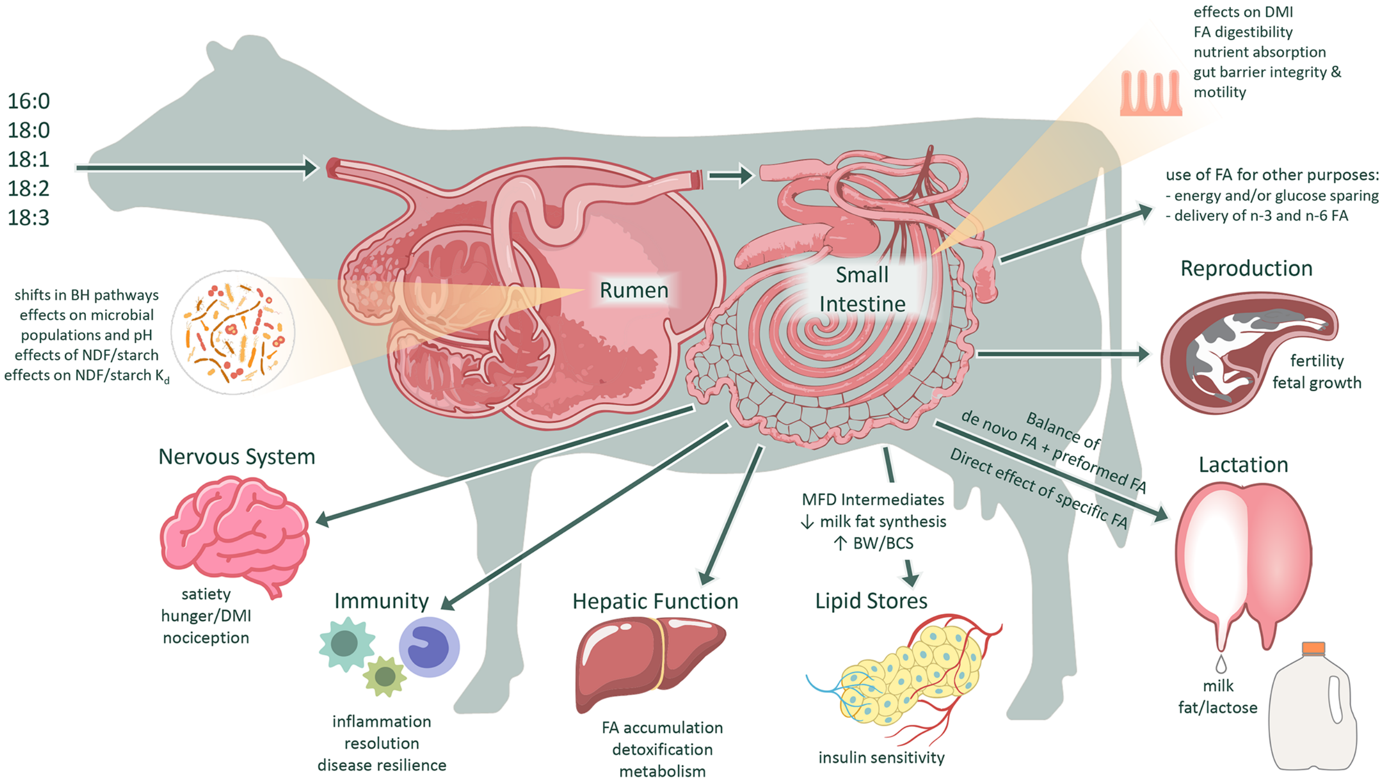

Figure 1. Schematic representation of metabolism of dietary FA. Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids are the main FA present in forage, concentrates, and supplements fed to dairy cows.

biohydrogenation (**BH**) of FA, which decreases the toxicity of UFA to rumen bacteria (Maia et al., 2010). After consumption and mastication, lipids are rapidly hydrolyzed. Microbial lipases release FA from their glycerol backbone through hydrolysis (Jenkins, 1993), and rumen bacteria are the main microbes that perform hydrolysis in the rumen (Harfoot and Hazlewood, 1997). Endogenous galactolipases and phospholipase in forage plant tissues can remain active for hours after ingestion and may also contribute to hydrolysis in the rumen (Van Ranst et al., 2009). However, the proportion of hydrolyzed lipids by plant-sourced galactolipases and phospholipases is unclear. Following hydrolysis, rumen bacteria biohydrogenate UFA to form SFA through a series of reactions, including isomerization, hydrogenation, double bond migration, and hydration, that produce many different intermediates (Toral et al., 2024). The profile of FA intermediates from rumen BH is associated with dietary conditions, microbial community composition, and rumen pH (Bauman et al., 2011).

The primary dietary UFA sources for BH are 18:1, 18:2, and 18:3, and the extent of rumen BH for these FA ranges from 60% to 80%, 80% to 95%, and 85% to 100%, respectively (Jenkins and Bridges, 2007; Jenkins et al., 2008). Conditions that might affect the extent of BH and increase the passage of UFA to the duodenum are increased rumen concentration of UFA, decreased rumen pH, and the presence of ionophores (Jenkins and Harvatine, 2014). Considerable opportunity remains for researchers to develop novel, improved, and more sophisticated models of ruminal lipolysis and BH of unsaturated FA and to continue to develop technologies to limit rumen BH and increase postrumen supply of dietary unsaturated FA (Lock and Bauman, 2004; Jenkins et al., 2008). Among the risk factors that may influence the production of intermediates of rumen BH, rumen pH is well characterized as a key factor (Bauman et al., 2011). For instance, the accumulation of both *trans*-10 18:1 and *trans*-10,*cis*-12 18:2 was greater with increasing corn oil levels in the diet at low pH compared with high pH in an in vitro model (Sun et al., 2019). Rumen bacteria can also synthesize FA from end products of rumen fermentation and AA, primarily producing odd- and branched-chain FA (Kaneda, 1991) and incorporating exogenous long-chain FA into bacterial lipids (Erwin, 1973). Total lipid in the bacterial mass usually ranges from 10% to 15% of bacterial DM (Jenkins, 1993; Mitchell et al., 2023). Absorption of long-chain FA in the rumen is considered limited (NASEM, 2021), and it has been associated with the low abundance of proteins responsible for FA transport and absorption at the rumen level (Hentz and Batistel, 2024). Microbial oxidation of long-chain FA is limited, but in vitro losses of <14-carbon FA and minimal changes for 16:0 and 18:0 have been reported (Wu and Palmquist, 1991). In one of our previous meta-analyses, the total duodenal flow of FA (g/d) was associated with FA intake (g/d), with a slope of 0.84 and intercept of 92 g/d (Boerman et al., 2015a). The slope indicates a disappearance of 16% of FA ingested due to absorption or direct microbial incorporation, while the intercept gives a net estimate of endogenous synthesis. Therefore, most of the material leaving the rumen is SFA, and the flow of FA to the intestine is slightly greater than intake due to microbial synthesis.

Effect of FA on Fiber Digestibility

Changes in digestibility of other nutrients, such as NDF, owing to FA supplementation may positively or negatively affect the digestible energy value of the fat supplement (Boerman et al., 2015a), and it has been widely accepted that FA supplementation negatively affects NDF digestibility. Several studies since the 1950s have addressed the effects of added vegetable oils on fiber digestion in the rumen, with results indicating negative effects of oils on cellulose digestibility (Palmquist and Jenkins, 2017). The potential reduction in fiber digestibility when oil is supplemented may arise from 1 or more of 4 proposed mechanisms: (1) coating of the fiber with fat preventing microbial interaction; (2) the toxic effect of UFA on certain microbial populations; (3) inhibition of microbial activity on cell membranes by FA; and (4) reduced cation availability due to the formation of insoluble complexes with FA (Palmquist and Jenkins, 1980).

However, recent research has refuted the prevailing dogma that incorporating FA into diets will result in reduced fiber digestibility. In a meta-analysis to evaluate the effects of FA supplementation on NDF digestibility in dairy cows, Weld and Armentano (2017) indicated that the effects are directly related to the fat source. They reported that supplementation of FA supplements high in medium-chain FA (12- and 14-carbon) and vegetable oils decreased NDF digestibility, whereas feeding a fat supplement with FA 16-carbons or greater in length had minimal effects on NDF digestibility. In our more recent meta-analyses, we assessed the influence of the most common commercially available FA supplements on nutrient digestibility (dos Santos Neto et al., 2021a,b). We observed that NDF digestibility increased by 1.6 and 4.5 percentage points when calcium salts of PFAD and 16:0-enriched supplements were fed, respectively. However, feeding SFA-mixed prills had no effect on NDF digestibility (Figure 2A).

Additionally, for this review, we performed a random regression analysis of individual cow data from 19 studies (descriptive information in Supplemental Table S1, see Notes) in which different supplements were fed to dairy cows to evaluate the relationship between FA intake and fiber digestibility. We observed that total-tract NDF digestibility was associated with 16:0 intake (g/d), with a slope of 0.0087 and an intercept of 40.6% (Figure 2B), but it was not influenced by intakes of 18:0 (Figure 2C) and 18:1 (Figure 2D). Our data also suggest that the increase in NDF digestibility when 16:0 supplements are fed to dairy cows is not explained by a decrease in DMI. Potential explanations for the effect of 16:0 on fiber digestibility may involve changes in gut peptides that are related to gastrointestinal motility and the direct effect of FA on microbial populations. Piantoni et al. (2013) suggested that the increase in NDF digestibility was related to an increase in retention time driven by enhanced cholecystokinin secretion. In a recent study, the positive effect of including 16:0 in the diet of continuous culture fermenters on fiber digestibility was linked to changes in the bacterial community composition and a tendency for increased microbial growth, especially for bacteria responsible for fiber digestion (Sears et al., 2024). Also, feeding increased levels of a 16:0-enriched supplement selectively enhanced the abundance of specific fiber-digesting bacteria and promoted the production of VFA, particularly propionate, valerate, and isobutyrate in continuous culture fermenters (Wenner et al., 2025). Protozoa contain proportionally more unsaturated FA than bacteria (Harfoot and Hazlewood, 1997). This is attributed to protozoa preferentially incorporating unsaturated FA and BH intermediates from ingested bacteria (Jenkins et al., 2008). Whether protozoa contribute to the effect of exogenous FA supply on fiber digestion remains to be determined. Therefore, the effect of FA supplementation on fiber digestibility is directly associated with the FA profile of the supplements, and improvements in fiber digestibility are observed when 16:0 rather than 18:0 and 18:1 is fed.

INTESTINAL DIGESTIBILITY AND ABSORPTION

FA Digestion in the Intestine

Under typical feeding conditions, 18:0 is the primary FA available for absorption by dairy cows. Long- and medium-chain FA are not significantly altered or absorbed in the omasum or abomasum, and the lipid composition available for absorption in the small intestine is thus similar to what exits the rumen (Moore and Christie, 1984). This material consists mainly of free FA (80%–90%) attached to feed particles, with the remainder be-

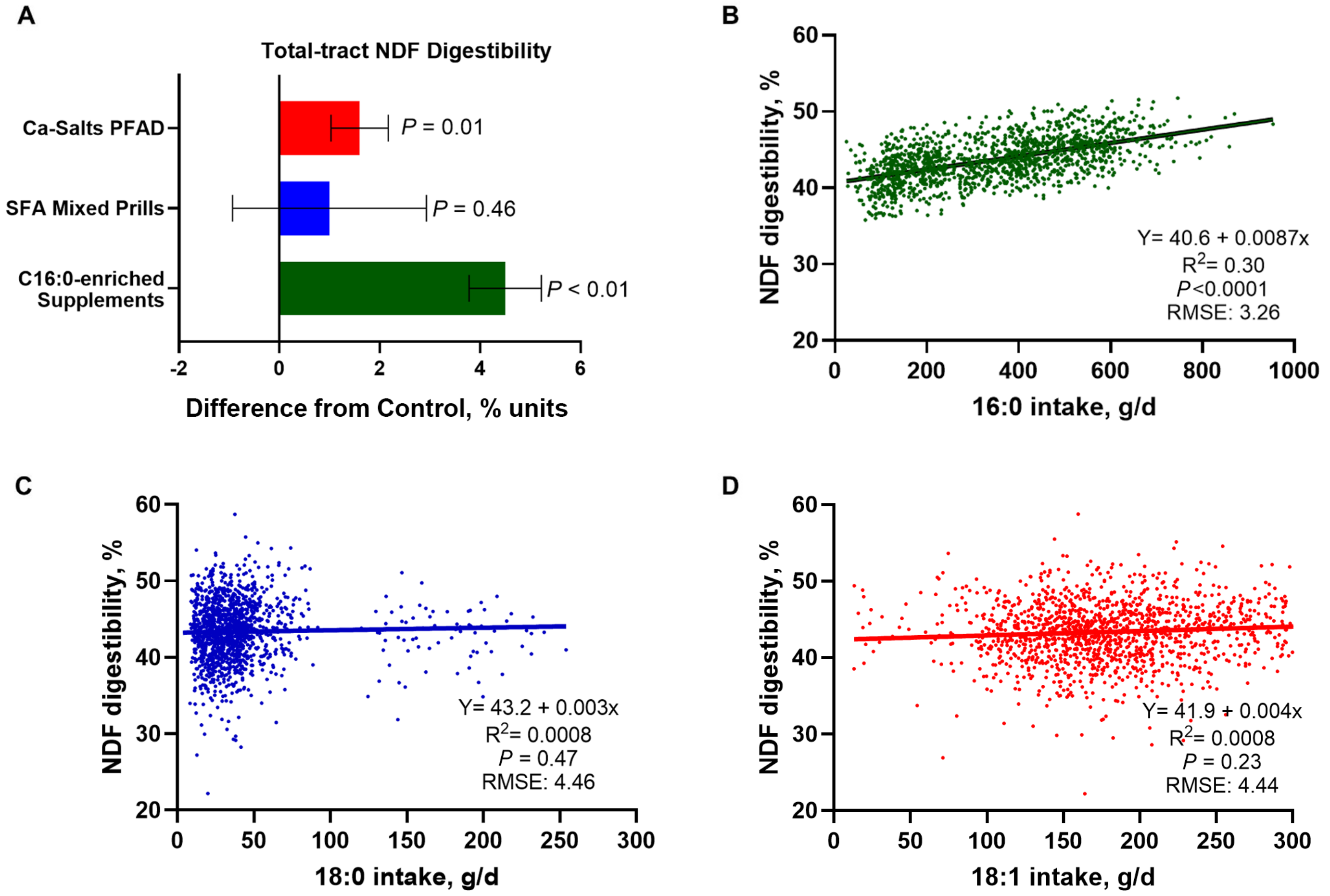

Figure 2. The relationship between FA intake and NDF digestibility of dairy cows. (A) Effect of feeding common commercially available FA supplements on NDF digestibility (dos Santos Neto et al., 2021a,b; *P*-value is the comparison of supplements against control). Meta-regression of 19 studies with 1,449 individual cow observations on the impact of (B) 16:0 intake, (C) 18:0 intake, and (D) 18:1 intake on NDF digestibility. Details on studies and meta-regression are presented in Supplemental Table S1 (see Notes). RMSE = root mean square error. Error bars are the SE of the difference.

ing microbial phospholipids, trace amounts of TG, and glycolipids from residual feed (Doreau and Chilliard, 1997). Esterified FA are hydrolyzed by intestinal and pancreatic lipases (Doreau and Ferlay, 1994). Efficient FA absorption in ruminants depends on micelle formation, which solubilizes water-insoluble FA by incorporating them into micellar structures (Freeman, 1984). This process requires bile and pancreatic secretions, which provide bile salts, lecithin, and enzymes that help form micelles (Davis, 1990). Once formed, micelles facilitate the transfer of water-insoluble lipids across the unstirred water layer of intestinal epithelial cells, allowing FA and lysolecithin to be absorbed.

Absorption of FA into intestinal epithelial cells is an energy-independent process that is facilitated by a concentration gradient being maintained in the cells (Drackley, 2000). The presence of key proteins responsible for FA trafficking and transport in the distal section of the small intestine suggests a protein-mediated absorption of FA in cattle (Hentz and Batistel, 2024). In intestinal cells, free FA are combined with glycerol to form TG, which are packaged with cholesterol, phospholipids, and apoproteins to form lipoproteins such as chylomicrons or very low-density lipoproteins (Bauchart, 1993). Owing to their size, the lipoproteins are first secreted into the interstitial spaces, where they enter the lymphatics and then the lymph for delivery into the bloodstream close to the heart and subsequently transported to other organs (Moore and Christie, 1984). Absorbed PUFA in intestinal epithelial cells are preferentially incorporated into phospholipids and cholesterol esters as a way to prevent PUFA from being oxidized as fuels or extensively incorporated into milk fat (Moore and Christie, 1984). Short- and medium-chain FA (<14-carbon) are primarily used in oxidation reactions in the liver and intestine (Palmquist and Jenkins, 1980).

FA Digestibility

Improving FA digestibility has received attention lately because it can directly influence energy intake (Boerman et al., 2015a). Looking forward, total dietary FA intake is likely to increase to meet energy demands as genetic selection progresses in dairy cows (NASEM, 2021). Based on our recent research, we identify the following potential factors as the most significant influencing factors for FA digestibility: (1) FA intake and total flow of FA to the intestine, (2) FA profile of the digesta flowing to the intestine, (3) sufficient secretion of emulsification compounds at the intestinal level, and (4) physical form and characteristics of FA supplements.

Typically, FA digestibility decreases as the flow of FA increases to the intestine, and it is well characterized that the flow of any nutrient is usually negatively related to its digestibility (Van Soest, 1994). In our analysis (Figure 3A) with intestinal estimates, total FA digestibility (%) was associated with FA intake (g/d) with a slope of −0.0084 and an intercept of 82.7% (Boerman et al., 2015a). Thus, the slope indicates a negative relationship between intake and digestibility, and we can estimate a decrease of about 8.4 percentage units in digestibility in a cow consuming 1,000 g of FA (assuming 1,000 g FA intake for a cow with a feed intake of 25 kg/d and 4% total dietary FA).

The profile of FA reaching the intestine has a critical effect on the total FA digestibility of long-chain FA in lactating dairy cows (Boerman et al., 2015a). A negative relationship between the total flow and digestibility of FA has been observed, and the decrease in total FA digestibility appears to be driven by the 18:0 digest-

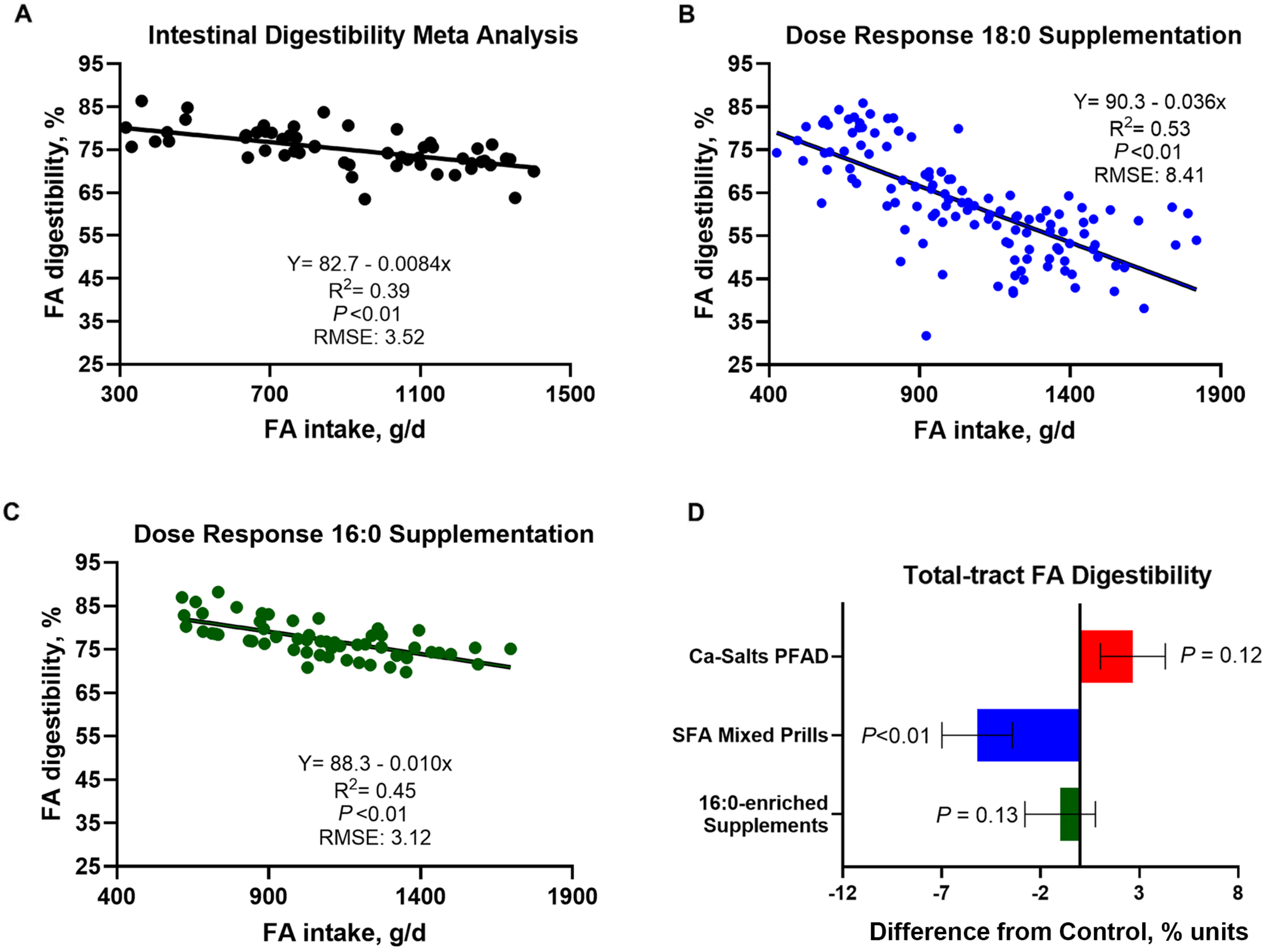

Figure 3. The relationship between FA intake and FA digestibility in dairy cows. (A) Total FA digestibility data from a meta-analysis evaluating intestinal digestibility of FA (Boerman et al., 2015a), and 2 studies in which cows were supplemented with either (B) an 18:0-enriched supplement (Boerman et al., 2017) or (C) a 16:0-enriched supplement (Rico et al., 2017). (D) The effect of feeding common commercially available FA supplements on FA digestibility (*P*-value is the comparison of supplements against control; dos Santos Neto et al., 2021a,b). Error bars are the SE of the difference.

ibility because of the pronounced negative relationship between the duodenal flow and its digestibility (Boerman et al., 2015a). In addition, a recent analysis estimating digestibility using a meta-regression model of total-tract apparent absorbed FA and total FA intake (Lucas test) did not show that total dietary FA and DMI-affected FA digestibility (Daley et al., 2020). The authors instead reported that FA sources strongly influenced FA digestibility, suggesting that FA profile and physical form have a more important role than FA intake. Boerman et al. (2017) fed increasing levels of an 18:0 supplement (93% 18:0) to dairy cows and observed no positive effect on production responses, which was likely associated with the pronounced decrease in total FA digestibility as FA intake increased (Figure 3B). Similarly, Rico et al. (2017) fed increasing levels of a 16:0 supplement (87% 16:0) to dairy cows, and even though a positive effect was observed on production response up to 1.5% diet DM, total FA digestibility decreased as FA intake increased (Figure 3C). Considering that the range in FA intake was similar across both studies and intercept values were also similar, the decrease in total FA digestibility was about 3 times more pronounced when there was increased intake/rumen outflow of 18:0 compared with 16:0. Furthermore, Glasser et al. (2008b) suggested a quadratic relationship between absorbed 18:0 and its duodenal flow, indicating reduced digestibility at higher duodenal flow of 18:0 on a gram per kilogram of DM basis. The authors suggest a saturation of absorption at high levels specific to 18:0 because the absorption of other 18-carbon FA was not modified by high duodenal flows. In previous meta-analyses, we assessed the influence of the most common commercially available FA supplements on FA digestibility and observed that supplements containing a mix of 18:0 and 16:0 decreased FA digestibility by 5 percentage units, whereas 16:0-enriched supplements and calcium salts of PFAD had no influence on total FA digestibility (Figure 3D; dos Santos Neto et al., 2021a,b). We also reviewed the effects of altering ratios of dietary 16:0, 18:0, and 18:1 on FA digestibility of dairy cows. Burch et al. (2021) reported that total FA digestibility increased by 9 percentage units and total absorbed FA by 120 g/d when a blend containing 60% 16:0 + 30% 18:1 was fed compared with a blend delivering 60% 16:0 + 30% 18:0. Further, increasing levels of 18:1 to replace 18:0 linearly increased total FA digestibility and total absorbed FA (Prom and Lock, 2021). Although 18:0 is the primary FA available for absorption in the intestine, increasing its intake or flow reduces FA digestibility and absorption; thus, increasing 18:0 digestibility potentially provides the greatest opportunity for strategies to increase FA absorption in cows.

In a series of studies infusing emulsifier compounds in dairy cows to improve FA digestibility, we abomasally infused 15, 30, or 45 g of a commercial emulsifier (Tween 80, Sigma-Aldrich, St. Louis, MO). Infusing up to 30 g of an exogenous emulsifier resulted in increases in the absorption of total, 16-carbon, and 18-carbon FA, as well as production performance (de Souza et al., 2020). Subsequently, our results suggested that the predominant FA present in the polysorbate affects its ability to improve FA digestibility because Tween 80 (polysorbate based on 18:1) increased FA absorption and performance compared with Tween 40 (polysorbate based on 16:0) and Tween 60 (polysorbate based on 18:0 + 16:0; Prom et al., 2022). Furthermore, our data indicated a linear increase in absorption of total, 16-carbon, and 18-carbon FA and greater production performance when we abomasally infused 20, 40, or 60 g of 18:1 (Prom et al., 2021). Response to emulsifiers and their ability to improve FA digestibility was greater when directly infused into the abomasum or rumen (dos Santos Neto et al., 2023b), but there was no additive effect of infusing Tween 80 and 18:1 in combination (dos Santos Neto et al., 2023a). Collectively, our results suggest that limited emulsification in the intestine is likely one of the reasons for reduced FA digestibility as the flow of FA to the intestine increases, and supplying emulsification compounds that reach the intestine will increase FA absorption. Interestingly, although polysorbates and 18:1 demonstrated their potential as emulsifiers, most studies feeding lecithin products (Fontoura et al., 2021) or abomasally infusing them (Gutierrez-Oviedo et al., 2024) reported no FA digestibility increase in ruminants. Overall, lecithin is more effective in nonruminants (Øverland et al., 1993; Zhang et al., 2011), as this phospholipid is not produced in considerable amounts by these animals. Consequently, supplying lecithin to ruminants could, to some extent, promote negative feedback, which would explain its commonly observed lack of effects in dairy cows. However, a recent study found that the dietary inclusion of lysophospholipids in diets containing 16:0-enriched supplements or mixed SFA prills increased FA digestibility in dairy cows (Porter et al., 2024). This topic deserves further investigation.

Physical and chemical characteristics (particle size, degree of esterification, purity, hardness) of fat supplements have been suggested as potential factors that influence digestibility and animal performance (NASEM, 2021). With regard to particle size, a previous study (Eastridge and Firkins, 2000) suggested lower FA digestibility for tallow flakes (mean particle size 1,180 μm) than for prills (mean particle size 600 μm). Because both fat supplements had similar FA profiles and iodine values, the finding was mainly attributed to particle size. We evaluated prill size of a 16:0-enriched supplement varying from 200 to 600 μm but did not observe major differences in FA digestibility or production responses (de Souza et al., 2017b). As most dry fat supplements generally maintain an average particle size under 1,000 µm, particle size likely has minimal influence on digestibility in most typical feeding conditions. To evaluate the influence of purity, FA profile, and degree of esterification, we used a Lucas test to determine the total-tract FA digestibility of 16:0-enriched supplements (Figure 4). Our analysis was based on individual observations (n = 385) of mid-lactation Holstein dairy cows from 7 previously published studies (Piantoni et al., 2013; Boerman et al., 2015b; Rico et al., 2017; de Souza et al., 2018; de Souza and Lock, 2018b, 2019b; Western et al., 2020a). Digestibility estimates (mean ± SE) for different 16:0-enriched supplements were: (1) 41.3% ± 1.3% for 16:0 high purity (prilled free FA supplements containing ~98% 16:0); (2) 69.0% ± 1.2% for 16:0 FA (prilled free FA supplements containing ~85% 16:0 and ~6.0% 18:1); (3) 58.0% ± 1.0% for 16:0 TG (prilled TG supplements containing ~80% 16:0 and ~13% 18:1); and (4) 76.0% ± 1.1% for 16:0 blend (combination of prilled FFA and calcium salt supplements containing ~80% 16:0 and ~10% 18:1). The low digestibility of pure supplements enriched with SFA has been previously reported (Piantoni et al., 2013, 2015a; Daley et al., 2020). The lower digestibility for highly enriched SFA prills could be associated with a small amount of UFA in the product that could increase its solubility, the physical changes in the structure of FA, or both. Changes in the angle orientation and stability of SFA due to purity may form structurally stable FA crystals that are more difficult to incorporate into micelles during the emulsification process compared with less rigid structures that may break apart more easily (Sato, 2001). Using differential scanning calorimetry, Shepardson et al. (2020) reported high enthalpy and melting temperature values as purity increased in SFA supplements, but FA digestibility was not measured or evaluated. Furthermore, the estimated digestibility for 16:0 was lower for TG than for FA. Previous studies have reported lower total FA digestibility for 16:0 TG supplements than for calcium salts of PFAD (de Souza and Lock, 2018b) and other supplements in TG form (Pantoja et al., 1995; Weiss and Wyatt, 2004). Lipolysis in the small intestine might be a rate-limiting step to TG supplement digestion because several lipases are only active at the distal end of the small intestine. When the supplements are fed, the lipases may be unable to hydrolyze such large amounts of duodenal TG, therefore possibly limiting intestinal digestibility (Jenkins and Jenny, 1992). Additionally, the improvement in digestibility in supplements containing a blend of prills and calcium salts may be associated with a potential increased supply of oleic acid postruminally. Abomasal infusion of 18:1 has increased FA digestibility (Prom et al., 2021), and increasing dietary levels of 18:1 using a calcium salt supplement has also resulted in improvements in FA digestibility in dairy cows (de Souza et al., 2019a).

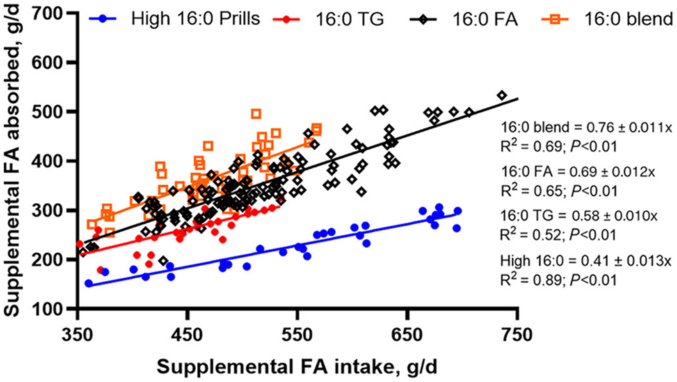

Figure 4. Physical and chemical factors that can influence the digestibility of 16:0-enriched supplements. We used a Lucas test to determine the total-tract FA digestibility of 16:0-enriched supplements that differed in their degree of esterification and FA profile. Our analysis used individual observations (n = 385) of mid-lactation Holstein dairy cows from 7 Latin square design studies (Piantoni et al., 2013; Boerman et al., 2015b; Rico et al., 2017; de Souza et al., 2018; de Souza and Lock, 2018b, 2019b; Western et al., 2020a). The 16:0 supplements were classified based on the degree of esterification and FA profile as follows: (1) 16:0 high purity (prilled FA supplement containing ~98% 16:0); (2) 16:0 FA (prilled FA supplement containing ~85% C16:0 and ~6.0% C18:1); (3) 16:0 TG (prilled TG supplement containing ~80% C16:0 and ~13% C18:1); and (4) 16:0 blend (combination of prilled FA and calcium salts of FA supplement containing ~80% C16:0 and ~10% C18:1). Slopes and SD are reported and indicate the estimated digestibility of each supplement.

Although unsaturated 18-carbon FA compose the majority of FA ingested by the cow, due to rumen BH, most of the flow of 18-carbon FA to the intestine is 18:0. To increase the dietary supply of 18-carbon FA, fat supplements, oilseeds, and some byproducts are available. We investigated if the source of 18-carbon FA in the diet influences FA digestibility as the intake of FA increases (Figure 5). We combined the data from 3 trials increasing 18-carbon intake by increasing levels of whole cottonseed (Bales et al., 2024c), feeding a supplement containing a mix of 18:0 and 16:0 (Western et al., 2020a), and feeding a highly enriched 18:0 supplement (Boerman et al., 2017). As expected, FA digestibility decreased overall as the intake of 18-carbon FA increased. However, the rate of decrease was influenced by the source. Digestibility was decreased to a greater extent when 18-carbon FA were supplied by a prilled supplement as opposed to oilseeds. In addition, as the level of 18:0 increased in the supplement, digestibility decreased. This outcome highlighted that not all 18-carbon sources have the same influence on digestibility, and nutritional models should consider the source of FA when assigning digestibility coefficients. Furthermore, when considering increasing the supply of 18-carbon FA, careful attention should be given to the source and, more specifically, to the individual FA supplied.

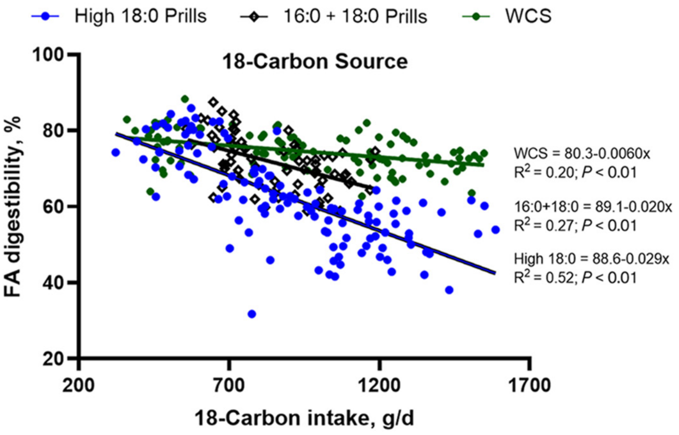

Figure 5. The relationship between 18-carbon intake from different sources of fat and FA digestibility. Influence of increasing 18-carbon intake by increasing dietary levels of whole cottonseed (WCS; Bales et al., 2024c), SFA mixed prills (Western et al., 2020a), and high 18:0 prills (Boerman et al., 2017) on total-tract digestibility of FA.

INFLUENCE OF FA ON PRODUCTION RESPONSES

Overall Impact of FA Supplements on Production Responses

Several authors have previously summarized the effect of supplemental fat on production responses (Onetti and Grummer, 2004; Rabiee et al., 2012; Hu et al., 2017). Although FA supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production in general, production performance varies greatly between different FA types and indeed, for the same supplement across different diets and studies. This pattern is evident in a meta-analysis examining the effect of FA supplementation on the diets of dairy cows (Rabiee et al., 2012). In general, milk production and milk fat percentage and yield increased, DMI and milk protein percentage decreased, and milk protein yield did not change with FA supplementation. A wide range of responses (~5 SD) for all variables indicated varied and marked biological effects of the different FA supplements (Rabiee et al., 2012). However, some of these studies had no limit on dietary FA inclusion or compared supplements with other sources of dietary fat, such as oilseeds and oils.

Calcium salts of PFAD and prilled saturated free FA are 2 common types of supplements used in the dairy industry, and they differ in FA content and FA profile. We conducted a meta-analysis and meta-regression on the effects of the most common dry fat supplements on the production responses of dairy cows (dos Santos Neto et al., 2021a,b). Our analysis limited the inclusion level to 3% DM and compared supplements against a nonfat control diet. Our final analysis included data from 33 studies supplementing calcium salts of PFAD (average inclusion of 2.20% diet DM), 18 studies supplementing SFA-mixed prills (average inclusion of 2.26% diet DM), and 15 studies supplementing 16:0-enriched supplements (average inclusion of 1.80% diet DM). Figure 6 presents a summary of the production responses to feeding these common dry fat supplements.

Feed intake was not affected by the addition of 16:0-enriched supplements and mixed SFA prills, but it decreased with feeding of calcium salts of PFAD (Figure 6A; dos Santos Neto et al., 2021a,b). Similarly, Allen (2000) observed in a review paper that hydrogenated SFA supplements did not affect DMI, whereas calcium salts of PFAD and tallow decreased feed intake. The hypophagic effect of feeding fat seems to be more pronounced for UFA than for SFA supplements (Harvatine and Allen, 2006), with DMI decreasing linearly as the degree of unsaturation increases (Drackley et al., 1992; Pantoja et al., 1995) and as the chain length of FA infused into the abomasum increases (Drackley et al., 1992). The more pronounced decrease in DMI for UFA is likely mediated in part by increased secretion of gut peptides such as cholecystokinin and glucagon-like peptide-1 related to satiety (Relling and Reynolds, 2007; Bradford et al., 2008). Milk yield increased with supplemental fat (Figure 6B), and the magnitude of response was similar across sources of supplemental fat. The inclusion of FA increases energy efficiency in lactating cows by generating more ATP per mole than glucose and protein, by promoting nutrient partition toward milk production, and by sparing energy by decreasing de novo milk FA synthesis (Palmquist, 2006). In addition, FA have a high energy density that can be incorporated into the diet without needing to considerably increase the heat increment (Wang et al., 2010). Thus, the effect of FA supplementation on milk yield is potentially driven by energetics rather than differences in metabolism driven by the FA profile of the supplement.

Milk fat yield responses to the different FA supplements were largely influenced by the FA profile of the fat sources (Figure 6C; dos Santos Neto et al., 2021a,b). The increase in milk fat yield was similar between calcium salts of PFAD and SFA-mixed prills (~40 g/d), but the response was greater for 16:0-enriched supplements (~100 g/d). Overall, the 3 sources of dry fat supplements decreased <16-carbon milk FA, with a similar magnitude of decrease between SFA-mixed prills and 16:0-enriched supplements (~20 g/d) and a greater decrease with calcium salts of PFAD (~41 g/d; Figure 7A; dos Santos Neto et al., 2021a,b). A 16-carbon milk FA yield was increased by supplying 16:0 supplements (~133 g/d), with a modest increase associated with calcium salts of PFAD (~10 g/d) and a tendency for SFA-mixed prills (~17 g/d; Figure 7B; dos Santos Neto et al., 2021a,b). The yield of >16-carbon milk FA increased with calcium salts of

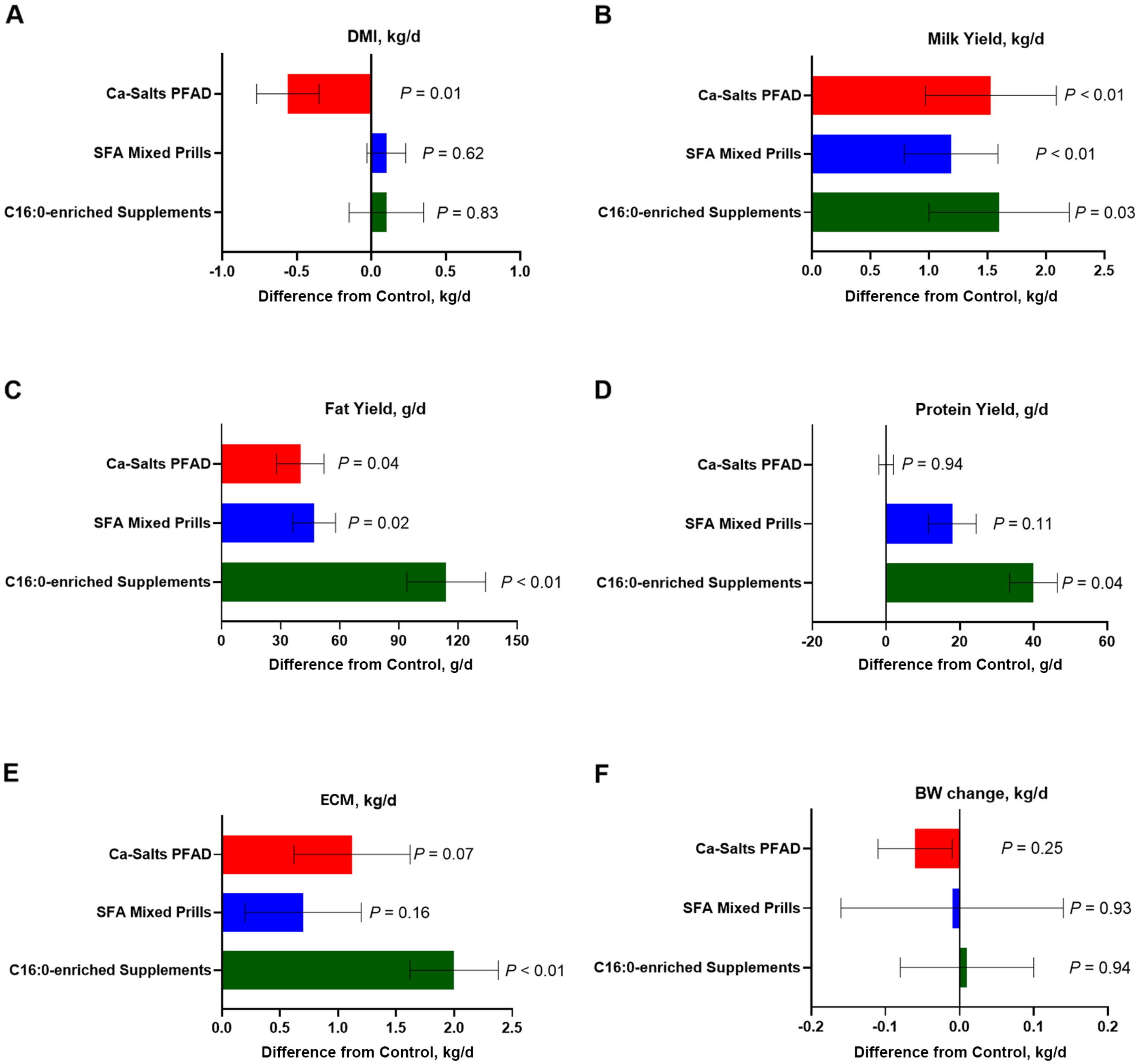

Figure 6. Effect of feeding common commercially available FA supplements on production responses of dairy cows (*P*-value is the comparison of supplements against control; adapted from dos Santos Neto et al., 2021a,b). Error bars are the SE of the difference.

PFAD (~68 g/d) and SFA-mixed prills (~40 g/d; Figure 7C; dos Santos Neto et al., 2021a,b). Collectively, our results indicate that the FA profile of supplemental fat largely influences milk fat responses. Milk TG synthesis is a highly coordinated process, and the location of FA along the glycerol backbone is not random, with specific enzymes preferentially locating individual FA at different positions (Jensen, 2002). Glasser et al. (2008a) proposed an interdependence between the FA in milk fat, wherein preformed FA would stimulate an increase of de novo FA in low-fat diets. However, as feeding levels of FA increased in the diet, they detected an inverse relationship and described a "substitution effect" of de novo FA by preformed FA. The mechanisms for FA substitution may be explained by the competition between de novo and exogenous long-chain FA to be incorporated into the glycerol-3-phosphate backbone. Thus, our results indicate that fat supplementation promotes a substitution

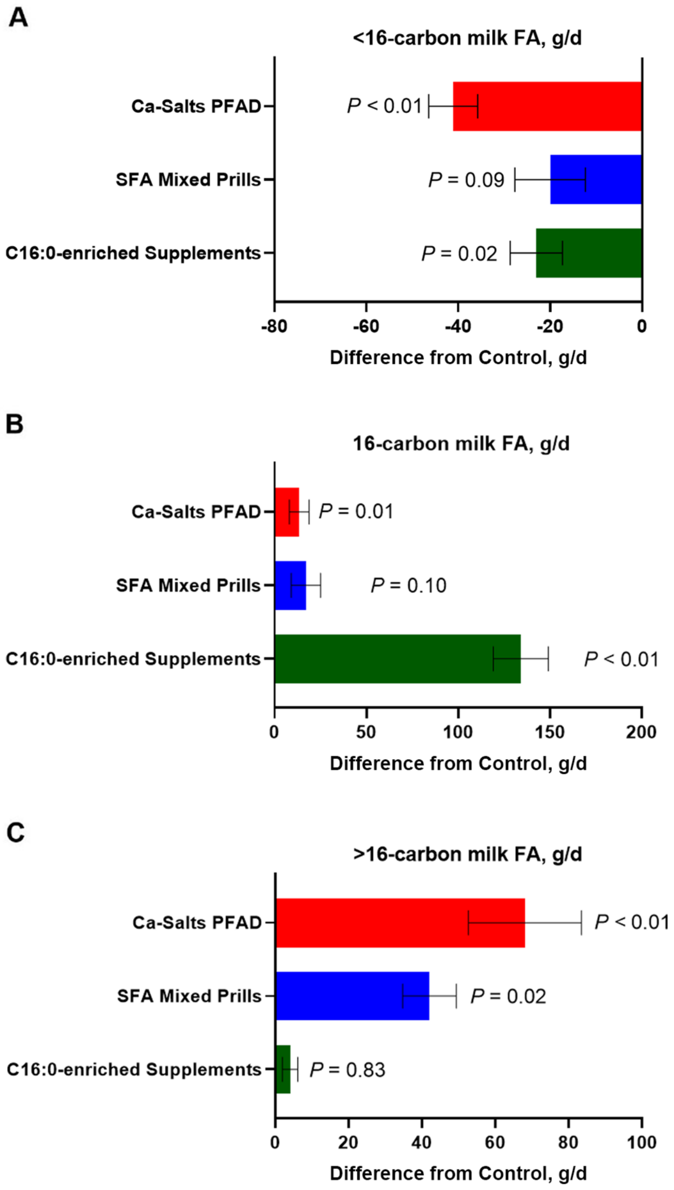

Figure 7. Effect of feeding common commercially available FA supplements on the yield of milk FA by source (*P*-value is the comparison of supplements against control; adapted from dos Santos Neto et al., 2021a,b). Fatty acids <16-carbon originate from mammary de novo synthesis (A), >16-carbon preformed FA originate from extraction from plasma (B), and 16-carbon FA originate from both sources (C). Error bars are the SE of the difference.

effect, but the mammary gland's utilization of preformed FA is associated with the FA profile.

Milk protein yield was mostly unaffected by the different FA supplements, with 16:0-enriched supplements having only a trend for increasing milk protein yield (~40 g/d; Figure 6D; dos Santos Neto et al., 2021a,b). In general, a reduction in milk protein content with no change in milk protein yield is fairly common when supplemental fat is fed (Rabiee et al., 2012). Interestingly, the yield of ECM increased only when 16:0 supplements were fed (~2 kg/d) and tended to increase with calcium salts of PFAD supplementation (~1.12 kg/d; Figure 6E; dos Santos Neto et al., 2021a,b). Feeding SFA-mixed prills did not result in increases in ECM, and indeed a lot of variation was observed across studies. In addition, we did not observe any effect of FA supplementation on BW change (Figure 6F; dos Santos Neto et al., 2021a,b). The effects of fat supplements on energy metabolism have been inconsistent across some studies, which may be related to different diets, stages of lactation, or physiological conditions. In general, we observed no effect of treatments on BW, BCS, or energy output for maintenance (dos Santos Neto et al., 2021a,b). However, we acknowledge that results can be influenced by the length of treatment periods within and across experimental designs and by the variability in how BW and BW change are measured and the frequency of measurement. These variables deserve further attention.

Impact of Individual FA on Production Responses

The effect of individual FA on the production responses of dairy cows has recently received renewed attention. In the 1960s, Steele and coworkers performed a series of studies using relatively pure sources of 16:0 and 18:0, and their findings suggested that 16:0 supplementation induces a higher milk fat response (concentration and yield) than 18:0 supplementation (Steele and Moore, 1968a,b; Steele, 1969). More recent work from Enjalbert et al. (1998) suggested that the uptake efficiency of the mammary gland is higher for 16:0 than for 18:0 and 18:1. Our group conducted a series of experiments to evaluate the effects of 16:0 and 18:0 on cow performance, with each experiment using very pure supplements (≥98% pure 16:0, 18:0, or both) and cows with a wide range of milk production. Piantoni et al. (2013) fed a 16:0-enriched supplement (2% diet DM; 99% 16:0) to cows with a wide range of milk production (31–65 kg/d) and reported increases in milk yield, milk fat yield, and feed efficiency among those receiving the supplement. However, production responses to 16:0 did not differ across the production level of cows. In contrast, Piantoni et al. (2015a) reported that 18:0 supplementation (2% diet DM; 98% 18:0) increased DMI and yields of milk and milk components only in high-producing cows (over 45 kg/d), indicating that there was an interaction between production levels and 18:0 supplementation. The reasons underlying this finding remain to be determined. Furthermore, in a direct comparison of 16:0 and 18:0 supplementation, 16:0 supplementation increased milk fat concentration, and the yield of milk fat and 3.5% FCM increased regardless of the level of milk production (Rico et al., 2014). Importantly, the above-mentioned studies used pure sources of FA as proof-of-concept studies. As described in the digestibility section of the current review, supplements that are highly enriched (>90% total FA) in a given FA usually have lower digestibility, which influences the magnitude of production responses. Commercially available supplements for 16:0 and 18:0 usually contain either a combination of 16:0 and 18:0 or a 16:0 concentration around 80% to 90% and 18:1 at 4% to 8%.

Limited research has examined the individual effects of 18:2 and 18:3, which represent n-6 and n-3 FA, respectively. Recently, we demonstrated that abomasal infusion of 43 g/d of 18:2 or 18:3 in dairy cows predominantly enriched plasma phospholipids and cholesterol esters with these FA (dos Santos Neto et al., 2024). Compared with plasma TG, these lipid fractions are less effective in delivering 18:2 or 18:3 to milk fat (Moore and Christie, 1984). Consequently, strategies to enhance PUFA absorption are likely to have a greater effect on cow health and well-being than focusing on enriching specific PUFA into milk fat for human consumption (dos Santos Neto et al., 2025b).

Impact of Blends/Combinations of 16- and 18-Carbon FA on Production Responses

Some research has focused on understanding the effects of different fat sources on milk production and energy partitioning. Typically, 16:0, 18:0, and 18:1 compose the majority of FA present in milk fat (Palmquist, 2006) and adipose tissue (Douglas et al., 2007) of dairy cows, and their proportions are affected by breed, lactation stage, seasonal variation, and dietary factors (Jensen, 2002). Therefore, determining an optimal dietary ratio for these FA may optimize their utilization. We conducted a series of studies to answer questions such as whether there is an ideal ratio that maximizes production responses and what conditions determine the appropriateness of combining these 3 FA.

Our first study evaluated the effects of different dietary FA combinations on the performance of mid-lactation cows (de Souza et al., 2018). We observed that all FA blends increased milk fat and ECM compared with the control, and the blend with the highest content of 16:0 increased milk fat yield and ECM compared with other FA blends, whereas feeding an FA blend with a combination of 16:0 and 18:1 increased BW gain. We also observed that diets supplemented with 16:0 improved fiber digestibility, whereas its combination with 18:1 improved total FA digestibility compared with the blend containing 18:0 and the control. Increasing 18:0 in an FA supplement reduced FA and NDF digestibility and did not increase digestible energy intake, which most likely explains its lower performance compared with the other FA treatments. These results suggested that 16:0 and 18:1 are able to alter nutrient partitioning between the mammary gland and adipose tissue, which may allow for different FA supplements to be used in different situations according to the metabolic priority of dairy cows and management needs.

Subsequently, we further explored feeding different blends of 16:0 and 18:1 to dairy cows. Feeding increasing levels of 18:1 in supplemental fat (from 10% to 30%) to replace 16:0 (from 80% to 60%) in mid-lactation cows did not affect DMI but increased FA digestibility, BW change, and BCS change (de Souza et al., 2019a). Interestingly, we observed an interaction between production level and dietary FA profile on production responses. Our results indicated that higher-producing dairy cows (averaging 60 kg/d) responded better to FA supplements containing more 18:1, whereas lower-producing cows (averaging 45 kg/d) responded better to FA supplements containing more 16:0 (de Souza et al., 2019a). Similarly, Western et al. (2020b) evaluated the effects of altering the dietary ratio 16:0 and 18:1 on milk production responses in dairy cows with a wide range in milk production (32–65 kg/d). Higher-producing cows (>55 kg/d) had increased DMI, milk yield, and ECM when fed with higher levels of 18:1, whereas lower-producing cows (<45 kg/d) showed a better response when a supplement higher in 16:0 was fed. In these trials, 18:1 was primarily provided using a calcium salt supplement; it can dissociate in the rumen (Sukhija and Palmquist, 1990) and undergo BH to 18:0. Because these blends increase the intake of 18:1, we can speculate that rumen outflow of other 18-carbon FA increased, so it is unclear whether these results were associated with an overall effect of 18-carbon FA or a specific FA. Thus, we investigated whether the effects we have observed were due to an increase in 18-carbon flow in general or specifically to 18:1. We tested whether low-producing (~42 kg/d) or high-producing (~56 kg/d) cows would respond better to supplements containing 60% 16:0 and 30% of either 18:0 or 18:1 (Burch et al., 2021). In general, 18:1 increased NDF and FA digestibility and did not influence feed intake compared with 18:0. It also increased milk yield, fat yield, and ECM compared with 18:0 in high-producing cows, whereas low-producing cows benefited more from 18:0 in terms of milk component yields. Bales et al. (2024b) evaluated the effects of feeding either a 60% 16:0 + 30% 18:1 blend or an 80% 16:0 + 10% 18:1 blend, considering the effect of the basal fat level. A high-fat basal diet increased milk production compared with a low-fat basal diet, and adding FA supplements to a low-fat diet also improved milk yield. Both FA blends enhanced production compared with no supplementation, regardless of the basal diet. Body weight was unaffected by treatments, but feed intake decreased with higher levels of 18:1 in the supplement. Potential benefits of increasing dietary 18:1 content on production responses of dairy cows have also been highlighted in recent research using high-oleic soybeans (Bales and Lock, 2024a,b). In summary, these studies emphasized the importance of considering production levels when designing FA supplementation strategies for dairy cows to optimize milk production and nutrient utilization. More research should be done considering basal fat levels and composition to evaluate how the supplemental FA profile would influence production responses.

The response to varying the levels of 16:0 and 18:0 in the supplemental fat has also been explored. In Figure 8, we summarize the influence of changing the ratio of 16:0 to 18:0 in FA supplements on intake and yields of milk, milk fat, and ECM. In general, feed intake was not influenced by changing the ratio of 16:0 to 18:0 across studies, although Shepardson and Harvatine (2021) reported reduced feed intake when cows were fed a supplement with 91% 16:0 compared with the control (Figure 8A). Overall, milk yield was increased in 3 of 5 studies when supplements with different ratios of 16:0 and 18:0 were fed compared with controls (Figure 8B). Furthermore, both milk fat yield (Figure 8C) and ECM (Figure 8D) increased with supplemental fat containing 16:0 and 18:0. Most importantly, across several studies, the yield of milk fat and ECM was greater as the amount of 16:0 in the supplement increased. This is supported by Chamberlain and DePeters (2017), who also reported a linear increase in milk fat yield and content when supplemental fat content of 16:0 increased and 18:0 decreased, while milk yield and intake remained unchanged. Glycerol-3-phosphate acyltransferase, the enzyme that esterifies FA at the *sn*-1 position to start milk TG synthesis, has shown a higher preference (8- to 10-fold) for 16:0 as a substrate compared with 18:0 or 18:1 (Kinsella and Gross, 1973). Tzompa-Sosa et al. (2014) suggested that an increase in the availability of 16:0 for lipid synthesis in mammary epithelial cells may increase the activity of glycerol-3-phosphate acyltransferase in the mammary gland, increasing the proportion of 16:0 acylated at *sn*-1 at the expense of *sn*-2. Other FA counterbalance the decrease in the amount of this FA at *sn*-2. In addition, the concept that the mammary gland "requires" differ-

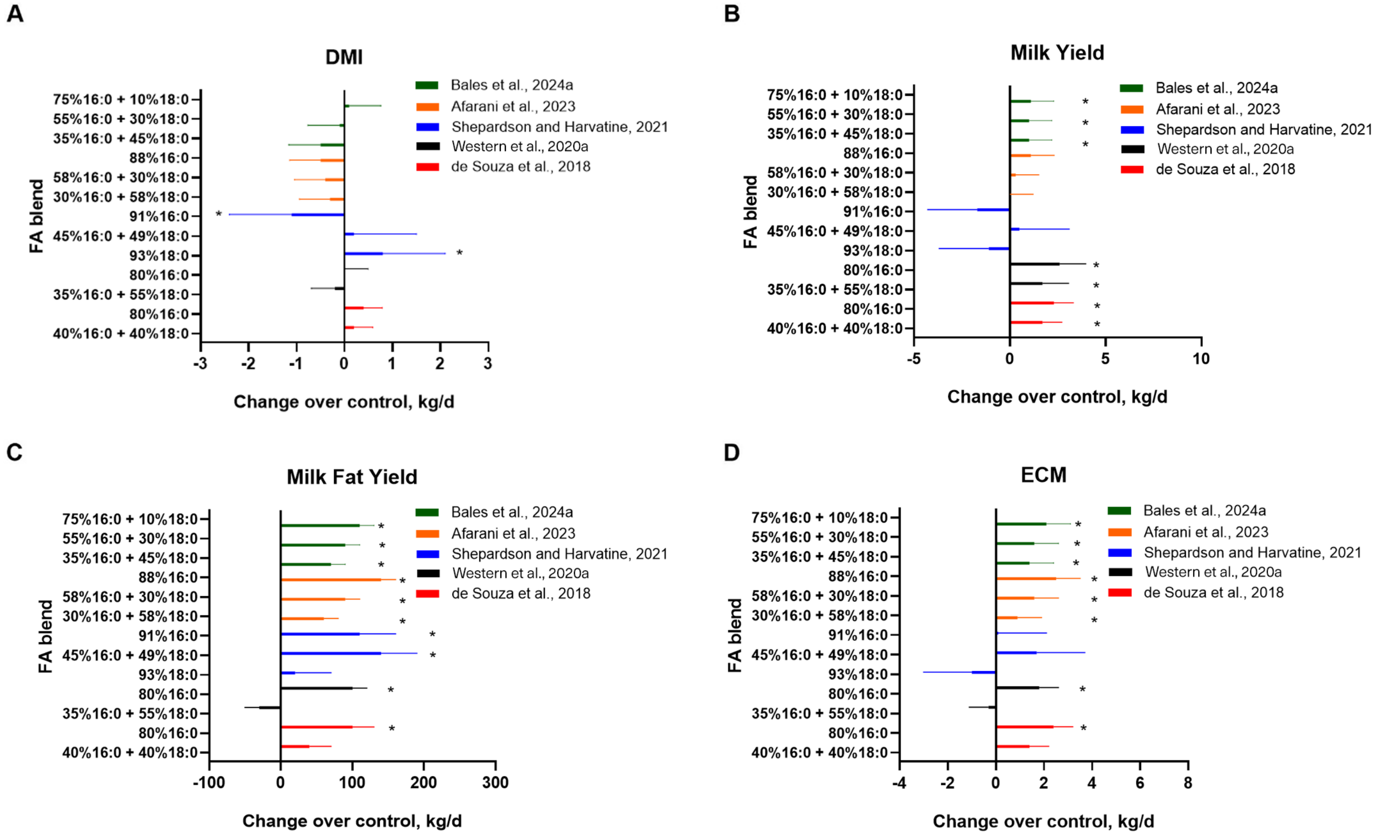

Figure 8. Summary of studies in which dairy cows were fed different ratios of 16:0 to 18:0 in the supplemental fat and the effect on intake and yields of milk, fat, and ECM. Differences between treatment and control are reported for each variable. *The comparison between treatment and control was different within the study ($P < 0.05$). Afarani et al. (2023) did not report ECM; for this reference, panel D shows 3.5% FCM responses for treatment diets, with control the treatment without supplemental palmitic acid. Error bars are SEM. References: de Souza et al. (2018); Western et al. (2020a); Shepardson and Harvatine (2021); Afarani et al. (2023); Bales et al. (2024a).

ent sources of FA to increase milk fat output has been previously proposed (Glasser et al., 2008a). Benoit et al. (2024) observed increased milk fat output when multiple sources of FA were provided in the diet, suggesting that a balance of short- and long-chain FA is needed for increased TG synthesis, thus representing a more interdependent relationship between sources of milk FA. Overall, this hypothesis could explain our finding that using 16:0-enriched supplement increased milk fat yield to a greater extent than other supplements by increasing TG synthesis and changing the FA interposition distribution in the TG.

Additionally, Western et al. (2020a) evaluated the production responses of dairy cows when fed commercially available supplements containing a mix of 16:0 and 18:0 (~33% 16:0 and 55% 18:0) or primarily 16:0 (~84% 16:0). Compared with the mixed supplement, the 16:0 supplement increased NDF, FA, and digestible energy intake while having no effect on DMI or BW gain. Similarly, Porter et al. (2024) reported increases in milk fat yield (+180 g/d), ECM (+2.9 kg/d), and fat content (+0.26 percentage units) when a 16:0 supplement (~82% 16:0) was fed compared with 16:0 + 18:0 (~28% 16:0 and 55% 18:0). Furthermore, a recent study reported that increasing 16:0 and decreasing 18:0 in FA blends linearly increased DMI, yields of 3.5% FCM, ECM, and milk fat and milk fat content, indicating that mid-lactation cows averaging ~40 to 50 kg/d of milk yield responded best to an FA ratio of 80% 16:0 + 10% 18:0 (Bales et al., 2024a). Likewise, Afarani et al. (2023) reported linear increases in milk fat content and yield and 3.5% FCM when increasing levels of 16:0 replaced 18:0 in the supplemental fat, whereas increasing the 18:0 level in the supplemental fat reduced FA digestibility in cows under heat-stress conditions. Shepardson and Harvatine (2021) reported that feeding a highly enriched 18:0 supplement decreased FA digestibility and did not influence production variables compared with a non-FA-supplemented control, whereas the yield of milk fat was increased with 16:0 and 16:0 + 18:0 supplementation.

Although Loften et al. (2014) proposed that combining 16:0 and 18:0 is essential to optimize their metabolic utilization and enhance milk production in lactating dairy cows, our findings challenge this assumption. In fact, the majority of studies that directly compared varying levels of 16:0 and 18:0 in supplemental fat provided no evidence to support the purported synergistic effect proposed by Loften et al. (2014). In general, adding 18:0 in the supplemental fat reduced FA digestibility compared with the other FA treatments. As indicated in our summary of published studies, increasing 18:0 over both 16:0 and 18:1 has not improved milk yield, yield of milk components, or body reserves. Because 18:0 is the end point of BH, the amount of 18:0 in the intestine from the basal diet is likely a factor limiting the response to 18:0 in FA supplements.

Supplemental 16- and 18-Carbon FA on Production Responses During Early Lactation

The high metabolic demands of lactation, combined with reduced DMI during the immediate postpartum period, lead to a negative energy balance in dairy cows. Strategies to increase energy intake include raising dietary starch levels or supplementing fat to increase the energy density of the diet (McCarthy et al., 2015; Piantoni et al., 2015b). However, high-starch diets that increase ruminal propionate production can suppress appetite, reduce DMI, and elevate the risk of ruminal acidosis and displaced abomasum (Allen, 2023). Some authors suggest exercising caution when using supplemental fats to increase the caloric density of diets in early-lactation dairy cows because a high lipid load may affect the endocrine system and feed intake and increase the risk for metabolic disorders (Kuhla et al., 2016). Based on studies conducted in the early 1990s, Grummer (1992) suggested that supplemental tallow had little benefit on cow performance when fed in the first 5 to 7 wk of lactation, which is likely associated with the high levels of supplemental fat included in the diet (5%–6% DM) and reduced DMI. However, as we have discussed previously, understanding of the effect of individual FA on animal metabolism and their direct influence on animal performance is increasing.

Although extensive research has been conducted with 16:0-enriched supplements, most of the data are from cows in mid lactation. van Knegsel et al. (2007) tested lipogenic (10.4% starch and 5.0% total fat) and glucogenic (26.6% starch and 3.1% total fat) diets, varying primary corn and a 16:0 supplement to cows from 3 wk precalving to 9 wk postcalving. Milk yield, feed intake, and BW loss were not affected by treatments, but cows fed the lipogenic diet had higher milk fat and milk fat yield, which was more pronounced in the multiparous cows. We conducted a trial to evaluate the effects of timing of 16:0 supplementation on the production responses of early-lactation dairy cows (de Souza and Lock, 2019a,b). We observed that when 16:0 was fed in the fresh period (1–24 DIM), it increased the yield of 3.5% FCM, ECM, milk fat yield, and protein yield, but it tended to increase BW loss. When 16:0 was fed during the peak period (25–67 DIM), it increased the yields of milk, 3.5% FCM, ECM, milk fat, and milk protein, but it tended to reduce BW compared with the control. Compared with other stages of lactation, adipose tissue lipolytic responses in dairy cows are enhanced immediately postpartum owing to hormonal changes associated with parturition and the onset of lactation (Contreras et al., 2018). Thus, it is possible that feeding 16:0 stimulates lipolysis in the immediate postpartum. Although lipolysis provides energy substrates required during the early postpartum period, when it becomes intense and prolonged, it can predispose dairy cows to inflammatory and metabolic diseases owing to a reduced capacity of the adipose tissue to buffer energy and may contribute to chronic increased plasma nonesterified FA (**NEFA**) concentrations (Bradford et al., 2015). Importantly, even though 16:0 increased BW loss and plasma NEFA concentration, NEFA levels were below the threshold that is considered critical for increased incidence of metabolic disorders (Ospina et al., 2013). We also measured digestible energy intake and calculated energy balance, and the cows supplemented with 16:0 returned to positive energy balance around the same time as the control group (de Souza et al., 2019b). Subsequently, Parales-Girón et al. (2025) evaluated if supplementation with 16:0 interacts with chromium propionate during the immediate postpartum and carryover period. Although no interaction was observed for production variables, supplementation with 16:0 during the immediate postpartum period (1–24 DIM) increased the yields of milk fat, ECM, and 3.5% FCM, and it did not affect feed intake, BW, or BW loss. The differences in the influence of 16:0 on BW loss may be associated with the lower magnitude of responses on ECM and precalving BCS because the average precalving BCS was 3.75 (de Souza and Lock, 2019a) and 3.50 (Parales-Girón et al., 2025). A higher BCS at calving has been associated with greater BW and BCS losses after calving (Roche et al., 2009).

Several studies have evaluated the influence of SFA containing 16:0 and 18:0 on the performance of early-lactation cows. Feeding a 16:0 + 18:0 supplement (~28% 16:0 and 51% 18:0) from precalving to 100 DIM reduced feed intake prepartum but not postpartum and increased milk yield but did not influence milk fat yield and 3.5% FCM compared with a nonfat control diet (Moallem et al., 2007). Similarly, Beam and Butler (1998) fed an SFA supplement (~40% 16:0 and 40% 18:0) and observed that FA supplementation decreased DMI but did not affect yields of milk and ECM in the first 4 wk after calving. Piantoni et al. (2015b) fed a similar SFA supplement (~37% 16:0 and 47% 18:0) and observed that FA supplementation during the immediate postpartum period (1–29 DIM) favored energy partitioning to body reserves rather than milk yield, especially in the lower forage diet. The high-forage diet with supplemental FA increased DMI and tended to decrease BCS loss compared with the same diet without FA supplementation. In addition, regardless of forage level, feeding supplemental FA increased DMI and decreased BCS loss but tended to decrease milk yield. When cows were fed a common diet during the carryover period, the low-forage diet with SFA supplementation fed during the immediate postpartum continued to decrease milk yield and maintained higher BCS compared with the other treatments. Weiss and Pinos-Rodríguez (2009) fed an SFA supplement (~40% 16:0 and 40% 18:0) to early-lactation cows (21–126 DIM) and observed that when a high-forage diet was supplemented with SFA, the increased NEL intake was partitioned toward body energy reserves as measured by higher BCS with no change in milk yield. However, when a low-forage diet was supplemented with SFA, milk yield increased (2.6 kg/d) with no change in BCS. Altogether, the results of feeding 16:0 + 18:0 supplements in early lactation are inconsistent. In the studies in which feeding 16:0 and 18:0 supplements increased energy partitioning to body reserves, this outcome was achieved owing to decreased milk yield and milk energy output.

Based on our work in postpeak cows, we tested whether varying the levels of 16:0 (from 60% to 80%) and 18:1 (from 10% to 30%) in the FA supplement would affect responses during the immediate postpartum and carryover periods (de Souza et al., 2021a,b). Our results indicated that feeding FA supplements containing 16:0 and 18:1 during the immediate postpartum period increased milk yield, ECM, nutrient digestibility, energy intake, and milk energy output compared with a non-FA-supplemented control diet. Increasing 18:1 in the FA supplement increased DMI and energy intake, reduced BW and BCS losses, and improved energy balance during the immediate postpartum period. Interestingly, increasing 18:1 in the FA supplement increased plasma insulin and decreased NEFA in this period (de Souza et al., 2021a). Piantoni et al. (2015c) reported that greater reductions in plasma NEFA concentrations after feeding were positively related to greater intakes in early postpartum cows, suggesting that decreased β-oxidation in the liver might allow for higher DMI. Plasma insulin concentration increased during and after meals, decreasing lipolysis and plasma NEFA concentrations (Allen, 2020). Therefore, the increase in DMI observed in our study as we increased 18:1 in the FA treatments may have been related to a decreased flux of fuels to the liver that could have potentially decreased satiety and increased DMI (Allen, 2023). The effect of 18:1 on feed intake most likely explains why energy output increased without increasing the negative energy balance. Additionally, the FA-supplemented diets fed during the immediate postpartum period had a positive carryover effect during early lactation when cows were fed a common diet. Similarly, previous studies with grazing cows reported that feeding calcium salts of palm FA supplement from 3 to 16 wk of lactation increased cumulative milk yield throughout lactation by 8% to 12% (Batistel et al., 2017; de Souza et al., 2017a). In contrast to our results, Hu et al. (2024) reported that increasing the 18:1 level in the supplemental fat did not influence feed intake or BW but increased milk yield when added to the diet of cows starting at 63 DIM, which could be associated with the timing that treatments were fed. These results reinforce that dairy cows may have distinct responses to similar nutrition strategies based on their stage of lactation or when the supplementation starts. The lactation stage is probably the most important factor that may affect energy partitioning. Marked changes in lipid metabolism occur during pregnancy and lactation in most mammals. Endocrine profiles change (Bauman and Currie, 1980), and lipolysis and lipogenesis are regulated to increase lipid reserves during pregnancy and the utilization of these reserves following parturition and the initiation of lactation (Roche et al., 2009). The positive effect of FA supplementation in early lactation and potential carryover effects are important factors to consider when evaluating the economics of feeding these supplements and when introducing them into the diets.

FA Effects on Energy Partitioning

Energy partitioning is the result of complex mechanisms that involve a variety of hormones and tissues, and it is affected by absorbed nutrients and the physiological state of the cow (Piantoni and VandeHaar, 2023). Fat is typically the most variable component in milk, and it is influenced by several factors (Jensen, 2002). Fat is the milk component with the highest energy content, and its production constitutes the major "energetic investment" in milk synthesis, accounting for over one-half of the milk's energy output (NASEM, 2021). Based on NASEM (2021) equations, every 0.25 percentage point change in milk fat concentration results in an ~3% to 4% increase or reduction in milk energy output (assuming no change in milk yield). If milk energy output is reduced, the spared energy can be used for other purposes and storage. For cows with a positive energy balance, a reduction in milk fat synthesis may result in a more rapid gain in BW and BCS, thereby reducing nutrient use efficiency for milk synthesis. However, an increase in milk energy output not followed by energy intake may result in body reserve mobilization. Ideally, adipose tissue reserves that are mobilized in early lactation when cows are in a lipolytic state are replenished as lactation proceeds. However, the inability to recover body reserves and the consequent inadequate body condition at parturition can limit milk yield and increase the risk of reproductive failure (Roche et al., 2009). Conversely, cows that gain excessive body condition in mid- and late lactation are at high risk for culling or an extended calving interval during the next lactation owing to an increased risk of metabolic disorders and reproductive failure (Roche et al., 2009). Therefore, an important goal for diet formulation and nutritional management of lactating cows is to achieve optimum milk energy output and improve body condition. Because FA supplements are often used to increase energy intake, yields of milk and milk components, and body reserves in dairy cows, understanding how different FA may affect energy partitioning is critical.

Individual FA can have an effect on the energy partitioning of dairy cows, and the impact will depend on the individual FA and the characteristics of the diet (Bauman et al., 2011). A classic example is dietary-induced milk fat depression (**MFD**), in which decreases in milk fat concentration and yield may redirect nutrients to the adipose tissue (Bauman et al., 2011). These changes in energy partitioning during MFD have been associated with a shift in ruminal BH pathways and increased production of several *trans* FA intermediates. Harvatine et al. (2009) evaluated adipose tissue gene expression in cows abomasally infused with *trans*-10, *cis*-12 18:2 and observed an upregulation in key lipogenic enzymes in adipose tissue. These findings suggest that the increase in BW usually observed in cows with MFD is due to an increase in adipose tissue lipogenesis either from a direct effect of *trans*-10, *cis*-12 18:2 on adipose tissue or from an indirect effect of increased fuel availability from decreased milk fat synthesis. Additionally, FA could also affect energy partitioning through an increase in plasma insulin concentration or modulation of insulin resistance, but results have been inconsistent. Previous studies reported that UFA increased insulin secretion in a perfused pancreas in rats (Stein et al., 1997), but increasing amounts of dietary UFA in dairy cows increased (Liu et al., 2020) or decreased plasma insulin (Choi and Palmquist, 1996). Chilliard (1993) suggested that the inconsistent insulin responses to fat supplementation might be related to the effect on DMI, which dietary ingredient is removed when FA are supplemented, the glucose-sparing effect that FA might have if they decrease milk fat synthesis, or a combination of these variables.

The FA profile is a critical factor influencing energy partitioning. For instance, Liu et al. (2020) reported that feeding a UFA supplement (soybean oil) increased plasma insulin and energy partitioning toward body reserves, whereas an SFA supplement (16:0 supplement) increased energy partitioning toward milk. We performed a random regression analysis of individual cow data (n = 978) from 16 studies (descriptive information in Supplemental Table S2, see Notes) that fed different FA supplements to mid-lactation dairy cows to evaluate the relationship between FA intake and energy partitioning. Energy intake and energy output were calculated from the individual cow data using the equations proposed by NASEM (2021). We observed that energy partitioned toward milk (as a percentage of energy intake) was associated with 16:0 intake (g/d), with a slope of 0.0029 and intercept of

60.4% (Figure 9A), but it was not influenced by intakes of 18:0 (Figure 9C) and 18:1 (Figure 9E). Feeding 16:0 has been associated with increased milk fat yield; thus, an increase in milk energy output in response to 16:0 is frequently observed (dos Santos Neto et al., 2021b). One mechanism proposed to explain these results suggests that 16:0 supplementation induces insulin resistance mediated through ceramides, reducing the utilization of glucose by adipose and muscle tissues (Mathews et al., 2016). Circulating ceramides are positively associated with the availability of NEFA in plasma, with very long-chain ceramides being the most responsive (Rico et al., 2018b). Feeding 16:0 increased circulating ceramides in cows (Rico et al., 2016), and because ceramides can

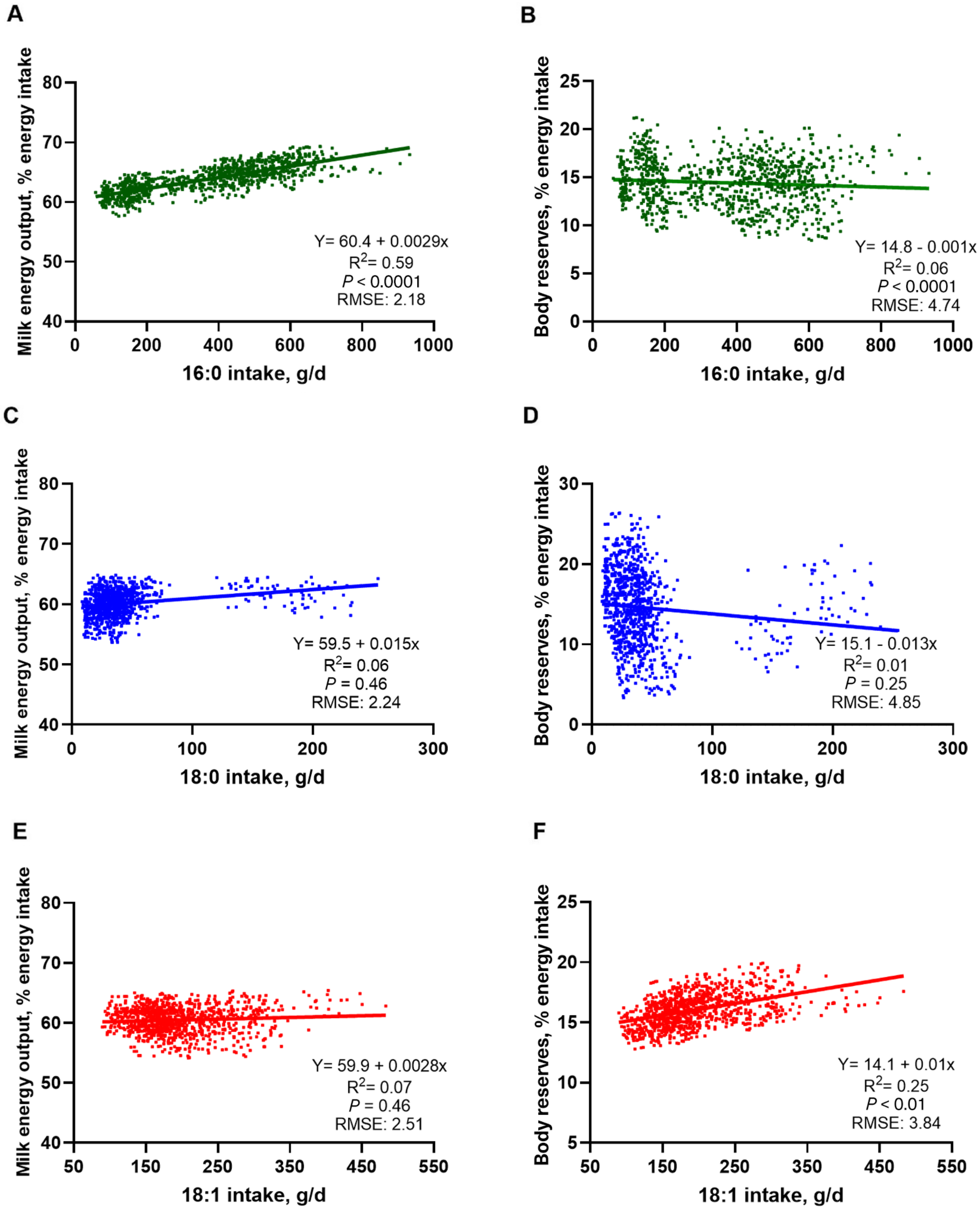

Figure 9. The relationship between FA intake and energy partitioning of dairy cows. Meta-regression of 16 studies with 978 individual cow observations on the effect of 16:0 intake (A and B), (C and D) 18:0 intake, and 18:1 intake (E and F) on energy partitioning to milk and body reserves. Energy intake and energy output were calculated based on NASEM (2021). Details on studies and meta-regression are presented in Supplemental Table S2 (see Notes).

decrease insulin sensitivity in adipocytes (Rico et al., 2018a), 16:0 might be able to shift energy partitioning toward milk production and away from body reserves (McFadden and Rico, 2019). Because the availability of lipolysis-derived 16:0 declines with the progression of lactation, feeding 16:0 supplements may increase the availability of 16:0 in circulation to tissues, thereby sustaining nutrient partitioning toward the mammary gland. Recently, Chivri et al. (2025) demonstrated that chromium propionate supplementation limits excessive lipolysis and enhances the antilipolytic effect of insulin and that including chromium along with supplemental 16:0 immediately postpartum may improve energy availability while minimizing adipose tissue loss.

Additionally, in our meta-regression, we observed that energy partitioned toward body reserves (as a percentage of energy intake) was associated with 18:1 intake (g/d), with a slope of 0.01 and intercept of 14.1% (Figure 9F), but it was poorly predicted by 16:0 intake (Figure 9B) and not affected by 18:0 intake (Figure 9D**)**. Similarly, previous studies indicated that feeding 18:1 not only increased BW gain in postpeak cows but also increased plasma insulin compared with feeding nonfat-supplemented diets and other FA supplements (de Souza et al., 2018, 2019b). In previous studies using rats, 18:1 stimulated insulin secretion from pancreatic β-cells (Itoh et al., 2003; Fujiwara et al., 2005). Emerging evidence highlights the role of 18:1 in modulating adipose tissue metabolism during early lactation. Abomasal infusion of 18:1 during the immediate postpartum period reduced adipose tissue lipolysis, improved systemic and adipose tissue insulin sensitivity, and improved markers of mitochondrial function, supporting a shift to lipogenesis in adipose tissue of dairy cows (Abou-Rjeileh et al., 2023). Elevated insulin concentrations would reduce plasma NEFA through inhibiting lipolysis or increasing lipogenesis (Vernon, 2005). In addition, higher concentrations of plasma TG could result from greater absorption of dietary FA increasing the supply of TG-rich lipoproteins available in circulation. As a result, increases in insulin secretion could partition circulating TG into other tissues and reduce lipolysis from adipose tissues. Furthermore, Yanting et al. (2018) reported that 18:1 increased adipocyte number and size through enhancing adipogenic commitment and lipogenesis compared with SFA (14:0, 16:0, and 18:0). In addition, the latter authors reported that in mature adipocytes treated with FA, the lipid content in the cells was affected by FA profile and ranked as 18:1 > 18:2 > 14:0 > 18:0 > 16:0. Mechanistically, 18:1 has recently been shown to activate PPARα signaling in bovine adipocytes, inducing downstream lipogenic gene expression and promoting lipid accumulation (Abou-Rjeileh et al., 2025b). This may contribute to improved energy balance and metabolic stability during early lactation. For an in-depth examination of these molecular mechanisms and their implications for improving health and lactation performance, readers are referred to Abou-Rjeileh et al. (2025a).

Fatty acid supplementation might also affect energy partitioning based on other physiological factors. We observed that 16:0 supplementation interacted with parity in mid-lactation cows by favoring energy partitioning to body reserves in primiparous cows and energy partitioning to milk in multiparous cows (de Souza and Lock, 2018a). However, in cows at mid- to late lactation, feeding a 16:0-enriched supplement has been shown to increase milk fat yield and ECM without increasing weight gain, regardless of starch level or parity (dos Santos Neto et al., 2025a). Interactions between nutrition and breed differences are poorly explored, but supplementation with 16:0 has increased milk fat yield in both Holstein and Jersey cows, but the transfer efficiency (additional fat yield/additional supplemental fat) was greater for Jersey than Holstein cows (Sears et al., 2020). The possible interactions between individual FA supplementation with parity, breed, lactation stage, and other nutrient components need to be further explored.

Importantly, these results support that a strong relationship exists between milk fat synthesis and energy partitioning and that different FA may be, at least partly, the mediators of changes in metabolism in adipose tissues and the mammary gland of lactating dairy cows. Altering the amount of 16:0 and 18:1 in the diet may be an effective strategy to manipulate energy partitioning. Developing strategies to increase insulin sensitivity of extrahepatic tissues and decrease mobilization of fat more quickly during meals in the fresh period might be beneficial; a decrease in hepatic oxidation of NEFA would decrease satiety signals and allow for longer meals, potentially increasing intake over a day and improving performance (Piantoni and VandeHaar, 2023). However, developing strategies to increase insulin resistance later in lactation could minimize excessive body reserve gain, while maximizing milk energy output.

CONCLUSIONS AND FUTURE DIRECTIONS

Our review integrates recent advances in understanding the distinct roles of individual FA in dairy cow nutrition, digestion, and metabolism, offering insights for optimizing dietary strategies and enhancing animal performance. It emphasizes the complexity of FA digestion and metabolism, underscoring the need to tailor FA supplementation strategies to the specific production stage and physiological status of dairy cows. Similar to the dairy industry embracing the importance of specific AA and their unique functions, it is now beginning to do the same with FA. The industry is recognizing that FA are far more than just energy sources and that individual FA can play crucial roles in ongoing efforts to enhance milk production, animal health, and efficiency and sustainability of the dairy industry.

We highlight the recent findings that challenge the long-standing belief that FA supplementation reduces fiber digestibility in dairy cows, showing instead that the effect depends on the specific FA. Although medium-chain FA and vegetable oils decrease NDF digestibility, feeding 16:0-enriched supplements increases NDF digestibility on average by 4.5 percentage points. These improvements are not linked to reduced intake but may result from shifts in microbial populations and bacterial metabolism. Because fiber is an important component of dairy cow diets and directly influences animal performance, understanding the mechanisms by which FA can positively or negatively influence fiber digestion will enhance current knowledge and improve feeding recommendations.

The digestibility of FA in dairy cows generally decreases as FA intake and intestinal flow increase, particularly for 18:0, which shows a strong negative relationship with digestibility. Fatty acid digestibility is influenced by the FA profile of the diet/supplement and the physical form and purity of the supplement. Supplements high in SFA, especially pure prilled forms, often result in lower digestibility owing to poor emulsification and micelle formation. Emulsifiers, such as polysorbates and 18:1, improve FA absorption when infused into the abomasum, suggesting that limited emulsification is a key barrier to FA digestion. Furthermore, the source of 18-carbon FA influences digestibility—oilseeds have higher digestibility than prilled FA supplements. Because 18:0 is the primary FA available for absorption in dairy cows, enhancing its digestibility offers the most promising avenue for improving FA absorption and overall performance.

The choice of what FA to use or supplement in dairy cow diets should be strategically tailored based on production level, lactation stage, and goals for feeding it. Supplementation with 16:0 consistently improves milk fat yield and ECM, especially in cows producing up to 45 kg/d of milk, whereas supplementation during early lactation in the context of increasing production may also lead to additional BW loss. Supplementation with 18:1 enhances FA digestibility, feed intake during early lactation, insulin sensitivity, and milk yield, showing greater benefit in high-producing cows (over 45 kg/d) when used in combination with 16:0. In general, 16:0 intake is positively associated with energy partitioning toward milk, whereas 18:1 intake is associated with energy partitioning toward body reserves. In contrast, blends containing 16:0 + 18:0 tend to reduce the digestibility of FA, resulting in limited or inconsistent production benefits compared with other supplements with higher digestibility. When feeding 18:1, it is likely that the absorption of 18:0 also increases due to rumen BH. However, the different production responses observed when feeding 18:1 instead of 18:0 indicate that the primary factor driving these outcomes is that the 18:1 that escapes BH results in differences in FA digestibility and postabsorptive metabolism of these FA at the mammary gland or other tissues.

Overall, increasing 16:0 and 18:1 while limiting 18:0 in FA supplements appears to be the most effective strategy to improve milk production, nutrient utilization, and metabolic balance. Ultimately, manipulating the balance of 16:0 and 18:1 offers promising tools to optimize milk energy output versus body condition, supporting performance and health across lactation stages. Future research should continue to refine dietary formulations to optimize energy partitioning, which could improve productivity and metabolic health across lactation stages. Research should also focus on how to balance FA to maximize milk fat output and health and how to improve models for FA digestion, metabolism, and partitioning for practical applications.

NOTES

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Nonstandard abbreviations used: 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid (*cis*-9 18:1); 18:2 = linoleic acid (*cis*-9,*cis*-12 18:2); 18:3 = linolenic acid (*cis*-9,*cis*-12,*cis*-15 18:2); BH = biohydrogenation; FA = fatty acid; MFD = milk fat depression; NEFA = nonesterified FA; PFAD = palm FA distillate; RMSE = root mean square error; Sm. = small; TG = triglyceride; WCS = whole cottonseed.

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