ENERGY SOURCES FOR YOUNG CALVES

Liliana Amado Barrantes

Propositions

1. Increasing the fat content in the starter reduces the post-weaning growth check in calves.

   (this thesis)

2. In young calves, considering body fat accretion as detrimental is not justified.

   (this thesis)

3. Latin American High Power Distance Culture (Hofstede, 1984) impedes both professional and personal growth.

4. Policies mitigating methane emissions from animal production are addressing the wrong issue (FAO, 2021).

5. There is a positive correlation between professional competence and being a good person.

6. Even individuals who believe they are free of prejudice about people's backgrounds unconsciously hold them.

Propositions belonging to the thesis, entitled

Energy sources for young calves

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Wageningen, 15 December 2023

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This research was conducted under the auspices of
the Graduate School of Wageningen Institute of Animal Science (WIAS)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 15 December 2023
at 11 a.m. in the Omnia Auditorium.

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Energy sources for young calves,
178 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2023).
With references, with summary in English.

ISBN: 978-94-6447-981-2
DOI: https://doi.org/10.18174/642174

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

BACKGROUND: NATURAL AND RESTRICTIVE FEEDING PRACTICES

Calves are the only farm animals subject to dietary restrictions during their early life stages. This practice diverges notably from how calves would naturally be fed by their mothers. This biological reference recognizes the nutritional, physiological and behavioral needs of calves, set to maximize their changes to survive and reproduce. However, this biological reference substantially deviates from contemporary production systems, which are marked by a restricted liquid feed supply and early weaning. While the biological reference comprehends extensive calf-mother interactions and a gradual weaning process, it does not match with present-day practices. The recognition of this discordance has encouraged reevaluating the nutritional approach, realizing that whole milk (**WM**) possesses superior nutritional composition that could enhance calf health and metabolic function when these characteristics are brought into milk replacer (**MR**) formulations.

The aims of early calf management in commercial settings should be centered on molding the long-term performance of future cows, striving to breed highly productive, resilient, and long-lived animals. Balancing these objectives entails recognizing the requirements of calves for health, growth, and overall well-being, while simultaneously accommodating the production-oriented objectives. In essence, calves offer a unique perspective on the intersection of biological needs and production goals, raising questions about how best to align these priorities for the optimal development of future cattle.

In nature, calves have an instinct to nurse shortly after birth, as the colostrum-rich milk provided by the mother is essential for acquiring passive immunity and vital nutrients (Weaver et al., 2000). Colostrum acts as the first line of defense, providing antibodies and immune factors that protect the calf from diseases during its early stages of life (Wells et al., 1996). During the first 3 weeks of life, young calves rely heavily on milk consumption, nursing 8 to 12 times daily (Albright and Arave, 1997), and consuming up to 12 liters of their dams' milk (de Veissier et al., 2013; Johnsen et al., 2015), while also adapting digestive enzymes for milk digestion (Baldwin et al., 2004). As the calf grows, the frequency of meals decreases, and the calf transitions to consuming large quantities of milk during each feeding as they undergo a transformative phase in which they shift from milk to other feed sources. Nevertheless, calves are naturally designed to evolve beyond a reliance on milk alone. Therefore, around 3 weeks of age, calves begin grazing and ruminating (Reinhardt and Reinhardt, 1981), this marks a significant step toward their independence from milk as they begin to explore and consume solid feeds, this transition plays a critical role in rumen development. While calves continue nursing until approximately 10 months of age

(Albright and Arave, 1997), the ultimate objective is gradually reducing their reliance on milk. This reduction aligns with their increasing ability to utilize energy from volatile fatty acids (**VFA**) and nutrients from the microbial biomass from their developing rumen. This process corresponds with the natural shape of the lactation curve with the declining milk production of the mother (Day et al., 1987).

Since humans began to utilize milk for their own consumption, calves have been reared in a restrictive feeding regime, which differs significantly from the natural biological reference. Over time, modern dairy and beef production systems have implemented this restrictive system, in which the amount of WM or MR fed to calves is severely limited. It is usually provided in 2 or 3 feedings, amounting to only 4 to 6 liters, approximately 10% of the calf's body weight (**BW**; Appleby et al., 2001; Tedeschi and Fox, 2009). This is only about half of what a calf naturally consumes when raised with its dam. The main reasons behind this approach are economic considerations and the desire to encourage early solid feed intake and rumen development, often leading to earlier weaning, usually before the calf reaches 2 months of age (Khan et al., 2011; Kertz et al., 2017). By limiting the amount of milk or MR, farmers aim to stimulate calves to consume more starter feed, accelerating the transition to a solid feed based diet. Additionally, the preference for restricting liquid feeds, such as MR or WM, is driven by economic factors. However, this economic incentive is not always clear, because while liquid feeds may have higher costs, when considering the cost per unit of BW gain, they can be more cost-effective than solid feed alternatives (NRC, 2021).

ENERGY INTAKE DURING THE PREWEANING PHASE

The energy intake of calves in natural or *ad libitum* systems is determined by factors as dietary composition, forage quality and availability, and calf age. Figure 1.1 illustrates the total metabolizable energy (**ME**) intake across two distinct feeding systems: *ad libitum* and restricted. The *ad libitum* system reflects both a natural scenario, while restricted represents the common rearing environment. In Webb et al. (2014), group-housed calves were granted unrestricted access to a diverse diet comprising five components (i.e., MR, concentrate, silage, hay, and straw). At 3 months of age, calves shift their diet in such a way that the proportion of MR in their diet was reduced to 52% of total dry matter intake (**DMI**), indicating the transition from pre-ruminant to ruminant digestion. As calves reach 6 months of age, their consumption of concentrate increases to account for 47% of the total DMI, leading to a corresponding reduction of MR intake to 23% of the total DMI. As clearly illustrated in Figure 1.1, calves following a restricted feeding regimen consistently display lower energy intake

before 4 months of age than their counterparts in an *ad libitum* system. Furthermore, it's noteworthy that during and after the weaning process, restricted calves are more susceptible to experiencing drops in energy intake, a pattern commonly observed in conventional rearing feeding systems (provision of milk or MR at about 10% of calf BW; Leal et al., 2021).

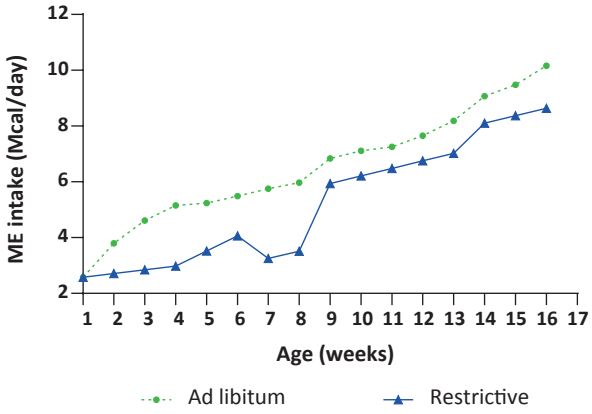

Figure 1.1 Total metabolizable energy (ME) intake (Mcal/day) in two milk feeding systems. *Ad libitum* milk replacer (MR) and starter intake (●dotted line; data adapted from Webb et al., 2014) and restrictive ME from MR and *ad libitum* starter feed (▲solid line; data adapted from Leal et al., 2021) over 4 months of life. The ME values for MR and starter feeds were assumed as 4.07 Mcal/day from MR and 2.7 Mcal/day from the starter feed.

Early-life nutrition plays a crucial role in bovine development and metabolic programming, significantly impacting the calf's health and future productivity. Over the past decade, alternative feeding schemes have been evaluated in rearing calves. It has been demonstrated that feeding elevated levels of WM or MR (~20% of birth weight) can improve calf health, growth rates, feed efficiency, and lifetime production. Both short- and long-term benefits of greater DMI have been described, including:

(i) *Greater growth rates:* Calves that have doubled their nutrient intake in comparison to those receiving 10% of their BW demonstrate a capacity to achieve significantly greater weight gain (~1 kg/d) compared to their restricted-fed counterparts (~0.45 kg/d) throughout the preweaning phase (Khan et al., 2011). For example, Jasper and Weary (2002) compared the growth rates of calves fed conventional milk (10% of body weight) with those consuming milk *ad libitum*. The *ad libitum*-fed calves consumed 89% more milk before weaning, resulting in a 63% weight advantage over the conventionally fed calves by day 35. Furthermore,

recent studies (Brown et al., 2005; Soberon and Van Amburgh et al., 2017) have demonstrated the connection between early-life feed intake and mammary gland development. Doubling the milk feed intake led to improved mammary weight (75.48 g in restricted Vs. 337.48 g in enhanced system) and accelerated growth of other organs, such as the liver (74% more weight in enhanced system; Soberon and Van Amburgh et al., 2017).

(ii) *Metabolic configuration:* Research has demonstrated that providing calves with high energy levels (ranging from 4.2 to 8.4 Mcal ME intake per day) as opposed to traditional nutrient-restricted diets (2.8 Mcal ME intake per day) have significant effects on gene expression profiles across various tissues, including muscle, liver, bone marrow, mammary gland, pancreas, and adipose tissue (Leal et al., 2021). In this study, Leal et al. (2021) identified distinct metabolic profiles at 49 days of age resulting from preweaning milk supply. Alterations in energy metabolism (fatty acid and tricarboxylic acid metabolites), protein metabolism (free amino acids, dipeptides, and the urea cycle), and liver metabolism (bile acid and heme metabolism) were the primary effects associated with these dietary interventions

(iii) *Health:* Nutrition significantly influences immune responses. Lower feeding rates correlate with compromised immune function, making calves more susceptible to diseases (Kooijman et al., 1996; Nonnecke et al., 2003). Furthermore, calves subjected to restrictive feeding systems often exhibit an elevated incidence of inflammatory events, marked by increased plasma fibrinogen and neutrophil responses before weaning (Khan et al., 2011; Ballou et al., 2012; Schaff et al., 2016). In addition, the *ad libitum* feeding approach has been associated with reduced therapeutic interventions required from birth until 77 days of age, as demonstrated by research conducted by Berends et al. (2020).

(iv) *Enhanced animal welfare:* Behavioral indications of reduced hunger have been observed, including enhanced activity or visits to the milk feeder without receiving a reward (Hammon et al., 2002; De Paula Vieira et al., 2008; Echeverry et al., 2021). Additionally, playful behavior has been noted (Krachun et al., 2010), along with reduced non-nutritive sucking (Jensen, 2006; De Paula Vieira et al., 2008), and certain studies have documented decreased cross-sucking behavior (Jung and Lidfors, 2001; Roth et al., 2009).

(v) *Reduced age at first calving:* Increased nutrient intake in heifers enables earlier breeding maturity, potentially lowering the age at first calving and reducing the cost of rearing replacement heifers. For instance, intensive feeding with high solids content (16.7%) led to calving occurring 27.5 days earlier than conventional MR regimens (Raeth-Knight et al., 2009; Rincker et al., 2011).

(vi) *Improved first-lactation performance:* Gelsinger et al. (2016) concluded that providing adequate nutrients from liquid and solid feeds, such as a 100 g/d

increase in liquid DMI among calves with an average daily gain (**ADG**) exceeding 500 g/d, could potentially yield an additional 66 kg of milk during the first lactation when combined with proper post-weaning practices.

While restricting milk feeding aims to reduce costs and encourage early weaning, adopting higher milk allowances with gradual weaning methods can successfully raise calves on a higher plane of nutrition (de Passillé and Rushen, 2016; Rosenberger et al., 2017). Although feeding greater volumes of liquid feed benefits calf health and performance, it is unclear whether current MR formulations are adequate for high feeding rates in preweaning calves, as MR presents nutrient imbalances compared with WM.

Milk Composition and milk replacer composition variability

Observing the composition of WM serves as a fundamental steppingstone to understanding the nutrient requirements of young calves. Table 1.1 presents the milk composition of various breeds for reference. On average, cow's milk comprises around 3.3% crude protein (**CP**), 3.9% to 5.0% fat, 4.7% lactose, and less than 1% minerals, as-fed basis (Bascom, 2002).

Table 1.1 Whole milk composition of five dairy cattle breeds (13% dry matter; DM) and a traditional 20-20 milk replacer (MR) composition (12.5% DM)

Breed	Protein	Fat	Lactose	Ash
Ayrshire	3.28	3.97	4.63	0.72
Brown Swiss	3.18	3.80	4.80	0.72
Guernsey	3.49	4.58	4.78	0.75
Holstein	3.02	3.56	4.61	0.73
Jersey	3.65	4.97	4.70	0.77
MR (20-20)	2.5	2.5	6.25	1.00

From Bascom, 2002 and Morrison et al., 2017

As shown in Table 1.1, commercial MR typically have higher levels of lactose and lower levels of fat compared to WM. Consequently, WM contains about 22.5 MJ of ME/kg DM, whereas commercial MR products range between 19.3 and 19.8 MJ of ME/kg DM (Drackley, 2008). This results in MR providing lower ME when fed at similar rates (Drackley, 2008). Additionally, commercial MR formulations often vary in their levels of CP, leading to a lower energy-to-protein ratio compared to WM (9.8 - 12.7 in MR vs. 12.2-13.7 g CP/MJ in WM). Based on Drackley (2011), under thermoneutral

conditions, a 45 kg calf requires approximately 1.75 Mcal/d for maintenance. Given estimated WM energy content of 5.37 Mcal ME/kg of solids, the same calf must consume about 325 g of milk solids, equivalent to approximately 2.5 L, just for maintenance. However, most MR have lower fat content than WM, providing less energy at approximately 4.6 – 4.7 Mcal/kg. Consequently, a 45-kg calf would require around 380 g (or 3.0 L) of MR for maintenance. Only any additional milk solids consumed beyond maintenance can be utilized for growth.

ENERGY SOURCES IN CALF DIETS

Protein

Dietary protein plays a crucial role in promoting the growth and development of various tissues and can significantly influence the ADG of calves (Barlett et al. 2006). Research by Diaz et al. (2001), Gerrits et al. (1996), and Donnelly and Hutton (1976) have highlighted the substantial impact of dietary protein levels on body composition. This influence goes beyond merely the protein content in MR; it extends to the relative protein intake in relation to energy consumption. When the intake of CP is restricted or limited relative to energy intake, the additional energy is stored as fat (Hill et al., 2009). For instance, Tikovsky et al. (2001) conducted a study involving Holstein calves fed MR with different levels of CP (23.5, 24.8 or 27%), fat (14.7, 21.6 or 30.6%), and lactose (55.2, 46.6 or 35.3%). Interestingly, when MR with equal CP and energy content were fed, but differed in energy sources, the total gain remained consistent, while the body composition exhibited marked differences. Understanding the influence of individual nutrient intake on growth rate and composition has been the subject of studies involving both young (Diaz et al., 2001; Blome et al., 2003) and older pre-ruminant calves (Gerrits et al., 1996; Labussière et al., 2008). These studies have shown that calves weighing between 65 and 105 kg generally exhibit a gross protein efficiency of approximately 60%. However, as their BW exceeds 110 kg, the gross protein efficiency tends to decrease and falls below 50%. Additionally, the incremental efficiency of protein utilization has been observed to decline significantly with increasing age, with values ranging from 20% to 40% for calves weighing above 150 kg (Gerrits et al., 1996; Labussière et al., 2008; Van den Borne et al., 2006a; Gerrits et al., 2019). In heavy veal calves (125 to 250 kg of body weight), our research group has observed that the incremental energy efficiency of protein, lactose, or fat supply in MR is approximately 39%, 74%, and 73%, respectively (Van den Borne, unpublished). However, it's worth noting that there is currently a lack of available data concerning such nutrient efficiency in calves during their first few weeks of life. The latest edition of the NRC (2021) uses a value of 0.70 at birth to calculate the efficiency of metabolizable protein for gain. Despite these findings, the metabolic

mechanisms by which these efficiencies are achieved and how nutrient and energy partitioning are modulated are insufficiently understood.

Fat

Dietary fat from WM provides about 50% of total dietary energy, while in the case of MR it only offers between 20 to 40% of dietary energy for young calves. Even greater is the difference in supply of essential fatty acids (**FA**). MR typically differs significantly in fat content, composition (FA profile), and structure compared to WM (Esselburn et al., 2013; Hageman et al., 2019). These differences in FA composition are linked to the fat sources used in MR, primarily vegetable oils (e.g., coconut, palm, and rapeseed) in Europe and animal fats (e.g., lard and tallow) in North America (Esselburn et al., 2013). Regarding heifer replacement, the nutritional objective has been promoting lean growth (NRC, 2021). Until recently, this has been the primary consideration. As a result, several publications have encouraged low-fat formulations (Tikofsky et al., 2001; Bartlett et al., 2006; Hill et al., 2008), based on the association of higher fat inclusion with increased fat deposition. Research has indicated that increasing the fat content of MR can impact the growth and performance of calves. The conversion of dietary fat to body fat has been found to be efficient, leading to increased body fat rather than increasing body protein mass (Bascom et al., 2007; Bartlett et al., 2006; Hill et al., 2008). Some studies suggest a link between increased dietary fat intake and higher fat deposition, which may impact mammary development (Sejrsen et al., 1982; Lammers et al., 1999; Radcliff et al., 2000). Reports have highlighted that an excessively rapid rate of gain during the prepubertal phase often pursued to achieve early pubertal BW, can negatively affect mammary development, and subsequently reduce future milk production (Sejrsen et al., 1998). However, research has shown that an elevated level of nutrition during the preweaning period does not impair parenchymal growth in heifers and even suggests the possibility of enhancing mammary growth (Brown et al., 2005; Meyer et al., 2006; Daniel et al., 2009; Geiger et al., 2016). For instance, during early development, Meyer et al. (2006) indicated a marked increase in mammary epithelial cell proliferation in heifers with elevated nutrient intake. Brown et al. (2005) established a connection between intensified nutrient intake and enhanced mammary growth, implying a potential influence on stem cell behavior. Furthermore, Geiger et al. (2016) examined the effects of nutrition levels on mammary gland development in young calves at 10 weeks of age. They demonstrated that high nutrition levels (MR: 28% CP, 25% fat, fed at 1.13 kg/calf per day) stimulate both mammary parenchyma (7.3-fold) and mammary fat pad (5.9-fold) growth without compromising the biochemical composition of the parenchyma. Nevertheless, whether fat deposition (within certain boundaries) is advantageous or detrimental for health and development of calves remains an open discussion.

Lactose

As previously mentioned, commercial MR formulations often contain high levels of lactose, which serves as the primary source of carbohydrates in these products. Lactose is hydrolyzed in the small intestine by the brush-border enzyme lactase. This enzyme breaks down lactose into its constituents, glucose, and galactose. Lactase activity is high and remains active during continuous lactose feeding, as observed in milk-fed calves (Gilbert et al., 2015). In the small intestine, lactase activity can reach levels as high as 190 U/g protein, indicating efficient digestion of lactose (Gilbert, 2015). However, lactase concentrations decrease as digestion progresses along the intestinal tract, with lower activity observed in the ileum compared to the small intestine (Huber et al., 1964; Gilbert et al., 2015). Therefore, the efficient enzymatic activity of lactase ensures high lactose digestibility and serves as an easily accessible source of energy for calves (~45% DE intake; Huber et al., 1964; Pantophlet et al., 2016), with over 90% of the intake being oxidized. Research conducted by Van den Borne et al. (2007) revealed that around 80% of the ^{13}C from orally supplied [U-^{13}C] glucose was recovered as $^{13}CO_2$, and a similar pattern was observed with feeding [2-^{13}C]glucose. Based on these findings, it was concluded that *de novo* synthesis of fatty acids is not a significant pathway for disposing of absorbed glucose in calves. Instead, when glucose absorption increases, heavy milk-fed calves tend to experience a reduction in fatty acid oxidation, resulting in the accumulation of fat as the main mechanism for fat deposition. Furthermore, studies have shown that calves younger than 3 weeks exhibit high insulin sensitivity, which gradually decreases after this period, regardless of the feeding strategy, reaching very low levels compared to other species (Stanley et al., 2002; Pantophlet et al., 2016). It has been suggested that prolonged intake of high lactose MR can have adverse effects on glucose homeostasis and insulin sensitivity in heavy veal calves older than 4 months of age, leading to conditions like hyperglycemia, hyperinsulinemia, and insulin resistance (Hugi et al., 1997). Van den Borne (2006b) explored the potential relationship between nutrients and their role in insulin resistance in heavy pre-ruminant calves. High lactose intake increases insulin resistance in pre-ruminant calves, while an increase in digestible protein intake with constant protein-free energy intake decreases insulin resistance, and an increase in fat intake is not perceived to increase insulin resistance. Understanding the impact of energy sources, such as lactose, on insulin sensitivity and glucose metabolism in calves is crucial for optimizing their growth and overall health.

Starch

Young calves possess a limited capacity to digest starch efficiently. The inclusion of starch in milk replacers has been explored to provide energy, yet it is important to note that the young calf's digestive system is not equipped to break down significant amounts of starch. Research indicates that while small quantities of starch can replace

lactose in milk replacers, post-ruminal starch disappearance is more attributed to fermentation rather than enzymatic digestion (Gilbert et al., 2015). This early phase of a calf's life introduces significant shifts in nutrient sources and digestion. As calves transition from a liquid-based diet to one that contains grains and forages, their gastrointestinal system must undergo fundamental adaptations to adapt ruminal fermentation and intestinal digestion of ingested nutrients (Baldwin et al., 2004; Guilloteau et al., 2009). Consequently, calf starters' nutrient composition becomes pivotal in nurturing healthy growth.

Calf starters are designed to play a central role in young calf nutrition, primarily characterized by a higher carbohydrate content than other nutrient categories. The quantity of starch within calf starter formulas can vary significantly, ranging from 22 to 38%, supplied mainly from cereal grains (NRC, 2021). The significance of starch in calf starters is underscored by the necessity for ruminal fermentation to produce VFA such as butyrate and propionate, partly responsible for the development of ruminal papillae and epithelium (Sander et al., 1959). However, it has been shown that the high inclusion of starch in starters (>32%) increased DE but decreased rumen pH (<5.5), changed ruminal digestion, and lowered animal performance (Pezhveh et al., 2014).

Fiber

Calf starter feeds play a crucial role in the early development of calves' digestive system, and including fiber is an important consideration in their formulation. Calf starters should contain a minimum level of fiber (~5% crude fiber, >13% NDF of DM; NRC, 2021); the source and amount of fiber can significantly impact the overall health and calf growth. Including roughage byproducts in calf starter feeds provides the necessary fiber content to promote proper rumen development and functioning. However, the balance of fiber intake is crucial. High intakes of roughage can lead to increased ruminal pH, which is beneficial for preventing ruminal acidosis and achieving optimal VFA production (Mertens, 1997). This balance is crucial for ensuring efficient diet utilization and maximizing animal performance. Like in dairy cattle, including too much fiber in calf starter rations can reduce intake and energy supply, leading to decreased ADG (Suarez et al., 2006). Conversely, a carefully managed dietary fiber content and physical structure of the feed can enhance rumen development. Manipulating the roughage-to-concentrate (R:C) ratio or altering the physical characteristics of the feed, such as particle size and bulk, can influence the rumen's development and function (Greenwood et al., 1997; Terré et al., 2015). These considerations underscore the significance of incorporating appropriate levels of fiber in calf starter feeds to support rumen health and overall growth.

ENERGY INTAKE AROUND WEANING

Under natural conditions, the weaning process of the bovine calf occurs gradually and is typically completed at approximately 10 months of age (Reinhardt and Reinhardt, 1981). In a free-choice-feeding setting, calves have been observed to continue consuming MR until at least 6 months of age (Webb et al., 2014). However, current practices often involve transitioning calves from milk to solid feed as early as possible to stimulate solid feed intake and promote rumen development (Eckert et al., 2015). While this approach is commonly used, the weaning period can be stressful for calves due to the immature state of their rumen. Therefore, the weaning method significantly influences calf performance and rumen development, especially when high amounts of milk are provided during the pre-weaning period. Sweeney et al. (2010) reported that calves gradually weaned starting at 19 days of age could not compensate for the reduced nutrient intake from milk by increasing their intake of starter feed. In contrast, calves weaned at older ages showed better ability to compensate for the reduced milk intake. The reduced nutrient intake from milk and the limited digestibility of starter feed by the immature rumen can negatively impact calf development, resulting in the loss of any growth advantage obtained before weaning after the weaning process (Leal et al., 2021). To address this weaning-related growth check, increasing the energy content of starter feed, such as through elevated inclusion levels of dietary fat, may prove beneficial in supporting calf growth and minimizing the negative effects of weaning (Araujo et al., 2014; Berends et al., 2018; Panahiha et al., 2022).

In mature ruminants, the inclusion of fat in diets can improve energy efficiency due to the direct use of long-chain fatty acids in the metabolic pathways of fat synthesis without the need for acetate and glucose (Machmuller et al., 2000). However, fat is generally perceived to be detrimental to rumen functioning due to its potential interference with bacterial fermentation (Enjalbert et al., 2017). Despite the benefit of providing fat to ruminants, using fat in starter diets is limited due to technological and nutritional limitations. On one hand, feeding rumen inert fats separated from the pellets is not an option for starter feeds because of poor palatability (Miller et al., 1959; Miller, 1962). On the other hand, physical mixing is unfeasible due to the significant difference in particle size between the fat prills and the pellets, which reduces the homogeneity of the mixture and stimulates the sorting of pellets by the calves (Berends et al., 2018). Moreover, evidence suggests that the inclusion of fat in single-pellet starters is limited due to altered pellet hardness and durability (Thomas et al., 1998), and can interfere with the digestion of other components (Berends et al., 2018).

Efforts to enhance the energy density in calf starter feeds have led to various studies exploring the effects of different fat sources and inclusion levels. Some studies found that adding up to 10% of fats like tallow, lard, butter, or hydrogenated cottonseed oil had no significant impact on calf growth and intake (Johnson et al., 1956; Miller, 1962). However, Miller et al. (1959) observed that feeding 10% brown grease or hydrogenated cottonseed oil reduced starter intake. Araújo et al. (2014) showed that including 11% fat from whole soybeans in calf starter did not affect DMI, in contrast to Kuehn et al. (1994), who reported negative effects when adding 7% added soybean oil. On the other hand, Hill et al. (2015) found that supplementing 2% of calf starters with either tallow or soybean oil decreased ADG. Berends et al. (2018) reported higher starter intake and ADG when using an extruded pellet containing about 40% hydrogenated palm fatty acids. Ghasemi et al. (2017) showed that supplementing with 3% soybean oil improved calf performance, while palm fat and tallow did not have the same effect. Doolatabad et al. (2020) found no growth improvements when feeding calves, a starter containing 7.5% whole soybean, and fractionated refined palm oil. Additionally, Ghorbani et al. (2020) observed that providing 2% of either soybean oil or extruded soy had no effect on the calf growth rate.

Including fat to increase energy density in dairy cow and feedlot diets is a well-established practice (Fluharty and Loerch, 1997). However, limited information exists regarding the supplementation of calf starter with rumen-inert fat. Rumen inert fat, also known as bypass fat or rumen-protected fat, is a dietary fat that resists lipolysis and biohydrogenation by rumen microorganisms but is digestible in the lower digestive tract (Naik, 2013). Early studies by Gardner and Wallentine (1972) reported growth responses in veal calves by adding tallow to starter rations. Contrarywise, Waldern and Fisher (1978) found no significant effect on feed conversion ratios when using unprotected tallow in calf diets. However, Fisher (1980) reported an increase in weight gains for calves receiving diets containing 20% vs. 10% of a protected lipid source (as a percentage of DM). More recent research by Berends et al. (2018) explored using an extruded high-fat pellet with hydrogenated palm-free fatty acids, resulting in higher starter and energy intake and BW gain around weaning compared to traditional single pellets or pellets with increased fat content. Additionally, Panahiha et al. (2022) investigated the inclusion of 3% rumen-inert palm FA in calf starter and observed increased DMI and growth. This study adds to the growing evidence that rumen inert fat supplementation in calf starter diets can positively influence calf performance. Overall, while rumen-protected fat to increase energy density in calf starter feeds is not as extensively studied as it is in dairy cow and feedlot diets, emerging research suggests that incorporating rumen-inert fat may hold promise for enhancing calf growth and performance.

OBJECTIVE AND OUTLINE

Dairy production systems have continuously evolved throughout history, adapting to new nutrition concepts, technologies, management strategies, costs, and market demands. In the current scenario, market pressures push for greater system efficiency and reduced replacement animal numbers, leading to the emergence of novel concepts in calf rearing and production methods. Calf rearing systems are particularly embracing higher allowances of WM or MR, equal to or exceeding 20% of birth weight in liters per day. The promise of enhanced later-life performance drives this shift. Despite the limited available data, it suggests that elevating the energy density in MR and starter feed has comparable positive long-term effects.

The aim of this thesis was to expand fundamental knowledge to improve feeding strategies, and to provide insights into the effects of protein, fat, and lactose inclusion in MR on the metabolism of calves younger than 21 days of age. In addition, to investigate the short-term impact of restoring fat intakes in early weaned calves, closer to the biological reference set by suckling calves, by combining higher inclusions of fat in MR and a starter feed during the first 120 d of life, that promote calf growth and development while minimizing adverse effects related to the shortage of energy intake during and after weaning. Specifically, the objectives of this research are as follows:

Chapters 2 and 3: Quantification of Macronutrient Contributions: The primary objective is to quantify the effect of protein, fat, and lactose content in MR on the accretion of protein and fat in young calves. This investigation will provide insights into the role of individual macronutrients in early calf growth.

Chapters 4 and 5: Lactose-fat exchange effects: This objective involves determining the effects of partially substituting lactose with fat in MR on composition by weight, on growth performance and gastrointestinal health in dairy calves. By expanding our understanding of these effects, the study seeks to optimize growth and health in calves.

Chapter 6: High-Fat Starter Feed Evaluation: The research aims to evaluate the consequences of incorporating a high-fat pellet into a conventional, highly fermentable calf starter. The effects on feed intake, overall performance, and health of rearing calves will be examined to determine any eventual benefits of this nutritional approach.

Finally, the general discussion (Chapter 7) integrates the findings of Chapters 2 through 6. This section explores the role of energy sources in the diets of rearing calves for their growth and development throughout the preweaning, weaning, and post-weaning phases. With an emphasis on protein and energy metabolism, and energy supply, while also discussing the role of dietary fat during the initial months of life. Ultimately, conclusions are drawn to summarize the findings of this thesis.

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CHAPTER 2

RESPONSES TO INCREMENTAL NUTRIENT SUPPLY ON ENERGY AND PROTEIN METABOLISM IN PRE-WEANING DAIRY CALVES

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ABSTRACT

Recently reviewed development objectives and feeding practices in young dairy calves require an adaptation of nutrient recommendations set for milk replacer (**MR**) composition. Nutrient requirements of calves younger than 21 days of age, and those of calves fed with high levels of milk replacer are insufficiently quantified. The efficiency at which macronutrients are utilized, particularly protein, substantially diminishes with age, and there is little data for the first weeks of life. In addition, in older (pre-)ruminants, protein and energy can be simultaneously limiting for protein gain. Whether this also applies to calves in the first weeks of life is unknown. Therefore, this study aimed to quantify the responses in protein and fat gain to incremental supply of protein, fat, or lactose to MR in very young calves. Thirty-two groups of 3 mixed-sex Holstein-Frisian newborn calves (3.4 ± 1.6 d of age), were randomly assigned to one of four dietary treatments applied for 19 days: a basal MR (23.3 % CP, 21.2% CF and 48.8% lactose of DM), provided at 550 kJ/kg $BW^{0.85}$ per day (**CON**; n = 24), or the basal MR incrementally supplied with 126 kJ of DE/$BW^{0.85}$ per day as milk fat (**+FAT**; n = 23), lactose (**+LAC**; n = 24) or milk protein (**+PRO**; n = 23). Calves were fed MR in 2 daily meals and had *ad libitum* access to water, but did not have access to calf starter nor any other solid feed. After 2 weeks of adaptation to their respective diets, groups of calves were placed for 1 week in an open-circuit respiration chamber for nitrogen and energy balance measurements (5 d). Data were analyzed using PROC MIXED with SAS software. Fixed effects included treatment, and arrival batch. The incremental nutrient efficiencies indicate what percentage of extra intake of nutrients is retained. In this study, we observed that with every 100 g increase in protein intake, 57% was converted into protein deposition, while 44% contributed to heat production. Similarly, a 100 g rise in fat intake led to 67% being stored as fat with 22% being released as heat. Likewise, a 100 g increment in lactose intake resulted in 49% being stored as fat with 38% being released as heat. Extra protein intake was not deposited as fat, and extra lactose and fat intake did not increase protein deposition.

Key words: Energy and nitrogen utilization, milk replacer, young calf

INTRODUCTION

Traditionally, the amount of whole milk (**WM**) or milk replacer (**MR**) fed to dairy calves has been severely restricted compared to their natural voluntary intake. Driven by the higher costs of liquid feeds, this strategy aimed to stimulate starter intake and rumen development and allow for early weaning (Warner, 1991). However, research over the past decade has demonstrated that increasing the supply of milk closer to *ad libitum* levels improves growth, health, and even long-term milk-production (Soberon et al., 2013, Davis Rincker et al., 2011). While the benefits of increased nutrient supply to dairy calves are now well recognized, the importance of macronutrient composition of MR is insufficiently understood for these feeding levels.

Although current feeding levels, in terms of volume, are very close to natural *ad libitum* intakes (Jasper and Weary, 2002; Webb et al., 2014) the feeding frequency and meal sizes remain different from the biological standard. As current guidelines were established to suit low planes of nutrition (Diaz et al., 2001; Quigley, 2010), there is a lack of data for nutrient requirements and reference recommendations for calves younger than 21 days of age, fed with high levels of MR. The effects of nutrient intake on growth rate and growth composition have been described for young (Diaz et al., 2001; Blome et al., 2003) and older pre-ruminant calves (Gerrits et al., 1996; Labussière et al., 2008). This research has shown that the gross protein efficiency in calves weighing between 65 to 105 kg is around 60% but decreases to below 50% when calves exceed 110 kg of body weight (**BW**). Besides of this, the incremental efficiency of protein has been demonstrated to diminish significantly with age, with values ranging between 20% to 40% for body weights above 150 kg (Gerrits et al., 1996; Labussière et al., 2008; Van den Borne et al., 2006a; Gerrits et al., 2019). Furthermore, unpublished data from our group has shown that the incremental energy efficiency of protein, lactose, or fat supply in MR for heavy veal calves (125 to 250 kg of BW) was found to be 39%, 74%, and 73%, respectively, regardless of the intake level of solid feed. To our knowledge, there is no such data available for calves in their first few weeks of life.

Both in calves fed with WM or MR, lactose and fat are the most important energy sources. It has been demonstrated that young calves have a high capacity to digest and metabolize lactose, as carbohydrate oxidation remained high (above 90%) when increasing lactose intake (57 kg of lactose; Huber et al.,1964). In the case of fat, Van den Borne (2006b) showed that when feed supply increases, the proportion of dietary fatty acids oxidized drops from 80% to about 30%. Extensive research has also been done investigating the relationship between production (either growth or lactation) with energy and protein supply in various animal species. In ruminants, it has been

suggested that energy and protein are often co-limiting factors (Gerrits et al., 1996; Zanton and Heinrichs, 2008). For instance, in Holstein steers, increasing energy intake through abomasal glucose infusions and intraruminal acetate infusions has been found to enhance nitrogen retention and improve the efficiency of amino acid utilization (Schroeder et al., 2006). Similarly, in a meta-analysis (Daniel et al., 2016), an increase in dietary net energy for lactation was shown to increase milk yield and milk protein efficiency in dairy cows, and the increase was independent of level of metabolizable protein fed. In calves, insulin sensitivity is quite high at birth, and decreases independent of feeding strategy in early age to very low levels when compared with other species (Pantoplhet et al., 2016). Consequently, the role of energy in these young calves and whether the simultaneous limitation of both protein and energy also applies to them remain unknown.

Most observations on energy and protein efficiency are performed on individually-housed animals, and it is known that this individual housing affects their energy metabolism, particularly when it can modify behavior and induce stress. These behaviors, such as repetitive oral manipulation of the pen or tongue playing, can impose considerable energetic costs on the animals (Van den Borne et al., 2004, 2006a). To date, no studies have been conducted on group-housed calves.

Therefore, the objectives of this study were: to quantify protein efficiency, both gross and incremental; to study the effect of energy supplied as lactose or fat on protein efficiency, and to quantify the fate of energy from different sources in group-housed calves up to 21d of age.

MATERIALS AND METHODS

This study was conducted at Carus, Research Facility of the Department of Animal Sciences, Wageningen University and Research (Wageningen, the Netherlands). Animal procedures complied with the Dutch Law on Experimental Animal in accordance with European Union Directive 2010/63 (European Commission, 2010; AVD2040020173425) and were approved by the Animal Care and Use Committee of Wageningen University.

Animals and Housing

Ninety-six Holstein-Frisian calves (39 females, 57 males) were sourced at birth from two commercial dairy farms and were selected based on clinical health, age, BW, and sex uniformity. Mean age and BW upon arrival were 3.5 ± 1.4 d and 44.5 ± 0.3 kg, respectively. At the farm of origin, a standardized protocol for colostrum management was followed wherein calves were fed 4 L within the first 1 h after birth followed by

one feeding of 2 L for a total of two feedings of colostrum in the first 12 h of life. From 24 h onward, calves were fed 5.25 L/d of MR (Sloten B.V., Trouw Nutrition; 48.8% lactose, 21.2% crude fat, and 23.3% CP of DM; 15% solids) in two or three meals. The MR was 40 to 42°C at feeding and was provided in a clean teat bucket. Calves arrived in 9 batches to the research facilities, and measurement periods were staggered. During the adaptation period, for the first 14 d after arrival, calves were housed in groups of 3 in pens of 3.0 × 3.0 m, equipped with rubber-slatted floors and open fences on all sides, with fresh straw as bedding material. Temperature was maintained between 15 and 21ºC, relative humidity between 55 and 75%, and air velocity at <0.8 m/s. Calves were exposed to natural light (400-500 lx) from 0530 to 1930h and to darkness (5 lx) during the remainder of the day. After the adaptation period, the first measurement period, which involved measuring a complete energy balance, including heat production (**HP**), lasted for 5 days. This was followed by the second measurement period, which involved measuring fasting heat production (**FHP**), lasting for 2 days. Before the FHP period, one calf from each group was randomly selected and euthanized for the collection of gastrointestinal tract tissues (the results of which have been reported elsewhere). Each group (n=3 for HP and n=2 for FHP) was housed in pens measuring 3.0 × 3.0 m, with tenderfoot® (Minneapolis, MN, USA) flooring and open fences only at the front side, without bedding material, in one of four identical 80 m3 indirect calorimetric chambers (described by Heetkamp et al. 2015). Temperature was maintained at 18ºC, relative humidity at 65%, and air velocity at <0.2 m/s. Calves were exposed to artificial light (420 lx) from 0530 to 1930h and to darkness (3.5 lx) during the remainder of the day.

Diets and feeding

Calves were fed according to their metabolic BW (**$BW^{0.85}$**), weekly adjusted as they grew at individual animal level. Groups were assigned to 1 of 4 dietary treatments: a control diet (basal, **CON**; Trouw Nutrition, Deventer, the Netherlands), or the control diet supplemented with butterfat (**+FAT**; anhydrous milk fat; Royal VIV Buisman BV, Zelhem, the Netherlands), lactose (**+LAC**; lactose powder; Arla Foods Ingredients Group, Viby, Denmark) or protein (**+PRO**; milk protein concentrate powder 80; Fonterra Ltd., Auckland, New Zealand), and were exposed to the dietary treatments for 14 d before the measurement period started. Supplemented treatments were isoenergetic, meaning that they were equal in estimated digestible energy intake (674 MJ/kg $BW^{0.85}$/d), provided at equal amount two times daily. The ingredient and analyzed nutrient composition of the experimental MR, as well as the designed nutrient intakes per treatment are shown in Table 2.1. Milk replacer was reconstituted with water (150 g/L) and supplied in a teat bucket at a temperature of about 40°C. Feeding times were 0600, and 1800 h. Calves had *ad libitum* access to water but did not have access to calf starter or any other solid feed.

Table 2.1 Ingredient and analyzed nutrient composition of the experimental milk replacers and design of the nutritional treatments

Item	Treatment			
	CON	+FAT	+LAC	+PRO
Ingredients (%DM)				
Milk replacer[1]	100	88.2	77.7	82.7
Anhydrous milk fat	-	11.8	-	-
Milk protein powder	-	-	-	17.3
Lactose powder	-	-	22.3	-
Nutrient composition (%DM)				
Dry matter (%)	96.2	96.5	96.9	95.6
Crude protein	23.4	20.7	18.2	34.5
Crude fat	21.4	30.6	16.6	18.0
Lactose	49.0	43.2	60.2	41.4
Crude ash	6.2	5.5	4.8	6.4
DE (MJ/kg of DM)[2]	20.6	22.3	19.6	20.6
Gross energy (MJ/kg of DM)	21.1	23.0	19.9	21.0
DM, DE, and nutrients provided (g/kg $BW^{0.85}$)				
DM	27	30	34	33
Crude protein	6	6	6	11
Crude fat	6	9	6	6
Lactose	13	13	21	13
Crude ash	2	2	2	2
DE (MJ/d)	548	674	674	674

[1]Premix, provided per kilogram of milk replacer: 25,000 IU/kg of vitamin A; 5,000 IU/kg of vitamin D3; 90 mg/kg of vitamin E; 158 mg/kg of vitamin C; 94 mg/kg of iron; 47 mg/kg of manganese; 124 mg/kg of zinc; 11 mg/kg of cooper; and 0.2 mg/kg of selenium. [2]DE = (0.396 × crude fat × 0.91) + (0.237 × CP × 0.93) + (0.17 × lactose × 0.98)

Measurements

Each calf was weighed at day 0, 7, 14, 19 and 21. The calves were in the respiration chambers for seven days, for which the first five days HP ($kJ/kg^{0.85}/d$) and the last two days FHP ($kJ/kg^{0.85}/d$) were measured. From day 14 and 19, gas exchange was measured in 10-min intervals by measuring the exchange of O_2, CO_2, and CH_4, as described by Heetkamp et al. (2015). To check the proper functioning of the chambers, a carbon dioxide recovery test was performed before the start of the experiment, according to procedures described by Heetkamp et al. (2015). In the 4 chambers, 99.8%, 100.4%, 100.5%, and 99.9% of the carbon dioxide released was recovered.

A radar device was mounted in the chamber above each pen completely covering the housing area to measure physical activity continuously during the respiration, and fasting period. During the first 5 d (respiration period), manure was quantitatively collected (mix of urine, feces and cleaning water) and stored at -20°C. After cleaning the respiration chambers, two of the three calves returned to the respiration chambers for two days (d19) for measuring the FHP. Aerial NH3 was collected from a quantified sample of the outgoing air in H_2SO_4, and NH4+ in water that condensed on the heat exchanger were collected quantitatively. MR was sampled during each measurement period, and milk refusals were recorded at an individual animal level.
At day 12 (two days before the start of the respiration chamber measurements) a marker cobalt-ethylenediamine tetraacetic acid (Co-EDTA; all batches) was mixed with at 0.5 g/kg MR for 48h. On day 13 and day 14, multiple rectal fecal samples were taken after manual stimulation of the rectum. One pooled sample were made for each experimental unit for the two sampling days and were transferred to a freezer (-20 °C) awaiting further processing.

Chemical Analysis

Feed refusals, and feces were freeze-dried for determination of DM content. Milk replacer, feed refusals, and feces were ground to pass 1-mm sieve. DM content was determined by drying to a constant weight according to ISO standard 9831 (ISO, 1998). Crude fat content was determined after acid hydrolysis in feed and in freeze-dried manure according to ISO Standard 6492 (ISO, 1999). Crude ash content was determined in feed and in freeze-dried feces. Samples were carefully incinerated in a muffle furnace by slowly increasing the temperature from 20 to 550°C to prevent foaming, and subsequent incineration took place according to ISO Standard 5984 (ISO, 2002). Lactose content was analyzed enzymatically in feed and in freeze-dried feces (Enzytec; Diffchamb Biocontrol, Nieuwerkerk aan den IJssel, The Netherlands). Nitrogen content was measured in fresh feed, feed refusals, manure, and aerial NH3 and water that condensed on the heat exchanger according to ISO Standard 5983 (ISO, 1997). Gross energy content was analyzed in feed, freeze-dried manure, using adiabatic bomb calorimetry (model C7000 calorimeter; IKA Werke GmbH & Co. KG, Staufen, Germany) according to ISO Standard 9831 (ISO, 1998). Cobalt was analyzed in feed and freeze-dried fecal samples after ashing and acid hydrolysis as described by Williams et al. (1962), using a SpectrAA 300 atomic absorption spectrophotometer (Varian B.V., Middelburg, the Netherlands). All analyses were carried out in duplicate.

Calculations

Gross energy (**GE**) intake was calculated as feed intake multiplied by the GE content of the MR. Intake of metabolizable energy (**ME**) was determined by subtracting energy excretion in manure and CH_4 from the gross energy intake. For each balance

period, Nitrogen retention (**NR**) of each group of 3 calves was calculated as the difference between ingested nitrogen and nitrogen lost in manure, condensed water and extracted air. Total HP was calculated from gas exchanges according to the formula of Brouwer (1965). Energy retention (**ER**) was calculated as the difference between ME intake and HP. Protein retained (N retention × 6.25) and energy retained as protein (protein retained × 23.7 kJ/g) were calculated from N retention. Energy retained as fat was calculated from the difference between total energy retained and energy retained as protein in the body.

Apparent total tract digestibility (**ATTD**) of nutrients were calculated from the following equation: ATTD = 100 -100 × ((Co_{feed}/$Co_{digesta}$) × ($Nutrient_{digesta}$/$Nutrient_{feed}$)) where $Nutrient_{digesta}$ is the nutrient concentration in the feces (g/kg DM), $Co_{digesta}$ is the cobalt concentration in feces (g/kg DM), $Nutrient_{feed}$ is the nutrient concentration in the MR (g/kg DM), and Co_{feed} is the cobalt concentration in the MR (g/kg DM).

Statistical Analysis

All statistical analyses were performed using PROC MIXED in SAS 9.4 (SAS Studio, SAS Institute, Inc., Cary, NC). A group of calves housed in one respiration chamber was considered the experimental unit for the N and energy balance and both the dependent variables and covariables were expressed as averages per group. Incremental responses differences were calculated by considering the CON treatment as a reference within each batch. The normal distribution of the residuals was checked to verify the model assumptions. The model included the fixed effects of diet, batch, and their interactions. All values are presented as least squares means ± SEM. Significance was declared when $P \leq 0.05$ and trends were declared when $P < 0.10$.

RESULTS

The data set included 32 observations, i.e., 8 replicates per treatment. Two replicates were based on 2, instead of 3 calves. The ATTD of the experimental diets is shown in Table 2.2. The ATTD of DM ranged between 88-91% and was not different between the groups. The ATTD of crude fat was lower for +LAC calves (-5% for +PRO and -7% for +FAT and CON; $P = 0.018$). The highest ATTD of crude protein (**CP**) digestibility was observed in calves fed the +PRO diet, and the lowest was reported for calves fed the +LAC diet ($P < 0.01$).

Treatment effects on performance are presented in Table 2.3. Calves in the +PRO treatment had a greater BW ($P = 0.01$), being 2.8 kg and 2.2 kg heavier than +FAT and +LAC calves, respectively. Consequently, +PRO calves had greater average daily gain (ADG; $P < 0.01$) than +FAT and +LAC calves (567 g, 420 g, and 449 g, respectively).

Table 2.2 Apparent total-tract digestibility coefficients of nutrients in young Holstein calves as affected by the dietary treatments[2]

	Treatments[2]					
***ATTD*[1], %**	**CON**	**+FAT**	**+LAC**	**+PRO**	**SEM**	***P*-Value**
Dry matter	91	91	88	91	1.2	0.12
Crude fat	93[a]	92[a]	86[b]	90[ab]	2.0	0.018
Crude protein	79[b]	78[b]	70[c]	85[a]	2.3	<0.001

[1]Apparent total tract digestibility. [2]Different superscripts between columns denote a significant difference between treatments

The effects of dietary treatments on energy and N balances are reported by the difference with the CON treatment, and shown in Table 2.3. As result of design, digestible N intake increased with the dietary CP intake from 895 to 1,538 mg of N/kg of $BW^{0.85}$ ($P < 0.01$). Fecal N excretion was affected by the diet being greater in the +LAC calves when compared with +FAT calves ($P = 0.03$), but no differences were found between +LAC and +PRO treatments. N retained in the body increased from 545 (CON) to 818 (+PRO) mg of N/kg of $BW^{0.85}$ per day and differed from +FAT and +LAC treatments ($P < 0.01$). Calves in the +PRO group retained 131 kJ/ kg of $BW^{0.85}$ per day as protein at GE intake of 659 kJ/ kg of $BW^{0.85}$ per day, whereas energy retained as fat was the highest for +FAT (148 kJ/kg of $BW^{0.85}$ per day) and the lowest for the +PRO group (61 kJ/kg of $BW^{0.85}$ per day; $P < 0.01$). Energy output with feces and urine did not differ between treatments and averaged 44 ±3.40 kJ/kg of $BW^{0.85}$ per day. Methane production was very low and unaffected by the dietary treatments. Total heat production was greater in +PRO than +FAT calves (Table 2.3; $P \leq 0.01$), however, there were no differences observed between +LAC and +PRO or +LAC and +FAT. Activity related heat production was not affected by dietary treatment ($P = 0.10$); however, calves in the +LAC treatment showed a marked numerical increase. The respiratory quotient was higher in the +LAC treatment when compared to +FAT and +PRO treatments. Additionally, the +FAT and +PRO treatments differed slightly but significantly from each other (Table 2.3; $P < 0.01$).

Differences in gross and incremental efficiencies are presented in Table 2.4. Treatment variations in N efficiency, expressed per gram of digested N intake, were 77% for CON calves, which increased for +LAC (89%) and +FAT (89%) calves, and decreased for +PRO calves (68%; $P < 0.05$). The gross efficiency of retaining GE, represented as a percentage of GE intake, was 24% for CON calves and significantly increased ($P < 0.05$) with all macronutrient additions. The highest energy efficiency was observed in the +FAT treatment (34%), followed by +PRO (29%) and +LAC (30%). The incremental nutrient efficiencies indicate the percentage of additional nutrient intake that is

Table 2.3 Body weight, nitrogen (N) and energy balance traits of calves receiving the control treatment, and effects of supplementation of 126 kJ of DE/kg of $BW^{0.85}.d^{-1}$ as fat, lactose, or protein on these traits, reported by difference with the control treatment, in calves younger than 21 days of age

			Differences between +FAT, +LAC, +PRO vs. CON				
Item	**CON**	**SE CON**	**+FAT**	**+LAC**	**+PRO**	**Pooled SE**	***P*-Value[1]**
Number of groups/ Number of animals	9/24	-	8/23	9/24	8/23	-	-
Initial BW, kg	45	0.4	0.2	0.1	0.8	0.13	0.46
Final BW, kg	51	0.4	1.5^{b}	2.1^{b}	4.3^{a}	0.4	<0.001
ADG, g/d	339	20.8	81^{b}	110^{b}	228^{a}	15.4	<0.001
N balance, mg of N/kg of $BW^{0.85}$ /day							
Total N intake	895	12.8	12^{b}	16^{b}	643^{a}	13.4	<0.01
Fecal N excretion	186	20.6	12^{b}	84^{a}	55^{ab}	21.7	0.03
Digestible N intake	709	22.9	-0.1^{c}	-68^{b}	588^{a}	0.04	<0.001
N retention	545	27.8	42^{b}	22^{b}	336^{a}	29.3	<0.001
Energy balance, kJ/kg of $BW^{0.85}$ /day							
GE intake from MR	556	7.2	134	118	103	8.0	0.08
Energy output feces+urine	36	4	8	16	6	3.4	0.12
Methane production	0.44	0.08	0.20	0.03	0.07	0.08	0.81
ME intake	520	6.9	126^{a}	101^{ab}	97^{b}	8.0	0.06
Total heat production, kJ/kg $BW^{0.85}$/d	382	4.8	29^{b}	35^{ab}	43^{a}	3.4	0.03
Activity-related heat production, kJ/kg $BW^{0.85}$/d	44	1.2	3	31	-2	14.2	0.10
Energy retention, kJ/kg $BW^{0.85}$/d							
Total	138	8.4	97^{a}	66^{ab}	54^{b}	9.3	0.02
As protein	81	4.1	6^{b}	3^{b}	50^{a}	4.8	<0.001
As fat	57	6.2	90^{a}	63^{b}	4^{c}	6.4	<0.001
Respiration quotient	0.89	0.002	-0.02^{b}	0.06^{a}	-0.01^{c}	0.001	<0.001

[1]Different superscripts between columns denote a significant difference among +FAT, +LAC and +PRO treatments

retained. With every 100 g increase in protein intake, 57% was retained as protein deposition, 44% contributed to heat production, and 2% to fat deposition. Similarly, for every 100 g increase in fat intake, 67% was stored as fat, 22% dissipated as heat, and 0% deposited as protein. Lastly, for every 100 g increase in lactose intake, 49% was retained as fat, 38% lost as heat, and 0% converted to protein.

Table 2.4 Gross and incremental efficiencies of N and energy of young dairy calves fed a milk replacer with increased intake of fat, lactose, or protein

	Treatment				Pooled	
Item	**CON**	**+FAT**	**+LAC**	**+PRO**	**SE**	**P-Value**
N efficiency (% of N intake)[1]	61	65	62	57	21	0.14
N efficiency (% of digested N intake)[2]	77^{bc}	83^{ab}	89^{a}	68^{d}	2.0	<0.001
Energy efficiency (% of GE intake)[3]	24^{c}	34^{a}	30^{b}	29^{b}	1.2	<0.001
Energy efficiency (% of ME intake)[4]	26^{c}	36^{a}	32^{b}	31^{b}	1.1	<0.001
Incremental energy efficiency GE (%)[5]	-	72	49	54	9.0	0.19
As protein deposition[6]	-	0^{b}	0^{b}	57^{a}	4.0	<0.001
As fat deposition[7]	-	67^{a}	49^{a}	2^{b}	6.0	<0.001
As heat[8]	-	22	38	44	9.0	0.26
Incremental energy efficiency ME (%)[9]	-	76	58	55	9.0	0.25
As protein[10]	-	0^{b}	0^{b}	52^{a}	2.0	<0.001
As fat deposition[11]	-	71^{a}	59^{a}	3^{b}	7.0	<0.001
As heat[12]	-	24	41	45	9.0	0.25

[1]Gross Nitrogen Efficiency = 100 × N retained / Total N intake
[2]Gross Nitrogen Efficiency = 100 × N retained / Digestible N intake
[3]Gross energy efficiency = 100 × Energy retention / GE intake
[4]Gross energy efficiency = 100 × Energy retention / ME intake
[5]Incremental efficiency of energy retention = 100 × extra energy retained / extra GE intake
[6]As protein deposition = 100 × extra energy retained as protein / extra GE intake
[7]As fat deposition = 100 × extra energy retained as fat / extra GE intake
[8]As heat =100 × Total heat production / GE intake
[9]Incremental efficiency of energy retention = 100 × extra energy retained / extra ME intake
[10]As protein deposition = 100 × extra energy retained as protein / extra ME intake
[11]As fat deposition = 100 × extra energy retained as fat / extra ME intake
[12]As heat =100 × Total heat production / extra ME intake

DISCUSSION

The present study investigated the incremental efficiencies of energy and protein deposition in young calves (21 days old) fed with a MR topped with either an isoenergetic amount of incremental protein, fat, or lactose. In this study, the apparent total tract digestibility of DM, crude fat, and crude protein, ranged from 88 to 91, 86 to 93, and 70 to 85%, respectively. In a meta-analysis examining the effects of age on the intestinal digestibility of liquids feeds in young calves conducted by Quigley et al. (2021), ATTD in calves before one month of age ranged from DM: 88-96%, crude fat: 89-99%, to CP: 85-99%. Our results fall within these ranges, although at the lower end. Nonetheless, our results seem to be consistent with earlier research by Terosky et al. (1997), in which the digestibility of CP in preweaned dairy calves of 2 weeks of age was between 61% to 73%. In that study, the effects of age were documented, and the low CP digestibility at this age was attributed to the presence of diarrhea (Terosky et al., 1997). In our study, calves in the +LAC group consistently had lower ATTD of DM, crude fat and CP compared to the other groups. This might be due to the higher concentration of lactose (60.2% in DM) in the +LAC group, which has been suggested to exceed the absorptive capacity of the calves and adversely affecting nutrient digestibility (Lodge and Lister, 1973; Hof, 1980). Additionally, compared to the other treatments (approximately 17%), the +LAC group displayed the lowest fecal DM content at 14%. It is worth noting that diarrhea is typically associated with a fecal DM content below 10% and clinical diarrhea was not observed in this group of calves. The elevated lactose content in the MR could potentially trigger an osmotic effect in the intestines, even without manifesting as loose feces (Hof, 1980).

Throughout the experimental period, calves fed +PRO grew more than calves on the other diets, with a higher BW and ADG (55.2 kg and 567 g/d, respectively). Our results are consistent with previous findings on effects of dietary CP content in calves (Blome et al., 2003), in which calves fed high protein MR (CP=26%) had an ADG between 560 and 620 g/d. Studies have shown that increasing CP content affects growth and body composition in milk-fed pre-ruminants which is in line with the higher BW and ADG observed in +PRO calves in this study (Gerrits et al., 1996; Blome et al., 2003).

Incremental efficiencies for energy and N retention were calculated based on nutrient digestibility and retention values. This study provides quantitative estimates of the efficiency at which increments in nutrient supply are utilized by young calves. The incremental efficiency of protein utilization, calculated as the percentage of additional nitrogen intake in relation to digested nitrogen, was found to be 57% for +PRO calves at 21 days of age. This value is higher than the values reported by Gerrits et al. (1996) for pre-ruminant calves weighing between 80 and 160 kg BW (40%) and by Donnelly

and Hutton (1976; 45%) for milk-fed calves weighing 40 to 70 kg. However, our results were not higher than the values observed for calves weighing between 50 and 83 kg BW in studies by Labussière et al. (2008; 64%) and Blome et al. (2003; 66%). Nevertheless, as the calves aged, meaning its live weight increases, the impact of CP concentration on protein deposition diminishes, resulting in a lower marginal efficiency of nitrogen utilization (Labussière et al., 2008), and increased urea excretion (Labussière et al., 2009. Previous studies have shown that as the BW of heavy pre-ruminant calves increases, there is a decline in the efficiency of nitrogen retention. According to Gerrits (2019), these efficiencies decrease from 50-65% when the BW is below 70 kg, to values ranging between 20-40% when the BW exceeds 150 kg. Consequently, heavy pre-ruminant calves exhibit lower efficiency in utilizing protein for growth compared to very young calves and other species. Van den Borne (2006b) concluded that multiple factors might contribute to the observed low nitrogen efficiency observed in pre-ruminant calves. These factors may include a decrease in the supply of amino acids or essential amino acids post-absorption, possibly due to milk fermentation or preferential use by intestinal tissues, insulin resistance, and a potential mismatch between the total nutrient supply and the total nutrient requirement for growth and maintenance. This mismatch could arise from factors like feeding frequency (twice daily) and differences in nutrient passage rates, ultimately resulting in varying post-absorptive availability of individual nutrients. However, the exact mechanism affecting protein efficiency in young calves remains unknown.

The gross efficiency of energy use, calculated as energy retention divided by gross energy intake, was found to be higher in calves fed with an additional supply of fat (34%), while no significant difference was observed between the +LAC and +PRO calves (30% and 29% respectively). The gross energy retention efficiency of the +FAT treatment was greater compared to that observed in calves with a body weight of 56 kg, which were fed an isocaloric whey protein-based MR with increasing CP concentration (14%-26%), resulting in values ranging from 27% to 29% (Barlett et al., 2006). These values are more similar to the efficiency obtained in the CON group (26%), and they align with the findings of other studies (Diaz et al., 2001). The incremental efficiency of energy retention, calculated as the additional energy retained per kilojoule of extra ME intake from the MR, was determined to be 76% in +FAT calves, 58% in +LAC calves, and 55% in +PRO calves. According to data collected from various studies (van Es et al., 1969; Neergaard et al., 1976; Vermorel et al., 1979; Aurousseau et al., 1984; Arieli et al., 1995; Blome et al., 2003; Barlett et al., 2006), the average incremental efficiency of ME utilization for growth is estimated to be 69% for milk-fed calves weighing between 45 to 60 kg. Similarly, van den Borne et al. (2006) and Gerrits et al. (1996) reported incremental efficiencies of 72% and 71%, respectively, for calves fed MR twice daily at 140 kg BW and for calves with a BW

ranging from 160 to 240 kg. The efficiency of utilizing ME for energy retention shows a considerable range, from 0.55 to 0.76. This variability could be influenced by factors such as the age of the calf, whether it is undergoing rapid protein accretion with limited fat deposition, or actively gaining fat in addition to protein. Moreover, the dietary fat content relative to total ME and protein also plays a significant role. It is important to note that while protein deposition demands higher energy expenditure, the process of fat deposition is energetically more efficient (NRC, 2021).

The partitioning of energy between protein and fat differed between treatments. Up to 57% of total energy was retained as protein in the +PRO group, where this percentage was 0% for +FAT and +LAC calves. Energy retention as fat accounted for 67% of the total energy retained in +FAT calves and 49% in +LAC calves, whereas only 2% was stored as fat in +PRO calves. Increases in protein supply have been associated with increased fat deposition, even when protein intake is low (Donnelly and Hutton, 1976; Gerrits et al., 1996). In the study conducted by Labussière et al. (2008), it was observed that varying protein intake while maintaining equivalent ME intake did not affect overall HP or energy retention in 70 kg BW calves. However, differing protein intake levels did result in variations in lipid deposition. In contrast, our study showed that the +PRO group exhibited greater total HP and energy retention compared to the +FAT group, with no significant differences observed in comparison to +LAC calves. Calves in the +PRO treatment either utilized the extra protein for growth, resulting in a higher BW and ADG, or lost it as heat (44%). As reported by Roy (1980), increased protein deposition has a greater impact on BW, as it is associated with lean tissue which is highly hydrated. Regardless, our data showed that the energy intake did not limit protein deposition in the young calves. The lack of an increase in fat deposition with elevated protein supply might be related to reduced incremental protein efficiency as calves age, which in turn increases the likelihood of extra protein leading to additional fat deposition (Gerrits et al., 1996).

Furthermore, additional fat and lactose intake did enhance fat deposition as expected but did not increase protein deposition. The absence of a response in protein deposition with increasing energy intake contradicts observations done in young calves (Donnelly and Hutton, 1976) and older (pre) ruminants (Gerrits et al., 1996; van den Borne et al., 2006a; Labussière et al., 2008). Altogether, the increase in ME intake coupled with reduced HP and RQ due to increased fat intake resulted in significantly increased energy retention in the form of fat. The conversion of dietary fat to body fat has been found to be efficient, leading to increased body fat rather than a significant surplus of additional protein (Bartlett et al., 2006; Bascom et al., 2007).

Furthermore, research conducted on preruminant calves has demonstrated that the energetic costs of stereotype behaviors, such as excessive oral manipulation of the pen or tongue playing, can be quite high in individually-housed calves. These behaviors can have a significant impact on the relationship between physical activity and total heat production (Van den Borne et al., 2004, 2006). In pigs, Gerrits et al. (2015) analyzed the effects of individual vs group housing on activity-related heat production and found that, even though group-housed animals have more room to move around, single housing increased activity-related heat production by 40 to 60%. Although in our study, the calves were housed in groups, we found similar values for activity related heat production (as a % of total HP) as those reported by van den Borne (2006). These results have been discussed in more detail in a paper reported by Amado et. al. (submitted) about heat partitioning and substrate oxidation with the same data.

Increasing lactose intake yielded a numerically higher activity related heat production in comparison to the other treatment groups. This observation quantitatively accounts for the elevated total HP in this group. While a link has been established between excess glucose intake and hyperactivity in humans (Wolraich et al., 1995; Schulte et al., 2015), studies investigating the impact of high glucose intake on activity or behavior in young calves remain limited.

CONCLUSIONS

These results describe responses of young calves to incremental supply of protein, fat, or lactose. In this study, we observed that with every 100 g increase in protein intake, 57% was converted into protein deposition, while 44% contributed to heat production. Similarly, a 100 g rise in fat intake led to 67% being stored as fat with 22% being released as heat. Likewise, a 100 g increment in lactose intake resulted in 49% being stored as fat with 38% being released as heat. Extra protein intake was not deposited as fat, and extra lactose and fat intake did not increase protein deposition. These findings offer an opportunity to revise nutritional recommendations taking into account responses to nutrients of young calves and to improve milk replacer formulations.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Annemiek Welboren, Eef Lovink, Tamme Zandstra, Marcel Heetkamp, Erika Beukers-van Laar, and all students involved in sample collection and analysis. Sincere thanks also to Sabine van Woudenberg, Bert Beukers, and other personnel of Carus research facilities (Wageningen, the Netherlands) for their involvement in the care of the animals. Chantal van den Hoven, Natasja Boots, and other personnel of the Calf and Beef research facilities (Trouw Nutrition, Boxmeer, the Netherlands) for the coordination of calf sourcing and transportation. Gert Klaassen is thanked for the expertise and production of the basal milk replacer (Sloten BV, Deventer, the Netherlands).

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CHAPTER 3

INCREMENTAL SUPPLY OF LACTOSE, PROTEIN OR FAT INFLUENCES THE DIURNAL PATTERN OF HEAT PRODUCTION AND SUBSTRATE OXIDATION IN PRE-WEANING DAIRY CALVES

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Submitted to Journal of Dairy Science

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ABSTRACT

Increasing nutrient supply to dairy calves has well known benefits; however, the effects of milk replacer (**MR**) composition when supplied in higher amounts are not fully understood, particularly in the first weeks of life. To better understand the metabolism of macronutrient supply in young calves (21 days old), we investigated diurnal patterns of heat production and substrate oxidation in young Holstein calves fed MR when fed an incremental supply of protein, fat, or lactose. Thirty-two groups of male and female Holstein-Frisian calves (3.4±1.6 d of age; n=3 per group) were blocked and assigned to one of four dietary treatments and studied for 19 days. Diets consisted of a basal MR (23.3 % CP, 21.2% CF and 48.8% lactose of DM) fed at 550 kJ/kg $BW^{0.85}$ per day (**CON**; n=24), or the basal MR supplemented with milk fat (**+FAT**; n=23), lactose (**+LAC**; n=24) or milk protein (**+PRO**; n=23) at 675 kJ/kg $BW^{0.85}$ per day. Milk replacer treatments were provided at 150 g/L, two times daily, and amounts were adjusted by measured BW weekly. Calves had *ad libitum* access to water but were not supplied any calf starter nor forage. After 2 weeks of adaptation to the diets, groups of 3 calves were placed for 1 week in an open-circuit respiration chamber for nitrogen and energy balance measurements (5 d). On day 3, glucose oxidation kinetics was estimated by using [U-^{13}C]glucose. Measurements included total heat production (total energy [**HP**], activity [$\mathbf{H_{act}}$] expenditure, resting metabolic rate [**RMR**]), respiration quotient (**RQ**), and carbohydrate (**COX**) and fat oxidation (**FOX**) in 10 min intervals and averaging these values per hour over days. Data were analyzed using PROC MIXED with SAS software. Fixed effects included treatment and batch. Incremental supply of lactose and fat increased body fat deposition, with observed patterns in RMR indicating that this increase occurred primarily after the meals. Specifically, the average daily RMR was highest in the +PRO group and lowest in the CON treatment. The HP was higher in the +PRO group and throughout the day, hourly means of HP were higher in this treatment mainly caused by an increase in H_{act}. The recovery of $^{13}CO_2$ from oral pulse-dosed [U-^{13}C]glucose was high (77%), and not significantly different between treatments, indicating that ingested lactose was oxidized to a similar extent across treatments. Increasing lactose supply in young calves increased fat retention by reduction in fatty oxidation. Calves fed a MR with additional protein or fat raised RMR persistently throughout the day, while extra lactose supply only affects RMR after the meal. Orally supplied glucose was almost completely oxidized regardless of nutrient supplementation. Extra protein supply increased HP and FOX compared to similar intakes of fat and lactose. FHP of young, group-housed calves is comparable to literature values and unaffected by energy intake. Overall, these findings deepen our understanding of how different nutrients impact metabolic processes, fat retention, and energy expenditure in young calves.

Key words: calf, heat partitioning, glucose and fat oxidation, milk replacer

INTRODUCTION

Traditionally, dairy calves have been fed either whole milk (**WM**) or milk replacer (**MR**), but in either case restricted at an approximate rate of 8-10% of their birth body weight (**BW**), aiming to encourage starter intake and anticipate rumen development (Drackley, 2008). Minimizing milk supply can spare milk for human consumption and MR being more expensive than starter by weight, is perceived as more costly way to feed calves, although this depends on costs and feed conversion efficiencies (Bach et al., 2013). However, *ad libitum* milk or MR intake of calves is naturally much higher (20% of BW; Jasper and Weary, 2002), most recent nutritional understanding accepts that higher supplies of milk or MR have short term benefits other than growth, and long-term positive effects on productivity of cows (Davis Rincker et al., 2011; Gelsinger et al., 2016).

This paper presents new data from a study that was reported previously by Amado et. al. (submitted), which reported the incremental efficiencies of energy and protein deposition in very young calves (21 days old) fed with a MR incrementally supplemented with isoenergetic amounts of protein, fat, or lactose. Incremental fat and lactose both resulted in increased fat deposition as compared to the incremental protein. In most non-ruminants, like humans and pigs, *de novo* fatty acid synthesis mainly derives from glucose supply (Bergen & Mersmann, 2005). In swine, glucose abundance in the postabsorptive state enhances *de novo* fatty acid synthesis and lipogenesis (van Erp et al., 2018; van Erp et al., 2019). The contribution of glucose to fat deposition increases with increasing feeding level and explains about 2/3 of total fat deposition in *ad libitum* fed pigs (van den Borne, 2006). In contrast, dietary glucose is almost completely oxidized in heavy pre-ruminant calves therefore glucose is not deposited as body fat. An increase in feed intake, increased glucose, but decreased fatty acid oxidation (van den Borne et al., 2007). This mechanism is also observed in adult ruminants where fatty acid synthesis from glucose is limited (Laliots et al., 2010). Furthermore, it has been suggested that changes in insulin sensitivity during early life, as observed in the study by Pantophlet et al. (2016), could potentially lead to alterations in how nutrients are distributed within the body. For instance, studies have shown high insulin sensitivity in calves younger than 3 weeks of age, decreasing with age, regardless of the feeding strategy, reaching very low levels compared to other species (Stanley et al., 2002; Pantophlet et al., 2016). Moreover, it has been shown that prolonged intake of lactose can have adverse effects on glucose homeostasis and insulin sensitivity in heavy veal calves older than 4 months of age, leading to conditions like hyperglycemia, hyperinsulinemia, and insulin resistance (Hugi et al., 1997). Understanding the impact of energy sources, such as lactose, on insulin sensitivity and glucose metabolism in calves is crucial for optimizing their growth and overall health.

In terms of protein deposition, the efficiency at which nitrogen intake was retained by young calves was 68%, only slightly higher than the 62% observed in preruminant calves between 60 to 100 kg of BW (Gerrits et al., 1996; Diaz et al., 2001; Blome et al., 2003; Labussière et al., 2008). The metabolic mechanism by which these efficiencies are achieved and how nutrient and energy partitioning are modulated remain unknown. Daily patterns of substrate oxidation and fasting heat production can help gaining understanding in the process of nutrient partitioning.

The present manuscript describes the diurnal patterns of energy expenditure by partitioning total heat production (**HP**) into heat production related to physical activity ($\mathbf{H_{act}}$), and resting metabolic rate (**RMR**) of young calves fed MR supplied with incremental lactose, fat, and protein. In addition, the diurnal patterns of the respiratory quotient (**RQ**), net rates of carbohydrate and fat oxidation and the fasting heat production (**FHP**) of these calves were also measured.

MATERIALS AND METHODS

This study was conducted at Carus, Research Facility of the Department of Animal Sciences, Wageningen University and Research (Wageningen, the Netherlands). Animal procedures complied with the Dutch Law on Experimental Animal in accordance with European Union Directive 2010/63 (European Commission, 2010; AVD2040020173425) and were approved by the Animal Care and Use Committee of Wageningen University.

Experimental Design

A detailed description of the experimental design has been described by Amado et al. (submitted). Briefly, ninety-six Holstein-Frisian calves (39 females, 57 males) were selected based on clinical health, age, BW, and sex uniformity and arrived in 9 batches. The average age and weight at arrival were 3.5 ± 1.4 d and 44.5 ± 0.3 kg, respectively. The experiment consisted of 3 consecutive periods: an adaptation period of 14 d after arrival; the first measurement period (HP, which lasted 5 d); and the second measurement period (FHP), which last 2 d. Before the FHP period, one calf from each group was randomly selected and euthanized for the collection of gastrointestinal tract tissues (the results of which have been reported elsewhere). Each group (n=3 for HP and n=2 for FHP) was housed in indirect calorimetric chamber without bedding material. The schedule was the same for all batches.

Diets and feeding

Calves were fed according to their metabolic BW (**$BW^{0.85}$**), adjusted weekly as they grew at individual animal level. Groups of calves (n=3) were blocked as they arrived and assigned to 1 of 4 dietary treatments: a control diet (basal MR, **CON**; (Trouw Nutrition, Deventer, the Netherlands), or the control diet supplemented with butterfat (**+FAT**; anhydrous milk fat; Royal VIV Buisman BV, Zelhem, the Netherlands), lactose (**+LAC**; lactose powder; Arla Foods Ingredients Group, Viby, Denmark) or protein (**+PRO**; milk protein concentrate powder 80; Fonterra Ltd., Auckland, New Zealand), and were adapted to their respective diets for 14 d before the measurement period started. The incremental nutrient treatments were defined on isoenergetic basis (digestible energy, 674 MJ/kg $BW^{0.85}$/d), and provided in two equal daily portions. The ingredient and analyzed nutrient composition of the experimental MR is shown in Table 3.1. Milk replacer was reconstituted with water at150 g/L and supplied with a teat bucket at about 40°C. Feeding times were set at 0600 and 1800 h. Calves had *ad libitum* access to water, but did not have access to calf starter nor forage.

Measurements and calculations

During the 7 days in the respiration chambers, nitrogen and energy balances were monitored and exchanges of oxygen (O_2), carbon dioxide (CO_2), and methane (CH_4) were measured over 10-minute intervals as described by Heetkamp et al. (2015). $^{13}CO_2$ production was measured during 10-minute intervals using a nondispersive infrared spectrometer (Advance Optima Uras 14, ABB group, Switzerland) as described by Alferink et al. (2003). The total energy expenditure (**HP**) was calculated using the formula of Brouwer (1965) based on gaseous exchange. HP was divided into activity energy expenditure (**H_{act}**) and resting metabolic rate (**RMR**) by use of penalized β-spline regression procedures (van Klinken et al., 2012) in Matlab (MathWorks), with 8 knots. For each group of calves, FHP was estimated as the asymptotic RMR during 24 h (including last feeding) of feed deprivation: $RMR = FHP + [b_0 \cdot c \cdot t^{(-c-1)} \cdot b_1{}^c] / [1 + (b_1/t)^c]^2$, where RMR = FHP (kJ ▪ kg $BW^{0.85}$) at time t (hour); b_0, b_1, and c (all > 0) are parameters that define the curve. The nonlinear least squares regression procedure (PROC NLIN, SAS Institute) was used for curve fitting. The respiratory quotient (**RQ**) was determined by calculating the ratio of CO_2 production to O_2 consumption. The net rates of carbohydrate oxidation (**COX**) and fat oxidation (**FOX**) were calculated from gaseous exchanges (in liters) as described by Van den Borne et al. (2015).

On the third day in the chambers, an oral dose of 11 µmol/kg BW of [U-^{13}C]glucose (99.0 atom%; Cambridge Isotope Laboratories) was added to the MR fed at 1800 to calculated the glucose oxidation from the amount of $^{13}CO_2$ exhaled. Bicarbonate sequestration was measured on day 4 and determined from the amount of $^{13}CO_2$ exhaled after a bolus dose of 32 µmol of [^{13}C] sodium bicarbonate ($NaHCO_3$, 99.0

Table 3.1 Ingredient and analyzed nutrient composition of the experimental milk replacers and design of the nutritional treatments

	Treatment			
Item	**CON**	**+FAT**	**+LAC**	**+PRO**
Ingredients (%DM)				
Milk replacer[1]	100	88.2	77.7	82.7
Anhydrous milk fat	-	11.8	-	-
Milk protein powder	-	-	-	17.3
Lactose powder	-	-	22.3	-
Nutrient composition (%DM)				
Dry matter (%)	96.2	96.5	96.9	95.6
Crude protein	23.4	20.7	18.2	34.5
Crude fat	21.4	30.6	16.6	18.0
Lactose	49.0	43.2	60.2	41.4
Crude ash	6.2	5.5	4.8	6.4
DE (MJ/kg of DM)[2]	20.6	22.3	19.6	20.6
Gross energy (MJ/kg of DM)	21.1	23.0	19.9	21.0
DM, DE, and nutrients provided (g/kg $BW^{0.85}$)				
DM	27	30	34	33
Crude protein	6	6	6	11
Crude fat	6	9	6	6
Lactose	13	13	21	13
Crude ash	2	2	2	2
DE (MJ/d)	548	674	674	674

[1]Premix, provided per kilogram of milk replacer: 25,000 IU/kg of vitamin A; 5,000 IU/kg of vitamin D3; 90 mg/kg of vitamin E; 158 mg/kg of vitamin C; 94 mg/kg of iron; 47mg/kg of manganese; 124 mg/kg of zinc; 11 mg/kg of cooper; and 0.2 mg/kg of selenium. [2]DE = (0.396 × crude fat × 0.91) + (0.237 × CP × 0.93) + (0.17 × lactose × 0.98)

atom%; Cambridge Isotope Laboratories) was injected into an ear vein. The injection took place 2 h after feeding and was completed within 2 minutes at 1800 h. The measurement of [^{13}C]-carbon dioxide enrichment was taken on days 2 (background) and 3. Because within-day patterns in background enrichment were observed, the enrichment levels in excess to background were determined by subtracting hourly background levels determined on day 2, which were subtracted from the measured enrichment of the corresponding hour on day 3. The recovery of ^{13}C was calculated by dividing the amount of ^{13}C exhaled in the form of carbon dioxide by the oral dose received on a per-hour basis.

A model described by van den Borne et al. (2007), was fitted to the 60-min means of $^{13}CO_2$ excretion in breath of each group of calves (corrected for background enrichment) after ingestion of [U-^{13}C]glucose, and after infusion of [^{13}C]sodium bicarbonate:

$$y = [b_0 \cdot c \cdot t^{(-c-1)} \cdot b_1{}^c] / [1 + (b_1/t)^c]^2,$$

where y = $^{13}CO_2$ production (micromoles per minute) at time t (minutes); b_0, b_1, and c (all > 0) are parameters that define the curve. The nonlinear least squares regression procedure (PROC NLIN, SAS Institute) was used for curve fitting. Oxidation of the tracer metabolites was calculated by integration of the area under the $^{13}CO_2$ excretion curve over a period of 24 h after administration of the isotope, $b_0 / (1 + b_1{}^c \cdot 1440^{-c})$ and expressed relative to the amount of ingested or infused isotope by dividing the area under the curve by the dose of isotope corrected for enrichment and chemical purity. The time to peak was calculated as $t_{max} = [b_1{}^c \cdot (1 - c) / (-c-1)]^{(1/c)}$. The maximum $^{13}CO_2$ excretion (mmol/h) was calculated as $y_{max} = b_0 \cdot c \cdot t_{max}{}^{(-c-1)} \cdot b_1{}^c / [1 + (b_1/t_{max})^c]^2$ and was expressed as percentage of the dose. Recoveries and maxima for the $^{13}CO_2$ excretion after the ingestion of glucose tracer were corrected for bicarbonate sequestration, using an average value to be subtracted from the glucose recovery test.

Statistical Analysis

All statistical analyses were performed using SAS Studio (version 3.2, SAS institute, Cary, NC). The group of calves housed in one respiration chamber were considered the experimental unit with treatment and batch as fixed effects. Continuous variables (i.e. figures presented of total heat production, activity related heat production, RMR, ^{13}C recovery, RQ, FHP, and the nets rates of COX and FOX) were analyzed using mixed-model analysis with PROC MIXED in SAS (SAS 9.4M6, SAS® Studio, SAS Institute). Time entered the model as a repeated statement in case of repeated measurements, and then the interaction between time and treatment and the SLICE command from SAS Studio (version 3.2, SAS institute, Cary, NC) to control Type I error were included. The effect of the diet on daily averages over the entire experimental period of total heat production, activity related heat production, RMR, ^{13}C recovery, RQ, FHP and the nets rates of COX and FOX were analyzed by ANOVA using a general linear model with treatment, batch, chamber, and their interactions as fixed effects. The normal distribution of the residuals was checked to verify the model assumptions. Treatment effects were studied by pairwise comparisons using the Tukey method. All values are presented as least squares means ± SEM. Significance was declared when $P \leq 0.05$ and trends were declared when $P < 0.10$.

RESULTS

The data set included 32 observations, 8 replicates per treatment in which 2 replicates were based on 2 calves per treatment, instead of 3 calves per treatment. For FHP measurement, each replicate was based on 2 calves per treatment.

Daily averages of heat production parameters are presented in Table 3.2, and hourly patterns are shown in Figure 3.1. The average HP over the 5-day period was lowest in the +LAC calves ($P < 0.001$; Table 3.2), and this difference was consistently observed throughout the day, with an HP peak following meal ingestion for all treatments (Figure 3.1A). The daily average of H_{act} was numerically higher in the +LAC group than in the other treatments (Table 3.2). Circadian patterns of H_{act} revealed meal-related behaviors, with calves displaying increased activity before meals (anticipation). +LAC calves exhibit higher activity levels, especially during the dark period ($P < 0.01$; Figure 3.1C). The average daily RMR was significantly higher in the +PRO group when compared with the +CON, +LAC, and to a lesser extent, the +FAT calves ($P < 0.01$;

Table 3.2 Heat production parameters and net rates of substrate oxidation over a 5d measurement period and RMR, RQ and net rates of substrate oxidation during the fasting period in young Holstein calves fed a milk replacer supplemented with additional fat, lactose, or protein at 21 days of age.

	Treatment					
Item	**CON**	**+FAT**	**+LAC**	**+PRO**	**SEM[3]**	***P*-Value**
Total heat production, kJ/kg $BW^{0.85}$/d	382[b]	411[a]	417[a]	424[a]	5.1	<0.01
Resting metabolic rate, kJ/kg $BW^{0.85}$/d	337[d]	363[b]	342[c]	382[a]	9.4	<.0001
Activity-related heat production, kJ/kg $BW^{0.85}$/d	44	47	75	43	10.1	0.09
RQ[1]	0.89[b]	0.87[c]	0.95[a]	0.89[b]	0.002	<0.01
Net rate of carbohydrate oxidation, kJ/kg $BW^{0.85}$/h	9.76[c]	9.61[c]	14.29[a]	10.57[b]	0.06	<0.01
Net rate of fat oxidation, kJ/kg $BW^{0.85}$/h	5.40[b]	6.66[a]	2.38[c]	6.30[a]	0.15	<0.01
Fasting period						
FHP[2], kJ/kg $BW^{0.85}$/d	291	311	312	313	8.4	0.22
RQ[1]	0.85[b]	0.84[b]	0.89[a]	0.85[b]	0.003	<0.01
Net rate of carbohydrate oxidation, kJ/kg $BW^{0.85}$/h	7.28[c]	7.64[c]	10.45[a]	8.29[b]	0.23	<0.01
Net rate of fat oxidation, kJ/kg $BW^{0.85}$/h	6.97[b]	7.63[a]	5.03[c]	7.59[a]	0.15	<0.01

[1]Respiratory quotient; [2]Fasting heat production; [3]Pooled standard error of the mean

Table 3.2). Hourly RMR means were lower in the +CON group than in +PRO calves, while consistently higher values were observed for +FAT and +PRO compared to CON and +LAC ($P < 0.05$; Figure 3.1D). Circadian patterns of RMR also displayed meal-related responses across all treatments, though these patterns were less clear for +PRO calves and most pronounced for +LAC calves, particularly after the morning meal. The RMR maximum (RMR_{max}) following the morning meal was reached 3 hours after the meal in the +CON, +FAT, and +PRO groups, but did not significantly differ between the groups. For the +LAC group, the RMR_{max} was reached 2 hours later compared with the other treatments, but it was not significantly differed between the groups ($P > 0.10$; Figure 3.1D). The RMR_{max} in response to the afternoon meal was reached at 19:00 in the +PRO and +FAT groups, and at 20:00 in the +LAC and +CON groups and were significantly different between the groups at these two time points ($P < 0.05$).

The average RQ over the 5-day period was highest among the +LAC calves ($P < 0.01$; Table 3.2), a difference that consistently persisted throughout the day (Figure 3.1B). Hourly RQ means were significantly greater in the +LAC treatment following both meals ($P < 0.01$; Figure 3.1B). RQ always remained consistently below 1. Circadian patterns of net carbohydrate and fat oxidation correspond with patterns observed for RQ, with a greater average daily net rates of COX in the +LAC group ($P < 0.01$; Table 3.2) and the lower for +FAT calves, and the opposite was observed for fat oxidation (Table 3.2; Figure 3.2). Hourly means of net rates of COX were significantly greater in the +LAC calves after the morning meal and lower in the +FAT and +CON group before the morning and afternoon meal ($P < 0.01$; Figure 3.2A). Average daily net rates of FOX over the 5 d period of measurements were lower in the +LAC treatment when compare with the other treatments ($P < 0.01$; Table 3.2). Hourly means of net rates of FOX were significantly less for all the treatments after the morning and afternoon meal. All hourly FOX values were lower in the +LAC ($P < 0.01$; Figure 3.2B). Recoveries of orally supplied [U-13C]glucose dose as $^{13}CO_2$ were high (mean 77%) and were unaffected by treatment (Table 3.3). Although, the maximum of $^{13}CO_2$ production was numerically greater for +PRO treatment. The time to maximum of the +PRO calves was 30 min shorter compared with CON and +LAC fed calves ($P < 0.05$), with values of +FAT calves being in between (Table 3.3).

No differences were found between the treatments in the estimate FHP at the end of the 24h measurement period (Table 3.2). FHP was 20 kJ/BW^{085} lower for CON calves compared to the other groups, but this difference did not reach statistical significance (Table 3.2). During the period of measurement of FHP, calves of all treatments exhibited a response to the last meal similar to the meal responses shown in Figures 3.1 and 3.2. During the final 10 hours of the fasting period, treatment differences were absent, except for the lower RMR in CON calves ($P < 0.01$; Figure 3.3A). Hourly

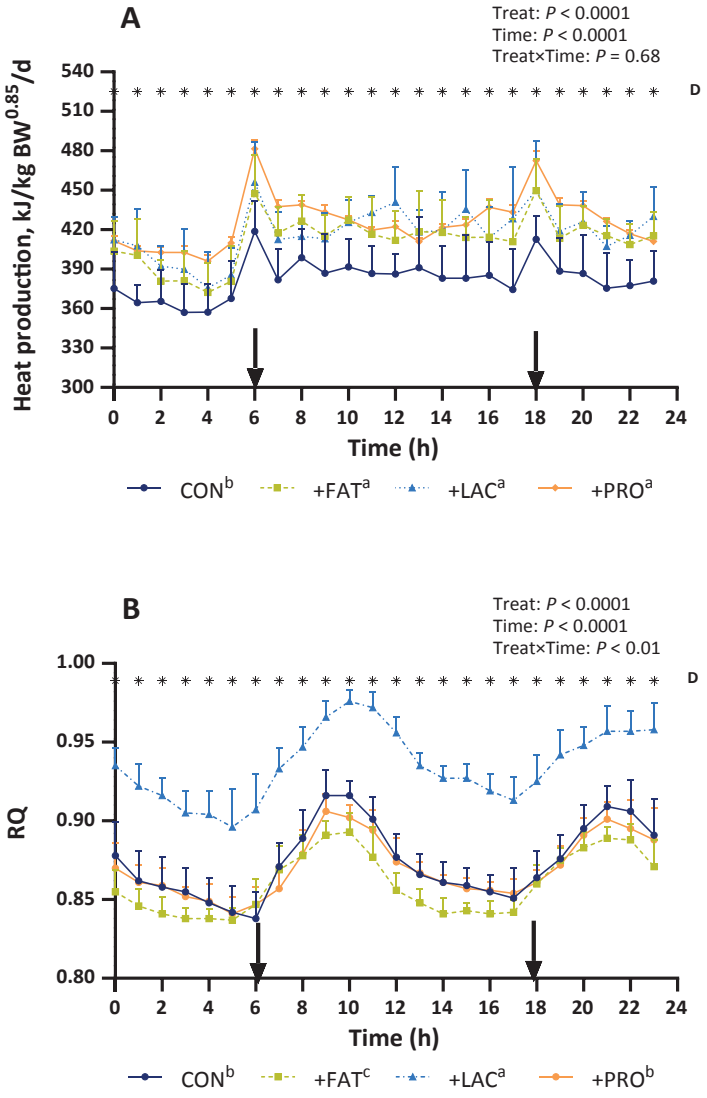

Figure 3.1 Circadian patterns of total heat production (A), RQ (B), activity-related heat production (C), and RMR (D) in young Holstein calves fed a milk replacer supplemented with additional fat, lactose, or protein at 21 days of age. Arrows represent feeding times. Values are least-square means ± pooled SD. *represents P < 0.05, ▲represents 0.05 < P > 0.1, - represents P > 0.1. D, dietary treatment effect.

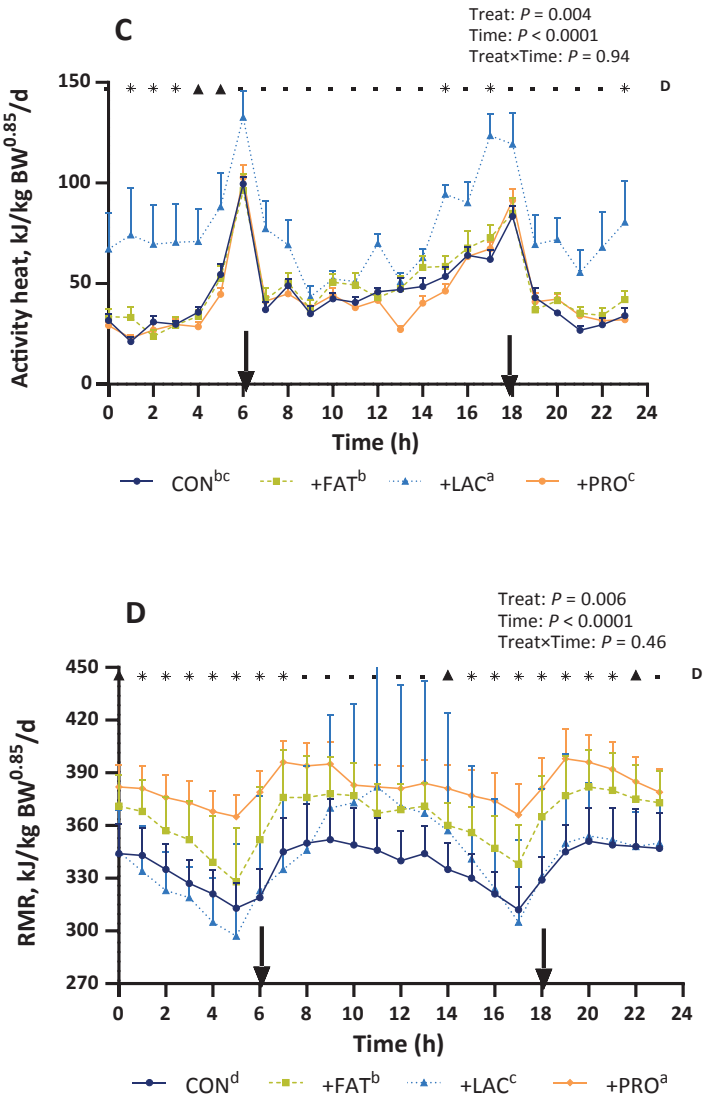

Figure 3.1 Continued

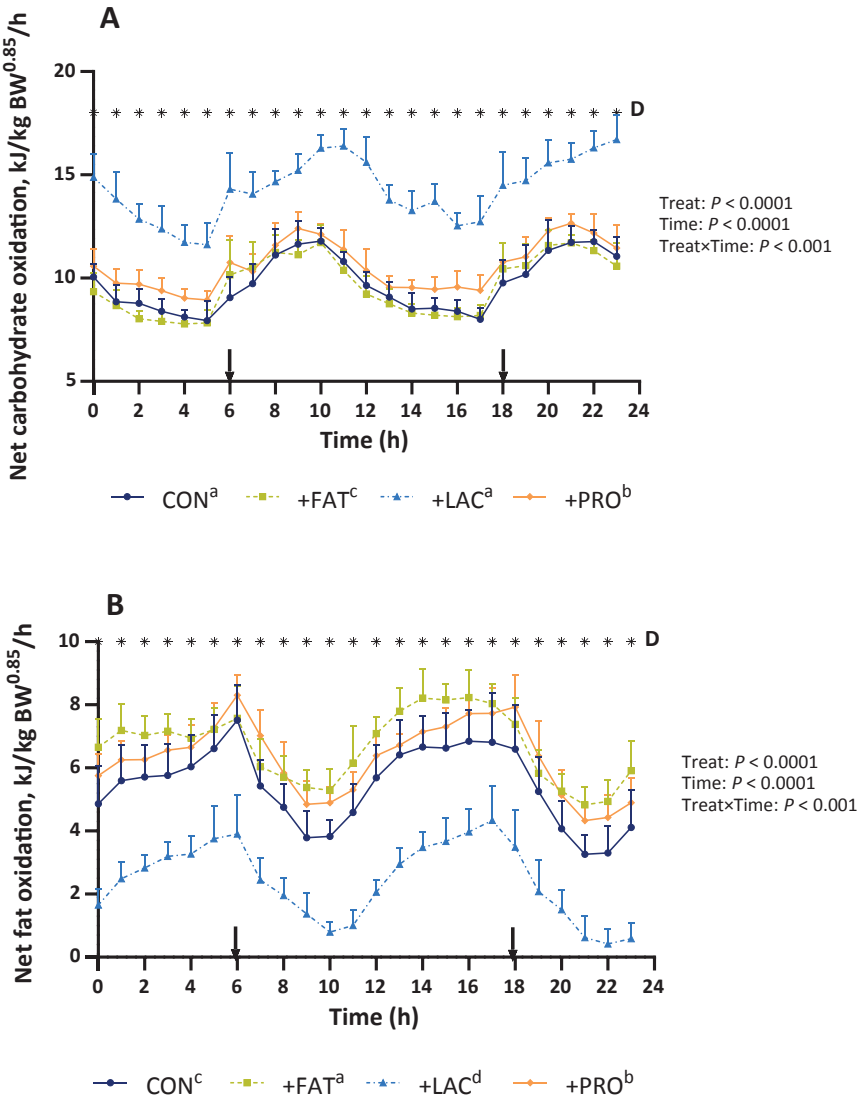

Figure 3.2 Circadian patterns of net carbohydrate (A) and fat oxidation (B) in young Holstein calves fed a milk replacer supplemented with additional fat, lactose or protein at 21 days of age. Arrows represent feeding times. Values are least-square means ± pooled SD. * represents $P < 0.05$, ▲represents $0.05 < P > 0.1$, - represents $P > 0.1$. D, dietary treatment effect.

Table 3.3 Recovery of 13C from [U-13C]glucose, provided with the afternoon meal, in exhaled carbon dioxide in calves at 21 days of age fed a milk replacer supplemented with additional fat, lactose or protein

	Treatment					
Item	**CON**	**+FAT**	**+LAC**	**+PRO**	**SEM[1]**	***P*-Value**
[U-^{13}C]glucose oxidation						
Recovery as $^{13}CO_2$, % of dose	67.2	65.7	69.2	66.1	1.55	0.38
Maximum $^{13}CO_2$ production, % of dose/h	6.7	6.5	7.0	7.3	0.23	0.01
Time to peak, min	351^{a}	335^{ab}	352^{a}	318^{b}	8.4	0.031
[^{13}C]sodium bicarbonate						
Recovery as $^{13}CO_2$, % of dose	76.3	89.1	93.6	88.6	5.72	0.30
Maximum $^{13}CO_2$ production, % of dose/h	22.5	22.7	25.3	23.2	2.36	0.81
Time to peak, min	111	123	97	109	9.0	0.47
[U-^{13}C]glucose oxidation						
Recovery as $^{13}CO_2$, % of dose, corrected for carbon sequestration[2]	77.2	75.5	79.6	76.0	1.78	0.37

[1]Pooled standard error of the mean; [2]Corrected for the average [^{13}C]sodium bicarbonate recovery as $^{13}CO_2$

means of RMR were lower in the +LAC calves and higher in the +PRO calves during the fed state ($P < 0.01$; Figure 3.3A). Hourly means of RQ were significantly higher in the +LAC group after the last meal, between 06:00 and 22:00 ($P < 0.01$; Figure 3.3B). However, RQ was not significantly different between the treatments after 22:00, when it decreased and reached an average value of 0.80 for all treatments (Figure 3.3B). Hourly means of net rates of COX over the 24-h fasting period were higher in the +LAC treatment than in the other treatments, particularly between 06:00 and 22:00 ($P < 0.01$; Figure 3.3C) and followed the same pattern as the RQ. Hourly means of net rates of FOX over the 24-h fasting period were higher in the +FAT and +PRO calves than in the +LAC and CON calves, specially 8 h after the last meal, and followed the same pattern for all treatments after 22:00 ($P < 0.01$; Figure 3.3D).

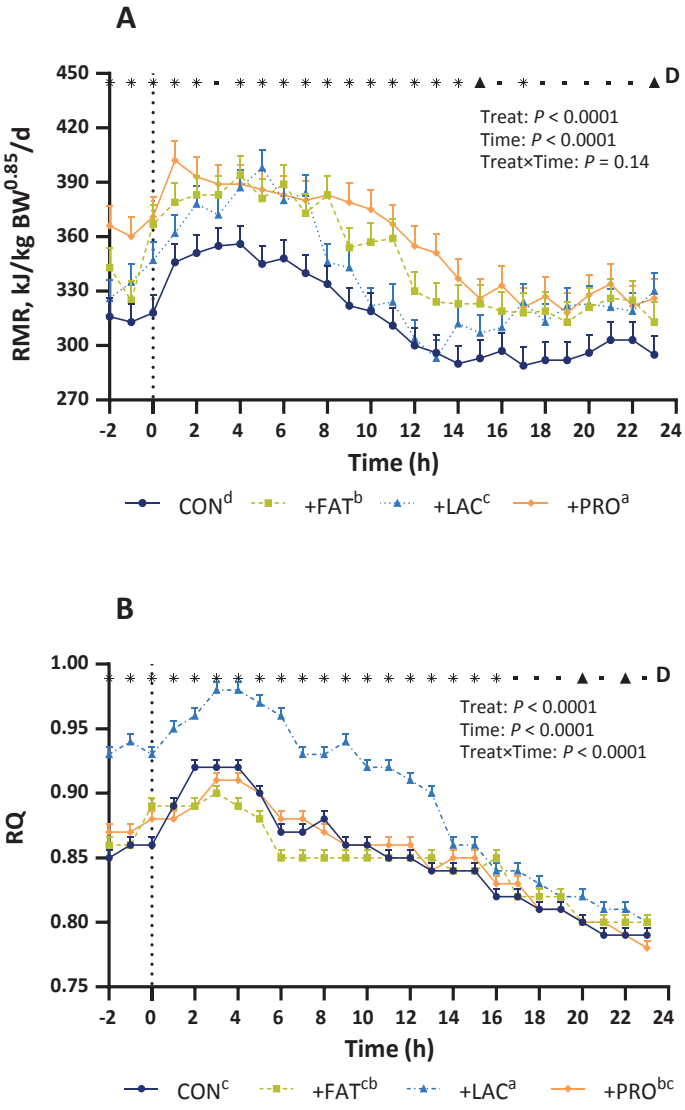

Figure 3.3 24-h patterns of RMR (A), RQ (B), net carbohydrate (C), and fat oxidation (D) during the fasting period in pair-housed, young Holstein calves following a single milk replacer meal supplemented with additional fat, lactose, or protein at 4 weeks of age. Dash line represent feeding time and start time of the fasting period1. Values are least-square means ± pooled SD. * represents P < 0.05, ▲represents 0.05 < P > 0.1, - represents P > 0.1. 1Fasting heat production was calculated from these data as described in the text.

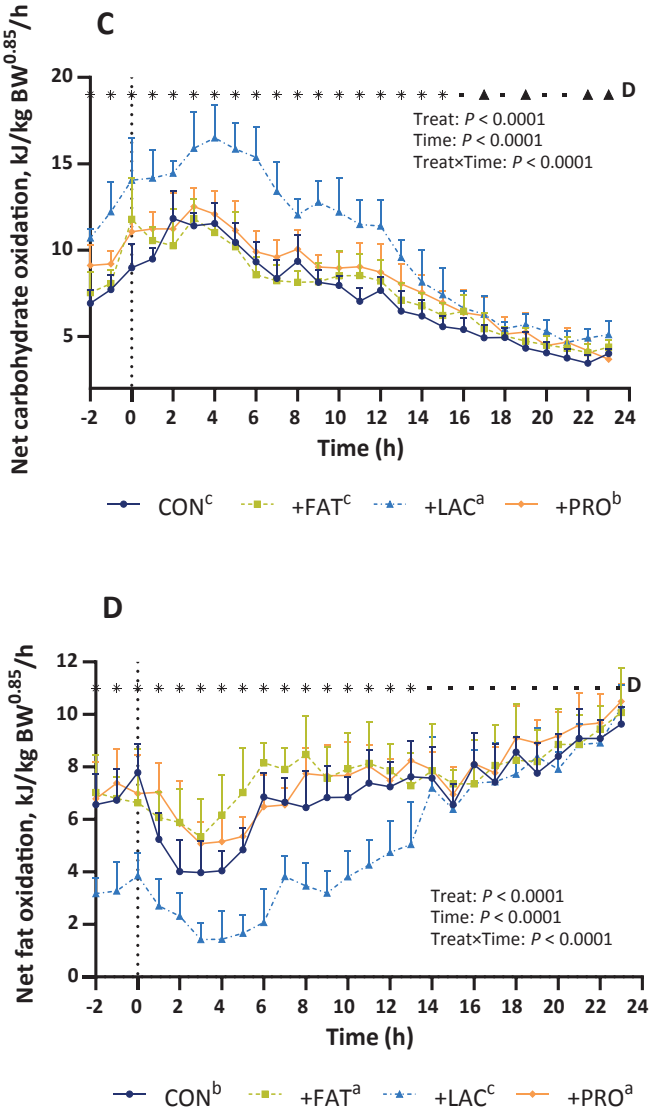

Figure 3.3 Continued

DISCUSSION

We investigated the diurnal patterns of heat production, RQ, FHP, and net rates of carbohydrate and fat oxidation of Holstein calves fed a MR supplemented with additional fat, lactose, or protein, with aiming to better understand the regulation and metabolism of macronutrient supply in 21-day old calves. Our study shows that calves receiving a higher protein intake had the highest HP and RMR. The circadian patterns of RMR for CON, +FAT, and +PRO treatments followed a similar pattern over time before and after feeding. However, notable distinctions in energy metabolic profiles were observed among the treatments, particularly with an increase in RMR during the postprandial state. The +LAC treatment displayed larger within-day variations in RMR, indicating substantial lactose oxidation between meals. In contrast, the circadian RMR patterns for the other treatments consistently remained higher throughout the day. Interestingly, the +PRO treatment exhibited less variation after the morning meal compared to +FAT and +LAC treatments, possibly due to differences in abomasal emptying kinetics.

When pre-ruminant calves are fed MR that formulated with skimmed milk, this can lead to a difference in the kinetics of protein and glucose digestion with variation in nutrient absorption. This is because casein, which accounts for 80% of the skimmed milk protein, coagulates in the abomasum and is gradually released during the day. In contrast, glucose and galactose are absorbed soon after milk ingestion and their levels peak within one hour after feeding (Verdonk et al., 1999). Lactose is rapidly hydrolyzed and absorbed, with a small quantity of triglyceride absorption (Gilbert et al., 2016). The remaining amount of the ingested nutrients is retained in the abomasal coagulum for a longer period and released more progressively (van den Borne et al., 2006; Ortigues et al., 1994).

Furthermore, calves in the +FAT, +PRO, and CON treatments exhibited a consistent pattern of physical activity before 6 p.m., displaying a gradual increase common to all treatments, possibly indicating anticipation before a meal. No differences were observed between the CON, +FAT, and +PRO groups. However, the +LAC group displayed higher activity levels. In the study by van den Borne et al. (2006), calves housed individually and fed MR with varying feeding frequencies and levels showed no significant effects on physical activity patterns. The proportion of HP spent on activity averaged 11% in that study, a value similarly observed for the +FAT, +PRO, and CON calves in our experiment (11%). The +LAC calves exhibited higher activity levels (18%) in comparison. Although in our study, the calves were housed in groups, we found similar values for H_{act} (as a % of total HP) as those reported by van den Borne (2006). In group-housed pigs, 65 to 70% of the variation in HP within a day was related

to physical activity (van Erp et al., 2019). Moreover, it is worth noting that the difference in activity levels observed in +LAC calves persisted throughout the day, becoming even more pronounced during the night. Calves within the +LAC group exhibited the lowest fecal DM content at 14% and consistently had lower apparent total tract digestibility of DM, crude fat, and CP compared to the other treatments (data shown in Amado et al., submitted). Although these calves did not present clinical diarrhea, calves with digestive issues might display signs of abdominal discomfort, such as kicking or increased vocalization due to discomfort or distress (Stull & Reynolds, 2008). Furthermore, it has been reported that calves fed restricted amounts of MR exhibit higher non-nutritive sucking and engage in unrewarded visits to the automatic feeder, both described as behavioral signs of hunger (De Paula Vieira et al., 2008). In a recent study comparing the effects of high lactose or high-fat MR on *ad libitum* calves, calves fed the high lactose formulation had approximately 41% more unrewarded visits to the automatic feeder (Echeverry et al., 2021). It may be possible that +LAC calves in our study were showing more hunger-related behaviors than the other calves, and this could influence H_{act}.

The RQ values reflect the net rates of oxidation of carbohydrates, fats, and proteins, and are approximately 1.0, 0.7, and 0.8, respectively, with slight variations depending on the specific nutrient source. These values are important because they provide insight into metabolic processes, like lipogenesis and carbohydrate oxidation. When RQ values exceed 1.0, it suggests the simultaneous occurrence of lipogenesis and carbohydrate oxidation. An RQ exceeding 1.0, corresponding to negative FOX values indicates that fatty acids are synthesized from carbohydrates (van den Borne et al., 2015). A high intake of energy from carbohydrates, mainly when animals are fed high levels of rapidly digestible sources of carbohydrates, such as in growing pigs, has been found to stimulate this process (Gerrits et al., 2012). Our study examined the effects of different treatments on RQ values and heat production in calves. Specifically, it was found that the circadian patterns of RQ and heat production followed the ingestion of meals for all treatments. Calves fed a MR with extra lactose had a higher RQ value (0.95) than those on other treatments, but it did not exceed 1. This finding suggests little synthesis of *de novo* fatty acids from glucose in the postprandial period because COX was almost complete. The increase in postprandial RQ coincided with reduced FOX and increased COX after the meals. Additionally, we observed that diurnal patterns for carbohydrate and fat oxidation were affected by treatment, with +LAC calves having the highest COX (14.29 kJ/kg $BW^{0.85}$/h) and CON and +FAT having the lowest (9.76 and 9.61 kJ/kg $BW^{0.85}$/h, respectively). However, it is important to note that the calculation of substrate oxidation from indirect calorimetry may lead to an underestimation of COX and an overestimation of FOX due to the contribution of noncarbohydrate precursors to glucogenesis (van den Borne et al., 2015). To address

this limitation, we used a stable isotope tracer in combination with indirect calorimetry to measure substrate oxidation. Specifically, we measured breath $^{13}CO_2$ excretion and calculated the proportion of the oxidized substrate, corrected for bicarbonate sequestration (van Hall, 1999). We found that the recovery of [^{13}C]bicarbonate averaged 86.9%, after an intravenous single dose, independently of the diet. This value is higher than the one reported by van den Borne et al. (2007), where calves with different feeding level of MR had a recovery of [^{13}C]bicarbonate of 72%. Additionally, the average recovery rate of 77% for [U-^{13}C]glucose as $^{13}CO_2$ suggests that ingested carbohydrates are predominantly oxidized independent of the diet. Although glucose oxidation by the tracer method was higher than that calculated from the gas exchange, this can be explained by the contribution of gluconeogenesis to gas exchange at a low RQ and that of lipogenesis at a high RQ (van den Borne et al., 2015). Based on the high recovery rate of [U-^{13}C]glucose as $^{13}CO_2$ and the high COX, there appears to be little fat deposition from carbohydrates, which is consistent with the low-fat oxidation found after feeding. This scenario is also true for milk-fed heavy calves (150 kg of BW) as shown by van den Borne et al. (2007), where dietary glucose was almost completely oxidized (80% based on [^{13}C]glucose and 94% from indirect calorimetry measurements), regardless of feeding level. Furthermore, Pantophlet et al. (2016) conducted a comparison between the effects of a high lactose (59% DM) and high fat (30% DM) MR on heavy milk-fed calves (120 kg BW), analyzing glucose homeostasis and insulin sensitivity, alongside energy and nitrogen balance measurements (unpublished data). Interestingly, in the high lactose treatment, the RQ of calves temporarily exceeded 1 during certain periods of the day. This coincided with an increase in the expression of genes related to lipogenesis in adipose tissue, liver, and muscle (as evidenced by mRNA expression of genes DGAT1-DGAT2). These findings suggest that calves have the capability to store glucose as body fat when exposed to high lactose intakes. However, calves in the +LAC treatment stored very little lactose as fat, even though they should theoretically be in an insulin-sensitive phase, and exposed to high lactose intake (60% DM). Our findings are consistent with the conclusions of the National Research Council (NRC, 2021), indicating that pre-ruminant calves utilize glucose similarly to adult ruminants, with minimal utilization of glucose for fatty acid synthesis and a significant amount used to fuel protein accretion.

Furthermore, the rate of nutrient delivery to the lower gut after a meal has been proposed to be a critical determinant of blood glucose regulation (Tong and D'Alessio, 2014; Gerrits et al., 2008). Although we have not studied insulin metabolism in these calves, calves in this study, measured at 3 weeks of age, were likely more insulin sensitive. Pantophlet et al. (2016) showed that during early life, there is a significant decrease in insulin sensitivity that is not influenced by the composition of the diet.

This reduction in sensitivity results in a change in the available substrate but does not prevent oxidative carbohydrate metabolism. Others have observed that prolonged feeding of a predominantly milk diet with high protein intake is associated with the development of hyperglycemia, hyperinsulinemia, and glucosuria in older milk-fed calves (Hugi et al., 1998; Gerrits et al., 2008). Further research is needed to elucidate the mechanisms underlying the effects of nutrient intake on glucose metabolism and insulin regulation in very young milk-fed calves.

This paper presents new data from a study that was reported previously by Amado et. al. (submitted), which reported the incremental efficiencies of energy and protein deposition in very young calves (21 days old) fed with a MR incrementally supplemented with isoenergetic amounts of protein, fat, or lactose. Interestingly, the study found differences between the additional protein supply and the supply of lactose or fat in terms of incremental efficiencies for protein deposition, and these distinctions could potentially be attributed to variations in fat or carbohydrate oxidation. Specifically, calves in the +PRO treatment were more efficient at protein deposition and had a higher FOX rate than those in the CON or +LAC treatments. Additionally, the study found that body fat deposition increased when fat or lactose supply increased. However, our results in FOX suggest that additional lactose supply reduces fatty acid oxidation rather than deposition.

In our experiment, we used non-linear regression modeling to estimate FHP, which involved calculating the horizontal asymptotic value of the decreasing HP kinetics during starvation while excluding the energy expenditure associated with physical activity. By using this method, we obtained an estimate of FHP that was not influenced by any behavioral disturbances that may have occurred during the starvation period (Labussière et al., 2011; Gerrits et al., 2015). Interestingly, no significant differences were observed in the estimation of FHP among the treatments, indicating that additional supply of fat, lactose, or protein did not affect the overall metabolic rate of the calves. These values are in agreement with previously observed estimates by Labussière et al. (2011) for veal calves with a BW of 73 kg and fed with different levels of MR. They obtained FHP values ranging from 276 and 310 kJ/(kg $BW^{0.85}$/d). As concluded by Labussière et al. (2011), the FHP value can be considered an estimate of the net energy requirement for maintenance and is independent of diet composition. However, studies involving pigs showed that FHP increased with higher feeding levels before the onset of fasting. This increase could potentially be linked to the occurrence where previous feeding leads to an increase in organ mass, particularly metabolically active tissues such as intestinal tissues (Labussière et al., 2011), as also numerically observed in our study. In our trial, FHP was determined over a 23 h fasting period following a morning meal. Although this fasting period was relatively short, and the

values did not reach a plateau, a longer period of fasting may increase abnormal behaviors that can affect the results (Labussière et al., 2008).

Furthermore, during the fasting period measurements, the hourly means of RQ were significantly higher in the +LAC group after the last meal, indicating a greater utilization of carbohydrates as the primary substrate for oxidation in pre-ruminants calves (Kuhla et al., 2015). This finding is consistent with the higher hourly means of net rates of COX observed in the +LAC treatment between 0600 and 2200 and the decrease in FOX during the same hours, demonstrating a reciprocal relationship between these measurements (Kuhla et al., 2015). After the meal, FOX declined for all treatments, with similar values in +FAT and +PRO calves without reaching a negative value. This finding indicates that the utilization of fat as an energy source was higher in these treatments, potentially due to differences in the availability and utilization of lipid substrates. However, after 2200, when RQ decreased and reached a value of 0.80 for all treatments, the hourly means of net rates of FOX followed the same pattern for all treatments. Between 3 to 4 hours after the meal, FOX started to increase again and remained increasing until the end of the fasting period. This indicates a shift in energy substrate utilization towards lipid oxidation during the later stages of fasting, regardless of the treatment.

CONCLUSIONS

In our study, the additional supply of lactose was observed to increase fat retention in young calves. This increase was not primarily attributed to an increase in fatty acid synthesis from glucose but was predominantly caused by a reduction in the oxidation of fatty acids. Moreover, the provision of extra lactose led to an increase in RMR during the postprandial state, whereas the supplementation of fat or protein resulted in a persistent increase of RMR throughout the day. Glucose oxidation remained nearly complete regardless of nutrient supplementation. Calves that were fed a milk replacer with additional protein showed the highest HP increase. This increase in HP was partly due to the oxidation of fatty acids. Additionally, the FHP of young, group-housed calves was found to be comparable to values in the literature, and this parameter appeared to be independent of energy intake.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Annemiek Welboren, Eef Lovink, Tamme Zandstra, Marcel Heetkamp, Erika Beukers-van Laar, and all students involved in sample collection and analysis. Sincere thanks also to Sabine van Woudenberg, Bert Beukers, and other personnel of Carus research facilities (Wageningen, the Netherlands) for their involvement in the care of the animals. Chantal van den Hoven, Natasja Boots, and other personnel of the Calf and Beef research facilities (Trouw Nutrition, Boxmeer, the Netherlands) for the coordination of calf sourcing and transportation. Gert Klaassen is thanked for the formulation and production of the basal milk replacer.

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CHAPTER 4

EFFECT OF ENERGY SOURCE IN CALF MILK REPLACER ON PERFORMANCE, DIGESTIBILITY, AND GUT PERMEABILITY IN REARING CALVES

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Journal of Dairy Science 2019: 102:3994–4001

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ABSTRACT

Current calf milk replacer (CMR) compositions significantly differ from whole milk in their levels of energy, protein, and minerals. Energy source is one of the major differences, as CMR contains high levels of lactose, whereas whole milk contains higher levels of fat. The aim of this study was to determine the effect of partially exchanging lactose for fat on performance, digestibility, and gut permeability in calves fed twice daily on a high feeding plane. Lactose and fat were exchanged in the CMR formulation on a weight–weight basis. The CMR were isonitrogenous but not isoenergetic. A total of 60 male Holstein-Friesian calves were assigned to 1 of 30 blocks based on serum IgG, body weight, and date of collection after birth. Within each block, calves were randomly assigned to 1 of 2 treatments: high fat and high lactose. The CMR was provided twice daily until 49 d of age, followed by a gradual weaning period of 14 d. Starter, straw, and water were available *ad libitum* throughout the complete study. Exchanging lactose for fat did not affect growth; intakes of starter, straw, water, crude protein, or total energy; or apparent total-tract digestibility of nutrients. Gastrointestinal permeability was assessed by measuring the recovery of lactulose and Cr in 24-h urine and the Cr concentration and lactulose: d -mannitol ratio in serum following an oral pulse dose. Urinary recoveries of Cr and lactulose were generally low in both treatments but were higher in calves fed the high-fat CMR. Accordingly, the serum lactulose: d -mannitol ratio and serum Cr concentrations were higher in calves fed the high-fat CMR. In wk 1 and during the weaning transition, calves fed the high fat CMR had significantly fewer abnormal fecal scores. In conclusion, exchanging lactose for fat in the CMR did not affect growth performance, total feed intake, or nutrient digestibility. The high-fat CMR was associated with an increase in permeability markers but positively influenced fecal scores in calves.

Key words: calf milk replacer, lactose, fat, gut permeability

INTRODUCTION

The current global trend in dairy calf rearing is to focus on growth performance using whole milk (**WM**) or calf milk replacers (**CMR**) of high nutritional quality. This is the consequence of recent insights suggesting that nutrient intake from WM or CMR during the preweaning period improves future milk yield (Moallem et al., 2010; Bach, 2012; Soberon et al., 2012). Feeding elevated levels of milk (~20% of birth weight by volume) to calves improves health, growth rates, feed efficiency, and lifetime production (Soberon et al., 2012). Despite economic consequences on farm milk output, many farmers are adopting this strategy using WM. This choice is generally motivated by the availability of WM on farm and by the fact that the nutrient balance of CMR significantly differs from that of WM. Compared with most commercial CMR, WM has higher energy content and contains bioactive components such as enzymes, hormones, and growth factors (Lee et al., 2009; Araujo and Bach, 2015). Compositional differences may result in increased BW at weaning, with some indications of long-term benefits on future milk production (Shamay et al., 2005; Moallem et al., 2010).

Commercial CMR usually contain higher levels of lactose (42–45% vs. 35%, DM basis) and lower levels of fat (16–20% vs. 30%, DM basis) than WM (Hof, 1980; Park, 2009). Furthermore, WM contains about 5.4 Mcal of ME/kg of DM, whereas commercial CMR products range between 4.6 and 4.7 Mcal of ME/kg of DM, indicating that CMR provides lower ME when fed at similar rates (Drackley, 2008). Typical commercial CMR formulations range from 20 to 28% of CP (Bartlett et al., 2006; Bascom et al., 2007; Hill et al., 2010), resulting in a lower energy: protein ratio compared with WM. Furthermore, CMR has a higher ash content, particularly when it consists of mineral-rich dairy by-products. In addition, energy type and content in CMR may affect the osmolality of the solution (Wilms et al., 2019).

To compensate for differences in energy supply between WM and CMR, several strategies have been used to accelerate the growth rate of calves during the milk phase. These strategies include higher feeding rates or modifying the milk replacer composition to increase nutrient intake (Silva et al., 2015). The CP and energy intake from specific CMR formulations strongly influences calf ADG (Bartlett et al., 2006). When CP is restricted or limited relative to energy intake, the extra energy is stored as fat (Hill et al., 2009). Several studies show that increasing the fat content in CMR can affect growth and performance of calves (Bartlett et al., 2006; Bascom et al., 2007; Hill et al., 2008). These studies show increased fat deposition in response to higher fat inclusion in CMR (Tikofsky et al., 2001). Whether fat deposition (within certain boundaries) is beneficial or detrimental remains an open question. Increased energy intake of calves in the first 2 mo of life showed no negative effects on

subsequent milk production at maturity (Brown et al., 2005), whereas fat deposition during the preweaning period may play a beneficial role in mitigating weaning stress in calves (Lee et al., 2009). Standard CMR formulations and lower feeding rates do not provide sufficient nutrients for the calf, which may influence immune function and disease susceptibility (Nonnecke et al., 2003).

Our hypothesis was that a weight–weight exchange of lactose for fat in CMR would positively affect performance and gastrointestinal health in rearing calves fed twice daily. To this end, the objective of the current study was to investigate the effects of exchanging dietary energy sources (fat vs. lactose) on growth performance, intestinal permeability, digestibility, and fecal score consistency in rearing calves.

MATERIALS AND METHODS

This study was conducted between January and May 2017 at the Calf Research Facility of Trouw Nutrition Research and Development (Sint Anthonis, the Netherlands). All procedures described in this article complied with the Dutch Law on Experimental Animals, which complies with ETS123 (Council of Europe 1985 and the 86/609/EEC Directive), and were approved by the animal welfare authority (DEC Utrecht, the Netherlands).

Animals, Housing, and Feeding

A total of 60 male calves between 0 and 3 d of age were collected from local dairy farms at a maximum distance of 14 km from the calf research facility. At the farm of origin a standardized protocol for colostrum management was followed wherein calves were fed 3 L within the first 3 h after birth followed by 2 feedings of 2 L for a total of 3 feedings of colostrum in the first 24 h of life. Successful application of this protocol was monitored by assessment of blood IgG within 48 to 72 h after birth of the calf at arrival at the research facility. Following colostrum feeding, calves were provided meals of 2.5 L of a CMR containing 150 g of CMR/L (Sprayfo Excellent, Trouw Nutrition, Deventer, the Netherlands) twice daily up to d 3 of life. Calves were assigned to 1 of 30 blocks (2 calves per block) based on IgG category (low: 1,000-2,000 mg/dL; high: >2,000 mg/dL; NAHMS, 2007), day of arrival, and BW. Mean BW upon arrival was 44.7 ± 0.86 kg and 45.5 ± 0.82 kg and serum IgG concentration was 1.871 ± 121 mg/dL and 2.003 ± 122 mg/dL for the high-fat (**HF**) and high-lactose (**HL**) treatments, respectively. Within each block, calves were randomized using the RAND function in Microsoft Excel (Microsoft Corp., Redmond, WA) to 1 of 2 isonitrogenous liquid feed treatments. Calves selected for the study were considered healthy after physical examination. Calves were housed indoors in individual pens (1.22 × 2.13 m), separated

by galvanized bar fences, and equipped with 50% rubber-slatted floors in the front and 50% lying area, including a mattress covered with flax straw, in the back. Milk replacer was reconstituted with water and supplied in a teat bucket at 40°C. Milk replacer concentration was 150 g/L and was provided daily in 2 equally sized meals at 0700 and 1600 h (Table 4.1). Meal size was 3.0 L until 14 d of age, increased to 3.5 L between 14 and 49 d of age, decreased to 2.5 L between 49 and 56 d of age, and decreased further to 1.5 L between 57 and 63 d of age. Calves had *ad libitum* access to water, chopped wheat straw (3–7 cm chop length; Ruwvoer Distributiecentrum, Gassel, the Netherlands), and starter feed (Table 4.1; ForFarmers, Lochem, the Netherlands), all of which were provided in separate buckets.

Table 4.1 Analyzed and estimated nutrient composition (g/kg of DM unless noted) of feeds used in the study

	Milk replacer			
Item	**High fat**	**High lactose**	**Concentrate**	**Straw**
DM, g/kg product	970	968	874	936
CP	235	232	204	54
Crude fat	233	173	38	11
Lactose	373	443	-	-
Crude ash	78	79	88	62
Starch	-	-	241	8
ME, Mcal/kg[1]	4.7	4.4	3.3	-
CP:ME ratio	50.1	53.1	62.8	-
Na	7.1	6.9	4.7	460
K	20	21.2	16	14.8
Ca	8.9	8.8	13.8	3.1
P	6.8	6.9	4.8	1.1
Cl	13	14	4.8	2.5

[1]Calculated following NRC (2001).

Calves were fed 1 of 2 experimental diets with an HF or HL milk replacer. Fat and lactose were exchanged on a 1:1 (wt/wt) basis (gram-per-gram substitution), subsequently altering energy content. The experimental diets were therefore isonitrogenous but not isoenergetic, whereas the rest of the CMR formulation remained unchanged. Nutrient composition of the diets is shown in Table 4.1. The experiment was designed to contrast an increase of crude fat from 17.3 to 23.3% associated with a reduction of lactose from 44.3 to 37.3%. The starter concentrate

had 20% CP and 3.8% crude fat. The minimum temperature in the calf facility was maintained at 15°C, and relative humidity was below 80%. Calves were exposed to daylight and artificial light from 0600 to 2200 h and to a night light during the remainder of the 24-h cycle.

Measurements

Health was monitored daily by caretakers, and a standard veterinary protocol was followed in case of disease. A veterinarian was consulted if the symptoms were not described in the standard protocol. Body weight was measured on the day of arrival and once per week thereafter until 10 wk of age. Individual intakes of milk replacer, water, concentrate, and straw were weighed and recorded daily during the entire experiment.

Total fecal collection was conducted for 48 h in 10 randomly selected blocks of calves ranging from 24 to 35 d of age. Feces were collected using fecal bags glued to the hindquarters of the animal. Fecal samples were analyzed for DM, CP, crude fat, ash, VFA, and mineral profile (Ca, P, Na, and K).

Gastrointestinal permeability was assessed in the same group of calves by measuring the appearance of indigestible markers in urine. Lactulose (0.4 g/kg of BW; Sigma-Aldrich, Zwijndrecht, the Netherlands), d-mannitol (0.12 g/kg of BW; Sigma-Aldrich), and Cr-EDTA (0.1 g/kg of BW; Masterlab, Boxmeer, the Netherlands) were dissolved separately in 100 mL of warm water. The solution was mixed together with the CMR meal at 0630 h. Calves were allowed to consume their meal for a maximum of 15 min. Urine was quantitatively collected during 24 h starting right after marker administration. A urine bag was glued to the underside of the calf, and bags were connected with a tube to a bucket. Urine was directly acidified to pH ≤2 with sulfuric acid to prevent microbial degradation. Urine samples were analyzed for lactulose and Cr. For technical reasons, d-mannitol analysis in urine was not successful. Calves were fit with jugular catheters (Intraflon 2 13G, Ecouen, France) on the day before scheduled blood sample collection. Samples were obtained at 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, and 420 min relative to marker administration. The catheters were flushed with 2 mL of heparinized saline (2% solution) before and after sample collection. Blood samples were collected using 20-mL syringes and then allocated into a potassium EDTA Vacutainer (Becton Dickinson, Franklin Lakes, NJ) and a sodium fluoride Vacutainer that contained a glycolysis inhibitor. All blood samples were centrifuged immediately at 2,800 × g for 30 min at room temperature, and 1.5 mL of plasma was pipetted into 2-mL cryotubes and stored immediately at −20°C. Blood samples were analyzed for Cr, lactulose, and D-mannitol.

All study personnel involved in calf handling and taking measurements were blinded to the treatments. Photos of feces were taken daily for all calves, and fecal scores were evaluated at the end of the study by a single observer who was blinded to treatments. The scoring system included 4 categories where 0 was considered normal and 3 was extremely loose. Fecal scores ≥2 were considered abnormal. Medical treatments were recorded daily.

Chemical Analysis

The feeds and feces were analyzed for DM content by drying to a constant weight in a 103°C stove for 4 h (EC 152/2009; EC, 2009). Crude protein was analyzed by combustion according to the Dumas method (Etheridge et al., 1998). Crude fat content was determined by treating the sample with hydrochloric acid and then extracting with petroleum (EC 152/2009; EC, 2009). Starch content was determined enzymatically as described by Rijnen et al. (2001). Crude ash content was analyzed by incineration in a muffle furnace by combustion for 4 h at 550°C (EC 152/2009; EC, 2009). Mineral profile (milk replacer and feces) was determined using inductively coupled plasma MS (PerkinElmer ICP-MS 300D, PerkinElmer, Waltham, MA) according to NENEN 15510 (European Union, 2017). For the preparation of Cr measurements, 0.5 mL of nitric acid was added to 1 mL of urine sample. After 4-h incubation at 95°C, MilliQ water (Millipore, Billerica, MA) was added to the solution until a final volume of 15 mL was reached. Samples were analyzed by inductively coupled plasma MS (PerkinElmer ICPMS 300D, Waltham, MA). For the quantification of samples, a calibration curve of Cr (0, 0.005, 0.02, 0.1, and 0.5 mg/L) was used, and the results were corrected using an internal standard (germanium 74). For the extraction of D-mannitol and lactulose, urine samples were diluted using maltitol as the internal standard. These extracts were then analyzed using a Phenomenex Luna 5u NH2 RP 18 250-mm × 4.6-mm column (Phenomenex, Utrecht, the Netherlands) on a Thermo Endura liquid chromatography tandem mass spectrometer (heated electrospray ionization source) with an Ultimate 3000 pump and autosampler (Thermo Fisher Scientific, Waltham, MA). The elution buffer was a blend of 75% acetonitrile and 25% water containing 1 mmol/L formiate.

Calculations and Statistical Analysis

A classical power analysis was conducted to determine the number of experimental units needed. The power ($1 - \beta$) was chosen to be equal to 80%. The α-level was 0.05. Growth performance was considered the most reliable parameter to determine power. Based on a previous study conducted at the same research facility (Calf and Beef Research, Sint Anthonis, the Netherlands) for 57 d after arrival at 0 to 3 d of age, a standard deviation of 100 g/d was assumed. The minimal meaningful difference in ADG was considered to be 75 g/d. The minimal sample size was 30 calves per

treatment when accounting for a maximum mortality rate of 10%. All statistical analyses were performed using SAS Studio (version 3.2, SAS Institute Inc., Cary, NC). Data on growth, intakes, and plasma concentrations of Cr, lactulose, and mannitol were analyzed with a mixed model procedure (PROC MIXED). Binary data (fecal score) were analyzed following a binomial distribution and proportions (apparent total-tract digestibility, minerals, and excretion in urine of Cr and lactulose) with a β-distribution using a generalized linear mixed model (PROC GLIMMIX). In all analyses, the individual calf was considered as experimental unit with treatment and block as fixed and random effects, respectively. Time entered the model as a repeated statement in case of repeated measurements, and then the interaction between time and treatment and the SLICE command from SAS Studio (version 3.2, SAS Institute Inc.) to control type I error were included. Treatment averages were presented as least squares means and standard errors of the mean. Significance was declared when $P \leq 0.05$, and trends were declared when $P < 0.10$.

RESULTS

The data set included 52 successful observations (24 calves in the HL treatment and 28 calves in the HF treatment). In total, 8 calves were not included in the data analysis based on the pre-established exclusion criteria for health (3 clinically sick calves, defined by a veterinarian, were excluded because therapeutic treatments may invalidate the results) or feed refusals (5 calves were excluded when they refused 3 or more consecutive CMR meals).

Body weight was not affected by dietary treatment at any time point (Table 4.2). Similarly, ADG (Table 4.2) remained unaffected by treatment. Total milk replacer intakes were also equal between dietary treatments (Table 4.2). Starter intake was higher during wk 7 to 9 for HL calves but was equal for the total course of the study (Table 4.2). Straw intake did not differ between the CMR treatments (Table 4.2). Metabolizable energy intake was identical between treatments over the entire experiment. However, intake of specific dietary constituents was different in that there was an increase of 13 MCal of ME/d from CMR ($P < 0.001$) and a numerically lower (13 MCal/d, $P = 0.19$) starter intake in the HF group. Crude protein and water intake did not differ between treatments (Table 4.2).

The apparent digestibility of DM, CP, fat, and ash was not different between treatments (Table 4.3). The apparent total-tract digestibility of Ca, P, and K in feces was not different, but it was higher for Na in the HL calves ($P < 0.05$; Table 4.3).

Table 4.2 The effect of exchanging fat for lactose in a calf milk replacer (CMR) on BW gain and intakes of CMR, total CP, total ME, starter, straw, and water (LSM) in calves during the first 10 wk of age

	Treatment				*P*-value	
Item	High fat (n=28)	SEM	High lactose (n=24)	SEM	T[1]	T x time
Bodyweight, kg						
Arrival	44.7	0.86	45.5	0.82	0.50	-
Week 10	98.4	1.9	99.7	1.8	0.60	-
ADG, kg/d	0.64	0.02	0.66	0.02	0.42	0.35
Total Intake, kg DM						
CMR	57.2	0.3	57.7	0.3	0.35	0.07
Starter	41.3	2.6	46.1	2.4	0.22	0.15
Straw DM	0.84	0.16	0.80	0.15	0.78	0.12
Water intake, kg	226.7	18.5	230.9	17.1	0.87	0.01
ME intake, Mcal						
From CMR	264	1.5	250	1.4	<0.01	-
From Starter	117	7.3	131	6.8	0.19	-
Total	381	8.0	381	7.4	0.84	0.18
CP intake, kg						
From CMR	13.0	0.08	13.1	0.07	0.35	-
From Starter	8.4	0.53	9.4	0.48	0.14	-
Total	21.5	0.56	22.5	0.51	0.17	-

[1]T = effect of treatment.

The 24-h urinary recovery of lactulose was lower ($P < 0.05$) with the HL treatment compared with the HF treatment at 1.4 and 2.2% of the oral dose, respectively (Table 4.4). The urinary recovery of Cr was 3.3% lower with HL compared with 4.6% in the HF group ($P < 0.05$). No treatment effects were found in the 6-h urinary recovery of lactulose and Cr.

Serum Cr concentrations and lactulose: D -mannitol ratio was lower ($P < 0.01$) for HL calves than for HF calves, although a treatment × time interaction was not detected (Table 4.4). There was a treatment × time interaction for fecal scores ($P < 0.05$; Figure 4.1). In wk 1, 3, 8, and 9, HF had fewer abnormal fecal scores than HL.

Table 4.3 The effect of exchanging fat for lactose in a calf milk replacer on apparent total-tract digestibility of nutrients (LSM) in calves measured at 4 wk of age

	Treatment				
Item	**High fat (n=9)**	**SEM**	**High lactose (n=10)**	**SEM**	***P*-value**
Apparent total tract digestibility, %					
Dry matter	91.6	0.69	93.0	0.74	0.13
Crude protein	88.0	1.04	89.5	1.10	0.36
Crude fat	93.8	0.81	94.1	0.84	0.80
Crude ash	83.2	0.13	86.2	0.13	0.14
Minerals, %					
Ca	77.7	0.27	81.1	0.26	0.38
P	84.9	0.17	87.9	0.16	0.23
Na	96.7	0.62	98.8	0.37	0.02
K	97.9	0.25	98.5	0.23	0.13

Table 4.4 Urinary recovery of lactulose and Cr-EDTA and serum concentrations of lactulose, D-mannitol, and Cr-EDTA (LSM) after an oral dose of the markers in calves fed the milk replacer treatments

	Treatment				
Item	**High fat (n=18)**	**SEM**	**High lactose (n=10)**	**SEM**	***P*-value**
0 to 6 h urine collection					
Cr EDTA (% of dose)	1.05	0.13	0.94	0.11	0.41
Lactulose (% of dose)	0.70	0.08	0.55	0.07	0.95
0 to 24 h urine collection					
Cr EDTA (% of dose)	4.59	0.25	3.31	0.03	<0.01
Lactulose (% of dose)	2.26	0.16	1.42	0.14	<0.01
Serum concentration					
Cr EDTA, mg/L	0.31	0.01	0.22	0.01	<0.01
Lactulose: D-Mannitol ratio	0.85	0.05	0.66	0.04	0.01

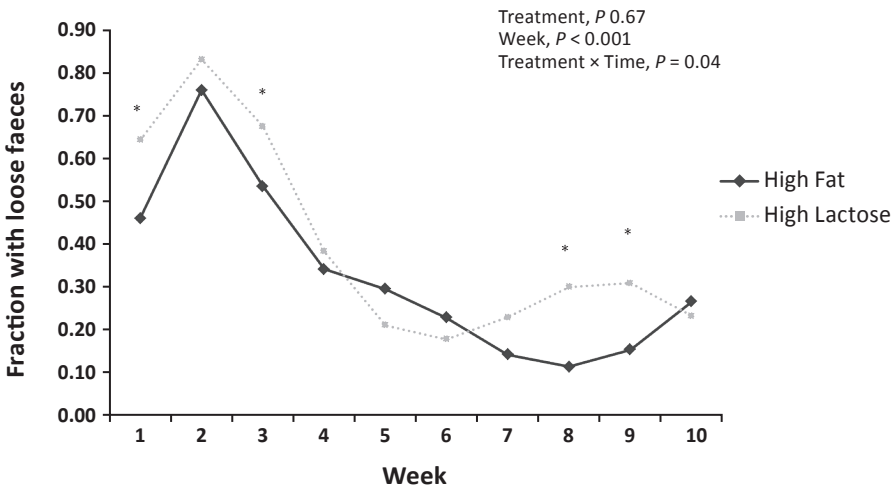

FIGURE 4.1 The effect of exchanging fat for lactose in a calf milk replacer on fraction with loose feces of 52 calves fed a high fat (solid) or a high lactose (dashed) calf milk replacer. * $P < 0.05$.

DISCUSSION

The main objective of this experiment was to study the effects of dietary energy source (fat vs. lactose) on growth performance, intestinal permeability, digestibility, and fecal consistency in rearing calves. In the current study, BW and ADG were unaffected by CMR composition, likely as a result of the equal total dietary energy and CP intakes. In the study of Tikofsky et al. (2001), different ratios of dietary fat and lactose were tested in dairy calves fed under isocaloric and isonitrogenous conditions, and rates of BW and empty BW gain were not different among treatments. Other studies have shown that increased fat and ME intake from CMR resulted in no change or reduced ADG (Bascom et al., 2007; Hill et al., 2009; Esselburn et al., 2013), although it can be expected that higher inclusion of fat in CMR would have increased fat deposition (Tikofsky et al., 2001; Bascom et al., 2007). However, a limitation of the present study is that body composition measurements were not included in the design. Because a fixed amount of CMR was provided in both treatments, all calves had a similar daily CMR intake (as DM), and for that reason the HF group consumed more fat and therefore more energy from the CMR. However, starter intake post-weaning was affected by a treatment × week interaction ($P < 0.05$), where HL calves consumed more starter in wk 6, 7, 8, and 9. Therefore, both treatments had similar total energy intake over the course of the study period. In the study of Kuehn et al. (1994), calves were fed a 20% CP CMR containing either 15.6 or 21.6% fat and a

texturized calf starter and report that the higher fat in CMR depressed starter intake before and after weaning. Preweaned calves gained more BW with the lower fat CMR, which is in contrast to the current study, where no effect was observed on preweaning gain or BW. To examine the effect of fat inclusion in CMR, Hill et al. (2009) used 48 Holstein calves fed 0.66 kg/d DM of CMR containing 14, 17, 20, or 23% fat and 27% CP. They concluded that preweaning starter intake responded quadratically to level of fat, being lowest at 14 and 23% fat in CMR. In contrast, we report here that a numerical increase in starter intake in calves fed the HL CMR was enough to sustain an equal growth rate between groups.

Despite similar intakes of CMR and starter during the week in which apparent total-tract digestibility was measured, there were numerical differences in nutrient digestibility in the HL group compared with the HF group. In the study comparing fat inclusion in CMR, Hill et al. (2009) determined apparent total-tract digestibility on d 19 to 23 and found that DM and fat digestibility decreased linearly as the concentration of fat increased in the CMR. Differences between these studies could be attributed to the fatty acid profile of the fat sources, as in our study the source of fat was palm and coconut oil, whereas the formulations reported in Hill et al. (2009) were based on lard, which has a higher SFA content.

The lactulose-d-mannitol and Cr-EDTA permeability tests were used in parallel for noninvasive monitoring of the mucosal integrity of the small intestine. These noninvasive tests are based on the ability of these molecules to permeate the intestinal mucosa. For large molecules (lactulose and Cr-EDTA), transport is paracellular and therefore takes place through the tight junctions. In contrast, smaller molecules such as mannitol are mostly absorbed transcellularly through epithelial cells by active or passive transport (Wijtten et al., 2011). Branco Pardal et al. (1995) assessed the intestinal permeability to markers, including lactulose d-mannitol and Cr-EDTA, in calves fed various milk replacers containing skim milk powder, whey, or soy protein. In this experiment, gastrointestinal permeability was assessed in 6 Holstein male calves. The authors showed that urinary lactulose recovered over a 6-h collection period was between 2.2 and 3.1% of the dose, depending on the treatment applied. In contrast, our recoveries were substantially lower (0.5 ± 0.07 for HL and 0.7% ± 0.08 for HF). This difference may be explained by the fact that calves in the study of Branco Pardal et al. (1995) were fed a diet containing antigenic components, which have been associated with impaired intestinal functions and digestive disorders (Lallès et al., 1998). Our results are in the range of those reported by Klein et al. (2007), where 24 healthy calves at 10 d of age fed WM presented a urinary lactulose recovery of 0.58% over 6 h. Changes in gastrointestinal permeability to markers occur in response to physiological, pharmaceutical, and nutritional stimuli (Branco Pardal et al., 1995;

Klein et al., 2007). Dietary fat (7% soybean oil and 23% lard) has been shown to increase small intestinal permeability to Cr-EDTA in rats (Suzuki and Hara, 2010). This increase was attributed to the suppression of tight junction protein expression (claudin-1, claudin-3, and occluding; Suzuki and Hara, 2010). Results for serum lactulose: d -mannitol ratio and serum Cr-EDTA were consistent with urine results and showed higher values in calves fed HF at 60 min (1.1 ± 0.06 and 0.9 ± 0.06, respectively; data not shown). As shown by Araujo et al. (2015), a high serum ratio (1.26 ± 0.16) is indicative of compromised gut barrier function, which may be more permeable to large molecules. However, in each of the 2 experiments, the assessment was performed on diarrheic calves and might therefore not be relevant to the current study, where healthy calves were used to determine differences in energy source on gastrointestinal permeability.

Despite there being no differences in performance or nutrient digestibility, the higher marker recovery is suggestive of an increase in gastrointestinal permeability in calves fed HF. In rodents and humans, dietary fat has been associated with decreased intestinal integrity, mainly in the context of postprandial endotoxemia (Boutagy et al., 2016). Intestine-derived LPS can be incorporated into micelles and chylomicrons (due to its insoluble component lipid A) and absorbed along lipids, transiently increasing its appearance in blood (Boutagy et al., 2016). Nevertheless, changes in intestinal integrity in adult humans have only been associated with dietary fat in the context of very high fat diets, and whether this occurs under physiological consumptions remains controversial (Vors et al., 2015). In the current study, the milk fat concentration in the HF diet was established to be similar to WM, and thus it is unlikely that dietary lipids had a major detrimental effect on gastrointestinal health. In agreement with this, none of the measured parameters (performance, nutrient digestibility, feces score, or diarrhea incidence) were negatively affected by HF. Reasons for the observed increase in marker recovery are currently not clear. We speculate that because markers were diluted with the dietary treatments, there could have been an interaction with milk fat (similar to what happens with LPS), thereby increasing their absorption. The effect of separating the marker from the diet (Klein et al., 2007) in a milk-fed preruminant model remains to be addressed.

The diet with HF had a lower frequency of abnormal fecal scores at 2 times points: in the first weeks (1–3 wk) and around the weaning period (8–9 wk). This could be associated with level of lactose, which increases the osmolality of CMR. This parameter can range from slightly hypertonic (just above 300 mOsm/kg) to highly hypertonic (>450 mOsm/kg; Wilms et al., 2019). Hypertonic solutions have been associated with delayed abomasal emptying rate (Bell and Razig, 1973; Sen et al., 2006), which was shown to increase the incidence of gastrointestinal diseases in

calves such abomasal bloat (Glenn Songer and Miskimins, 2005) and diarrhea (Foster and Smith, 2009). Moreover, lactose intakes exceeding nutritional tolerance increase the incidence of gastrointestinal dysfunction in the first 4 wk of age, referred to as "nutritional diarrhea" (Hof, 1980). In the weaning period beginning in wk 7, HF calves had fewer abnormal fecal scores than HL calves. A possible explanation was the weaning stress due to differences in ME content and subsequent CMR energy intake. Energy shortage can depress immune function and increase susceptibility to diseases in calves (Nonnecke et al., 2003).

CONCLUSIONS

A weight–weight exchange of lactose for fat does not affect calf growth and performance, total starter intake, or apparent total digestion of macronutrients. High-fat CMR was associated with an increase in permeability indicators with a corresponding improvement in fecal scores. The effect of high-fat CMR formulations on intestinal health remains to be further elucidated.

ACKNOWLEDGMENTS

The authors thank Natasja Boots, Mieke Langen-Thijssen, and the staff of the Trouw Nutrition Calf and Beef Research facility (Amersfoort, the Netherlands) for their skilled technical assistance.

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CHAPTER 5

EFFECT OF PARTIAL EXCHANGE OF LACTOSE WITH FAT IN MILK REPLACER ON PERFORMANCE AND BLOOD METABOLITES OF HOLSTEIN CALVES

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Journal of Dairy Science Communications 2023; 4:19–24

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ABSTRACT

The objective of this study was to determine the effect of dietary energy source (fat vs. carbohydrate) in calf milk replacer (**MR**) on growth performance parameters and feed intake in rearing calves. In a randomized complete block design, 68 Holstein calves [40 females and 28 males; (mean ± SD) body weight (**BW**): 43.7 ± 1.43 kg] were assigned to 17 blocks of 4 calves based on birth date and parity of the dam. Within each block, calves were randomly assigned to 1 of 2 treatments: a high-lactose MR (**HL**; 17% fat; 44% lactose; n = 34), or a high-fat MR (**HF**; 23% fat; 37% lactose; n = 34). Lactose was exchanged for fat on a weight per weight basis, resulting in a 6% difference in metabolizable energy density per kilogram of MR. The feeding plan started with 6 L/d for 7 d, then 8 L/d for 35 d, 6 L/d for 7 d, and finally, 4 L/d for 7 d. Milk replacer allowances were offered in 2 meals per day at 140 g/L. Measurements included daily MR, starter and straw intakes, weekly BW, and blood metabolites, including nonesterified fatty acids (**NEFA**) and glucose, on wk 4, 6, 8, and 10. Increasing fat at the expense of lactose did not affect MR intake or solid feed intake during the preweaning and weaning periods. However, HF calves tended to consume more solid feed than HL calves during the postweaning period (2.63 ± 0.08 vs. 2.52 ± 0.08 kg/d). Additionally, average daily gain (HF = 0.78 ± 0.02, HL = 0.77 ± 0.02 kg/d) and final BW (HF = 98.8 ± 1.53, HL = 97.7 ± 1.57 kg) were not affected by MR composition. Nevertheless, NEFA concentration was higher in HF calves than in HL calves (0.21 ± 0.01 vs. 0.17 ± 0.01 mmol/L), and glucose concentration was higher in HF calves (6.52 ± 0.23 vs. 5.86 ± 0.23 mmol/L). Under the conditions of this study, HF calves consumed similar amounts of solid feed and grew comparably to the HL calves; however, the isonitrogenous replacement of lactose by fat had evident metabolic effects, such as increased blood NEFA and glucose concentrations.

Rearing calves have traditionally been offered ~10% of birth BW by volume of whole milk (**WM**) or milk replacer (MR; ~20% CP and ~20% fat DM basis; Jasper and Weary, 2002). With the global trend in the dairy industry moving toward enhanced feeding programs (>20% of birth BW by volume), the feeding of MR higher in protein content (up to 30%, DM basis; Diaz et al., 2001) has been proposed because it can benefit body composition (i.e., Blome et al., 2003). Nevertheless, fat levels remain similar to those in traditional formulations (~20% fat DM; Raeth-Knight et al., 2009). For both situations, lactose levels usually range between 42 and 45% (DM basis; Park, 2009); however, compared with WM (33 to 38% lactose; 30 to 40% fat DM; Pantophlet et al., 2016; Berends et al., 2020), these MR formulations provide a lower dietary energy density, different fat composition in terms of fatty acid profile and triglyceride structure, and a lower energy: protein ratio. Although information is limited, it has been reported that high-fat MR (21.6% of DM; Kuehn et al., 1994), and additional milk or milk solids in the diet (Jaster et al., 1992) can depress solid feed DMI before and after weaning. Studies comparing low-fat MR (<20% fat) with WM (~30% fat), which naturally presents a higher fat content (including a lower lactose content than MR), reported better growth rates (Moallem et al., 2010) and enhanced structural development (Esselburn et al., 2013) when calves were fed WM.

Researchers have been investigating the effects of altering the macronutrient compositions of MR formulations, especially lactose and fat, in an attempt to formulate MR that more closely resembles WM (Amado et al., 2019; Echeverry-Munera et al., 2021; Welboren et al., 2021). Interestingly, more recent studies have reported no differences on BW and ADG of calves fed restricted (Amado et al., 2019) or *ad libitum* (Berends et al., 2020; Echeverry-Munera et al., 2021) high-fat MR (23% fat DM; vegetable oil) compared with diets containing lower dietary fat levels (17% fat DM; vegetable oil). Notwithstanding, it has been demonstrated that partially replacing lactose with fat (vegetable oil) in MR formulations can increase BW gain as well as gain:ME intake during the first 7 d of life (Welboren et al., 2021). It has also been suggested that greater dietary fat inclusion could be beneficial for glucose homeostasis, because smaller fluctuations in postprandial glucose and insulin concentrations have been observed (Welboren et al., 2021). Thus, under study conditions and based on previous studies where high-fat diets were offered, we hypothesized that a high-fat diet (1:1 wt/wt lactose by fat exchange) would decrease solid feed consumption; however, calf growth is not expected to be affected by the partial exchange of lactose for fat. The objective of this study was to evaluate the effects of a high-fat MR and a high-lactose MR on growth performance, feed intake, and blood metabolites in rearing calves under isonitrogenous conditions.

This study was conducted at Trouw Nutrition Ruminant Research Facility (Boxmeer, the Netherlands). All experimental procedures and animal care were conducted in accordance with animal welfare legislation and were approved by the animal experimentation committee (DEC Dierexperimentencommissie, Utrecht, approval #2013.III.11.120). A classical power analysis was conducted to determine the number of experimental units needed. The power (1 − β) was chosen to be equal to 80%, and the α-level was 0.05. Solid feed intake was considered the most reliable parameter to determine the power of this study, based on the outcome of a previous study conducted by Echeverry-Munera et al. (2021) with 32 calves allocated to 2 treatments and fed *ad libitum* milk allowances. At 84 d of age, a standard deviation of 0.130 kg/d was assumed for solid feed intake. The minimal meaningful difference in solid feed intake was 0.190 kg/d. Therefore, the minimal sample size to detect differences at this assumption was calculated to be 34 calves per treatment group when accounting for maximum mortality of 10%.

A total of 68 Holstein-Friesian calves (40 females and 28 males) born at the research facility, with a mean initial BW of 44.1 ± 4.3 kg (mean ± SD) and apparently healthy (no respiratory, heart, skin irregularities, or blindness) were used in this study. Calves were immediately separated from their dams after birth and housed in individual hutches (≥2.5 m2) composed of 50% outdoor area and 50% indoor with straw bedding until 70 d of age. Calves received a total of 4 L of pasteurized colostrum (previously frozen and thawed) with a reading of 22% Brix or greater in 2 separate meals. The first colostrum meal (2 L) was given within 1 h of birth, and the second meal (2 L) was given 6 h after birth. Passive immunity was evaluated between 48 and 72 h after birth using the portable Multi-Test Analyzer (DVM Rapid Test II-Multi-Test Analyzer). Calves were blocked based on birth date and parity of the dam (17 blocks, 4 calves of the same sex/block). Within each block, calves were randomly assigned to 1 of 2 treatments: a high-fat (HF; 23% fat, 37% lactose, 23.5% CP) or a high-lactose (HL; 17% fat, 44% lactose, 23.2% CP) milk replacer. The HF treatment was designed to have similar fat content to WM, and the HL treatment was formulated to resemble commonly available high-lactose MR formulations. Fat and lactose were partially exchanged on a weight per weight (wt/wt) basis, based on spray-dried fat kernels (Trouw Nutrition), with the fat being 35% coconut oil and 65% palm oil. The rest of the MR formulation components remained unchanged and have been previously described by Echeverry-Munera et al. (2021). Because of the weight per weight exchange of lactose for fat, the experimental diets were isonitrogenous but not isoenergetic (HF = 4.7 and HL = 4.4 Mcal/kg of DM), therefore; the CP:ME ratio of the 2 MR was different: 50 versus 53 g of CP/Mcal of ME for HF and HL, respectively.

Calves were fed a fixed allowance of the designated treatment in a teat bucket following a step-up and step-down protocol. Therefore, from d 2 to 7, calves were fed 3 L of MR twice daily; from d 8 to 42, 4 L twice daily; from d 42 to 49, 3 L twice daily; from d 49 to 56, 2 L twice daily; and on d 56 the MR supply was finished. Milk replacer was reconstituted at 140 g of MR/L and offered at 0700 and 1600 h. Calves had constant *ad libitum* access to water, and fresh calf starter (analyzed composition DM basis: 87.4% DM, 20.4% CP, 24.1% starch, 3.8% fat; ForFarmers B.V.) and dry chopped straw (analyzed composition DM basis: 93.6% DM, 5.4% CP, 1.1% crude fat, 6.2% crude ash, 40.8% fiber; 3- to 7-cm chop length; Ruwvoer Distributiecentrum) were offered daily in separate buckets from d 4 after birth. Calves were weighed with a custom scale (W2000; Welvaarts Weegsystemen) and body measures (wither height, hip height, body barrel, and chest girth) were taken on the day of birth and then once per week. Individual intakes of MR, water, concentrate, and straw were recorded daily by weighing the leftovers.

To evaluate blood metabolites, blood samples were obtained from the jugular vein of a convenience sample of 28 heifer calves (14 calves/treatment). Blood samples were taken in wk 4, 6, 8, and 10 at 1000 h (3 to 4 h after first meal) into one 10-mL EDTA tube for serum, and one 6-mL sodium fluoride (NaF) evacuated tube containing a glycolysis inhibitor for plasma glucose (BD Vacutainer). The EDTA tubes were kept at room temperature for 15 min, and all tubes were centrifuged at 1,500 × g for 15 min at 20°C. Samples were stored in 2-mL cryotubes (2 per sample) and stored immediately at −20°C. General calf health was monitored by caretakers daily, and a standard health protocol was followed. All study personnel involved in calf handling and taking measurements were blinded to the treatments.

Blood samples were analyzed at GD Animal Health (Gezondheidsdienst voor Dieren). Serum nonesterified fatty acids (NEFA), urea, and cholesterol were analyzed using enzymatic methods. Plasma glucose was determined with an enzymatic method based on hexokinase. Colorimetric methods were used to analyze total bilirubin (dimethyl sulfoxide method), haptoglobin, and albumin (bromocresol green method). Aspartate aminotransferase and gamma-glutamyl transferase were analyzed using enzymatic methods according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Plasma glutamate dehydrogenase was analyzed using an enzymatic method (Deutsche Gesellschaft für Klinische Chemie method).

One bull calf from the HL treatment was excluded from the study due to severe metabolic acidosis and dehydration associated with diarrhea. Data collected from this animal before removal were excluded from the analysis. Continuous variables (i.e., intakes, ADG, feed efficiency, and blood parameters) were analyzed using mixed-model analysis with PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc.). The model included the fixed effects of block, treatment, time, and the interaction between treatment and time. The experimental unit was the calf and time (week) was used as a repeated measure. The autoregressive covariance structure was applied as time points were equally spaced. Initial body weight was used in the model as a covariate for ADG and feed efficiency. Blood parameters and intakes were analyzed without initial BW as a covariate due to a lack of significance of this factor. For BW, the initial and the last measurement (d 70) were used. Body weight data were then analyzed with PROC MIXED with MR treatment as a fixed effect, initial BW as a covariate, and the fixed effect of block. Treatment averages were presented as least squares means (LSM) and standard errors of the mean (SEM). Statistical significance was declared at $P \leq 0.05$, and trends toward statistical significance were noted when $0.05 < P < 0.10$.

As a starting point, no differences were observed in plasma IgG (HL = 16.3 ± 1.65 g of IgG/L; HF = 16.0 ± 1.69 g of IgG/L; P = 0.62). Initial BW did not differ between the 2 treatments (HF = 44.5 ± 0.78 kg; HL = 43.4 ± 0.80 kg; P = 0.33). Final BW was comparable between the 2 treatments, with HF calves being 1.1 kg heavier than HL calves (P = 0.62; Table 5.1). Similarly, ADG was not affected by dietary treatment ($P > 0.05$; Table 5.1). Feed intake data are presented in Table 5.1. Milk replacer intake did not differ between the 2 groups during the preweaning (P = 0.68) and weaning (P = 0.11) periods. Similarly, solid feed intake during the preweaning (P = 0.27) and weaning (P = 0.84) periods was not affected by dietary treatment. However, during the postweaning period, HF calves tended to consume more starter than HL calves (P = 0.08). Additionally, by design, dietary treatments influenced ME intake, with HF consuming more ME than HL calves during the preweaning (P = 0.003) and weaning (P = 0.05) periods. No differences were detected in straw intake during the study ($P >$ 0.05). Nevertheless, feed conversion was comparable between treatments during the whole study ($P > 0.05$).

Blood data from 28 heifer calves (14/treatment) are summarized in Table 5.2. Treatment (P = 0.02) and time (P = 0.03) effects were observed for NEFA concentration, with greater concentrations in HF than HL calves during wk 6 and 8 ($P < 0.05$; Figure 5.1A). Similarly, treatment (P = 0.04) and time (P = 0.002) effects were detected for glucose concentration, with greater concentrations in HF than HL calves during wk 6 and 8 ($P < 0.05$; Figure 5.1B). No differences ($P > 0.05$) were observed in any of the other blood parameters analyzed.

Table 5.1 Growth performance, intakes, and feed efficiency (LSM ± SEM) for Holstein calves (n = 67) fed milk replacers (MR) differing in dietary energy source during the preweaning (d 1 to 42), weaning (d 43 to 56), and postweaning (d 57 to 70) phases

	Treatment[1]				*P – values*[2]		
Item	***HF***	***SEM***	***HL***	***SEM***	***TRT***	***Time***	***TRT × Time***
Growth performance							
Final BW, kg	98.8	1.53	97.7	1.57	0.62	-	-
ADG (kg/d)	0.78	0.02	0.77	0.02	0.62	<0.001	0.55
Intake							
MR, kg/d DM							
Pre-weaning	1.01	0.001	1.01	0.01	0.68	<0.001	0.44
Weaning	0.68	0.001	0.67	0.002	0.11	<0.001	0.24
Starter, kg/d							
Pre-weaning	0.13	0.02	0.12	0.02	0.58	<0.001	0.53
Weaning	0.98	0.05	0.88	0.06	0.19	<0.001	0.32
Post-weaning	2.63	0.08	2.52	0.08	0.32	0.01	0.08
ME, Mcal/d							
Pre-weaning	5.07	0.05	4.85	0.05	0.03	<0.001	0.26
Weaning	5.95	0.16	5.51	0.16	0.05	<0.001	0.61
Post-weaning	7.48	0.23	7.15	0.23	0.32	0.02	0.08
Straw, kg/d							
Pre-weaning	0.02	0.003	0.01	0.003	0.54	<0.01	0.70
Weaning	0.08	0.01	0.08	0.01	0.90	0.005	0.51
Post-weaning	0.11	0.02	0.10	0.02	0.83	0.07	0.10
Mcal/kg gained							
Pre-weaning	8.11	0.26	7.83	0.26	0.45	<0.01	0.13
Weaning	9.51	0.48	8.66	0.50	0.23	0.55	0.68
Post-weaning	7.13	0.32	7.34	0.33	0.65	0.67	0.55

[1]Treatments included a high-fat MR (HF; 23% fat, 37% lactose, and 23.5% CP; n = 34) and a high-lactose MR (HL; 17% fat, 44% lactose, and 23.2% CP; n = 33). [2]TRT = treatment effect; T = time effect (week); TRT × T = treatment by time interaction.

Table 5.2 Effect of calf milk replacer (MR) composition on selected blood metabolites (LSM ± SEM) in dairy Heifer calves (n = 28) on wk 4, 6, 8, and 10 at 1000 h

	Treatment[1]			*P*-value[2]		
Item	**HF**	**HL**	**SEM**	**TRT**	**Time**	**TRT × Time**
Non-esterified fatty acids, mmol/L	0.21	0.17	0.01	0.02	0.03	0.73
Urea, mmol/L	2.90	2.82	0.12	0.65	<0.001	0.22
Glucose, mmol/L	6.52	5.86	0.23	0.04	0.002	0.37
Cholesterol, mmol/L	2.94	2.87	0.13	0.68	<0.001	0.19
Albumin, g/L	31.4	31.6	0.50	0.71	<0.001	0.92
Haptoglobin, g/L	0.07	0.06	0.01	0.52	0.18	0.74
Total Bilirubin, µmol/L	1.80	1.75	0.06	0.52	<0.001	0.46
Glutamate dehydrogenase, UI/L	45.0	51.6	6.73	0.49	<0.001	0.08
Gamma-glutamyl transferase, UI/L	12.6	12.3	1.55	0.91	<0.001	0.63
Aspartate aminotransferase, UI/L	44.7	46.1	1.30	0.46	<0.001	0.38

[1]Treatments included a high-fat MR (23% fat, 37% lactose; n = 14; HF) and a high-lactose MR (17% fat, 44% lactose; n = 14; HL). [2]TRT = treatment effect; T = time effect (week); TRT × T = treatment by time interaction.

The objective of this study was to evaluate the effects of a high-fat and a high-lactose MR on growth performance, feed intake, and blood metabolites in rearing calves. By design, the dietary treatments in this study provided different amounts of energy due to the partial replacement of lactose by fat (HF = 4.7 and HL = 4.4 Mcal/kg of DM). Despite the macronutrient composition of the MR, calves consumed similar amounts during the preweaning and weaning periods. In a previous study, similar dietary treatments were fed *ad libitum*, and a 12% (150 g MR/d) reduction in the intake of high-fat MR was observed during the preweaning period (Echeverry-Munera et al., 2021). Therefore, the lack of difference in MR intake in the current study might be attributed to the restrictive nature of the feeding program implemented. Despite the similar intakes, HF calves consumed, on average, more total ME during the preweaning and weaning periods. This difference was a direct consequence of differences in the caloric density of the diets. Although it has been reported that energy intake regulates the consumption of MR in *ad libitum*-fed calves (Berends et al., 2020), in the current study, this might not be the case due to the restrictive feeding program implemented. After the initial weeks of life, preweaning starter and liquid feed intake are inversely correlated (Gelsinger et al., 2016). It has been well documented that higher feeding rates of WM or MR might decrease solid feed intake and have adverse effects on rumen development and, consequently, postweaning performance (i.e., Jasper and Weary, 2002). In addition, feeding high-fat diets can have a negative effect on feed

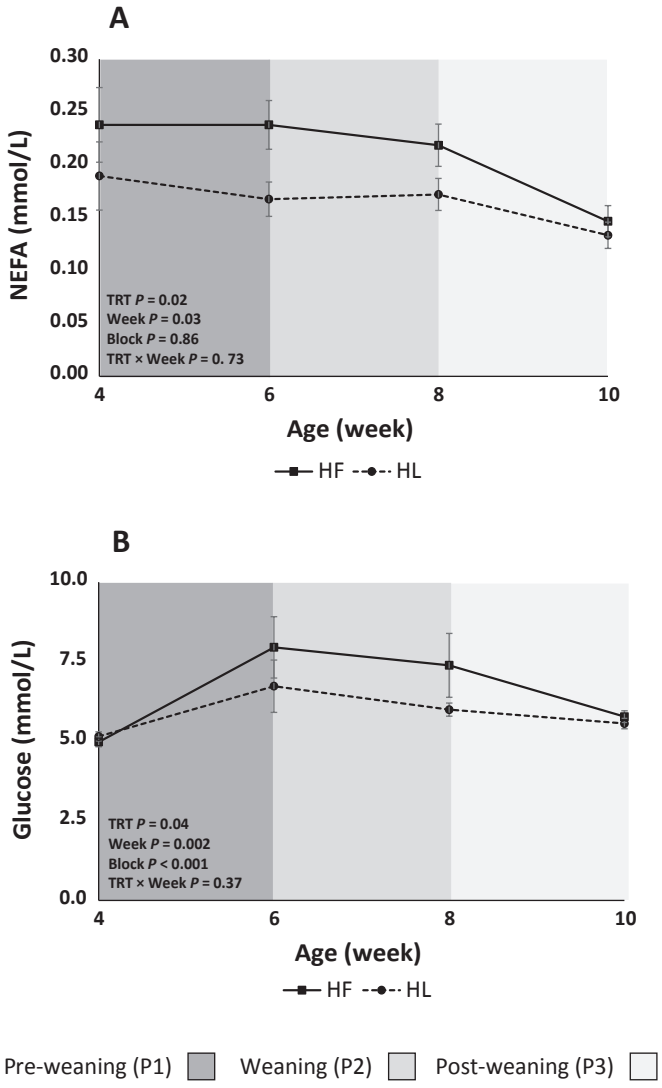

Figure 5.1 Plasma concentrations of (A) nonesterified fatty acids (NEFA), and (B) glucose measured in heifer calves (n = 28) fed restricted (8 L/d) levels of a high-fat milk replacer (■, HF; 23% fat, 37% lactose, and 23.5% CP; n = 14) or a high-lactose milk replacer (●, HL; 17% fat, 44% lactose, and 23.2% CP; n = 14). Samples were taken on wk 4, 6, 8, and 10 of the study. Treatment (TRT) × time differences at each time point are indicated by *($P \leq 0.05$). Standard errors were computed on raw data to better illustrate the observed difference in variability between treatments.

intake by exceeding the metabolic capacity of the animal and generating satiety signals (Palmquist, 1994; Choi et al., 1996). Therefore, when implementing enhanced feeding programs, it is important to have an appropriate weaning protocol that allows the animals to maintain the BW gained (Khan et al., 2007). Nevertheless, the lack of difference in starter intake during the preweaning and weaning periods between treatments in the current study was in line with earlier findings (Hill et al., 2008) and suggests that solid feed intake in the presence of enhanced WM or MR is potentially regulated by the rate of rumen development (Meale et al., 2017).

During the postweaning period of the current study, calves in the HF treatment consumed, on average, 110 g/d more starter and greater ME than the HL group, resulting in 1.1 kg greater final BW. These results suggest that calves can grow well under the provision of greater dietary fat levels. Beyond the effects of greater dietary fat inclusion on feed intake and growth, it has been suggested that feeding additional fat usually increases NEFA concentrations in cattle (Choi and Palmquist, 1995). Although the liquid diets in this study were isonitrogenous, they were not isocaloric. Therefore, markers for fat metabolism (e.g., NEFA) were expected to be affected by diet, and thus, were higher during the milk phase (up to wk 8) in heifer calves consuming the high-fat milk replacer. Although Bascom et al. (2007) attributed the elevated NEFA concentrations in calves fed a high-fat MR (33% vs. 16% fat) to the fat source in the diet (edible lard as opposed to milk fat), in the current study, the increased NEFA concentrations in HF calves seemed to be a direct result of the greater inclusion of fat in the diet. These results agree with more recent studies where similar compositions were fed *ad libitum* (Berends et al., 2020; Echeverry-Munera et al., 2021).

Plasma glucose concentration in heifer calves was also affected by dietary treatment. During the preweaning period, glucose is obtained primarily from the consumption of MR and digestion of lactose. However, during the weaning and postweaning periods, the liver nutrient supply starts to change from glucose to short chain fatty acids from ruminal fermentation in the ruminating calf (Suarez-Mena et al., 2017); therefore, plasma glucose concentration is expected to decrease due to shifts in the hepatic metabolic activity of the calf from glycolytic to gluconeogenic. Although HL calves were expected to have greater glucose concentrations due to the nature of the diet, calves consuming the HF treatment showed higher blood glucose levels. Nevertheless, in the current study, blood samples were taken from the heifer calves after the first month of life (at wk 4), which differs from other studies (Welboren et al., 2021) that have looked at the effect of MR composition earlier in life. Although sampling was performed 3 to 4 h after MR feeding on average, calves in the current study still did not experience hyperglycemia (blood glucose concentrations >8.3 mmol/L; Hostettler-Allen et al., 1994). While differences were detected for some blood metabolites

(i.e., NEFA and glucose), there is the possibility of a type I error because metabolic indicators were measured using a convenience sample size, rather than a calculated formal sample size.

Increasing fat content at the expense of lactose resulted in an increase of 6% in energy density of the HF treatment; as a consequence, ME intake was different throughout the study. Regardless, under the experimental conditions, dietary composition did not affect MR acceptance or starter intake. Growth rate and BW were similar despite energy intake differences, but we did not evaluate body composition. Balancing the lactose-to-fat ratio of MR toward that of WM by increasing fat and reducing lactose could have metabolic effects, as shown by greater NEFA and blood glucose concentrations in HF calves.

NOTES: The authors thank Natasja Boots and the personnel from Calf and Beef Research Facility, Trouw Nutrition, for their technical assistance. Additionally, the authors thank the Canadian Dairy Commission (CDC; Ottawa, ON, Canada), the Natural Science and Engineering Research Council of Canada (NSERC, Ottawa, ON, Canada), and Alberta Milk (Edmonton, AB, Canada) for their funding. The present study was funded and several of the authors are employed by Trouw Nutrition (Amersfoort, the Netherlands), a company with commercial interests in milk replacers for calves. Trouw Nutrition R&D is committed to high standards of research integrity and adheres to the principles of the European Code of Conduct for Research Integrity (Drenth, 2011). The authors have not stated any other conflicts of interest.

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CHAPTER 6

EFFECTS OF MIXING A HIGH-FAT EXTRUDED PELLET WITH A DAIRY CALF STARTER ON PERFORMANCE, FEED INTAKE, AND DIGESTIBILITY

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Journal of Dairy Science 2022-22065

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ABSTRACT

During weaning, withdrawal of milk replacer is not directly compensated for by an increase in solid feed intake. Therefore, greater fat inclusion in the starter might mitigate this temporary dietary energy decline. However, fat inclusion in solid feeds may generally limit rumen fermentability and development. To address these potentially conflicting outcomes, we conducted 2 experiments to evaluate the effect of supplementing a high-fat extruded pellet mixed with a calf starter on feed intake, performance, and nutrient digestibility in calves. In experiment 1, 60 Holstein bull calves were blocked by serum IgG (2,449 ± 176 mg/dL) and date of arrival (2.5 ± 0.5 d of age). Within each block, calves were randomly assigned to 1 of 3 treatments: a standard control calf starter (**CON**; 3.1% fat) and mixtures of CON with 10% inclusion of 1 of 2 different high-fat extruded pellets containing 85% of either hydrogenated free palm fatty acids (**PFA**, 7.1% fat) or hydrogenated rapeseed triglycerides (**RFT**, 6.7% fat). Calves were offered milk replacer up to 920 g/d until 42 d of age, followed by a gradual weaning period of 7 d. Calves had *ad libitum* access to the starter diets, straw, and water. No differences were observed between CON, PFA, and RFT calves on body weight (**BW**) or average daily gain (**ADG**) until 49 d of age. From weaning (50 d) until 112 d, PFA calves had a greater BW and ADG than RFT and CON animals. Moreover, PFA calves had the highest intakes of starter, straw, calculated metabolizable energy, and crude protein after weaning. Overall, no differences were present in blood β-hydroxybutyrate and glucose concentrations between treatments; however, calves in the RFT treatment had a higher concentration of insulin-like growth factor-1. In experiment 2, 24 Holstein bull calves at 3 mo of age were assigned to 1 of 8 blocks based on arrival BW and age. Within each block, calves were randomly assigned to 1 of the 3 treatments previously described for experiment 1. Calves on the RFT treatment had the lowest total-tract apparent dry matter and fat digestibility, potentially explaining the differences in performance observed between PFA and RFT calves. Inclusion of the PFA pellet at 10% with a calf starter improved BW, solid feed, and energy intake after weaning. However, these benefits were conditioned by fat source and its digestibility.

Key words: calf weaning, fat inclusion, solid feed

INTRODUCTION

Over the last decade, the industry has been progressively changing to higher liquid feed allowances (~20% of birth BW by volume; Khan et al., 2010). This feeding strategy has been reported to increase growth rate, decrease age at first calving, and increase first-lactation milk yield (Hill et al., 2006; Soberon et al., 2012; Leal et al., 2021). However, greater preweaning milk allowances have been associated with reduced preweaning solid feed intake and delayed rumen development (Khan et al., 2007; Terré et al., 2007; Weary et al., 2008), making weaning more challenging and affecting calf growth and development around the weaning period (Cowles et al., 2006; Hill et al., 2010; Davis Rincker et al., 2011).

Weaning is stressful for calves due to factors such as rapid microbial changes and gastrointestinal structural adaptations (Meale et al., 2017); therefore, the method of weaning can influence calf performance and gastrointestinal development. The immature state of the rumen to ferment solid feeds, combined with the transition from a high-energy-density diet, such as whole milk (~5.4 Mcal of ME/kg of DM; Drackley, 2008) or milk replacer (MR; ~4.5 Mcal of ME/kg of DM) to a relatively low-energy-density solid diet (~2.48 Mcal/kg of DM) can result in lesser growth performance around this period, which is also known as the weaning gap (Leal et al., 2021). It has been suggested that total ME intake can decrease by as much as 39% due to a decline in DMI from MR, which is not compensated for by an immediate increase in starter consumption (Leal et al., 2021). Nondairy calves combine solid intakes with milk, resulting in a gradual exchange between these 2 nutrient sources, thus gradually reducing their intake of milk fat. When given the choice, this transition from milk to solid feed can take over 6 mo (Webb et al., 2014). In contrast, traditional rearing programs for dairy calves abruptly reduce their fat intake at weaning. Therefore, nutrient intake depends on total feed intake and diet composition. The above factors suggest that the relatively low energy intake during the weaning and early postweaning periods could be mitigated by increasing the dietary energy density of the starter feed through fat, as previously demonstrated by Araujo et al. (2014) and Berends et al. (2018). The opportunities and limitations of inclusion of rumen-stable fat to increase energy density in dairy and feedlot diets are well established (Fluharty and Loerch, 1997), but information regarding rumen-inert fat inclusion in calf starters is limited. Gardner and Wallentine (1972) reported growth responses by veal calves to the addition of tallow to starter rations. Waldern and Fisher (1978) found no effect on feed conversion ratios with unprotected tallow in calf diets, whereas Fisher (1980) reported an increase in weight gain for calves receiving diets containing 20% versus 10% (of DM) of a protected lipid source. Berends et al. (2018) reported greater performance in calves fed a mixture of a traditional low-fat pellet (90%) with an

extruded high-fat pellet containing hydrogenated fatty acids (**FA**) from palm oil (10%), compared with the low-fat control pellet. Despite the benefits reported in the previous study, palm oil use in animal nutrition is questioned because of issues related to environmental sustainability, which has led to animal feed manufacturers wanting to reduce its inclusion in feeds. To evaluate alternative sources of fat to increase raw material flexibility in feed production, in this study, we evaluated a 90:10 mixture of a calf starter and a high-fat extruded pellet containing either hydrogenated FA from palm oil, as described in Berends et al. (2018), or an alternative formulated with hydrogenated triglycerides (**TG**) from rapeseed oil. Although most commercially available fat supplements for ruminants are based on hydrogenated free fatty acids (**FFA**), hydrogenated TG are also available. However, TG are generally considered less digestible (Elliott et al., 1999; Weiss and Wyatt, 2004). Esterification and FA profile of fat supplements are important factors affecting FA digestibility. Based on differences in melting point, FA profile, and chemical structure, a C16: 0 -rich hydrogenated FFA product could be expected to have higher digestibility than a C:18-rich hydrogenated TG (Doppenberg and van der Aar, 2017). Yet, the magnitude and relevance of this difference is difficult to predict for such a specific and novel application of hydrogenated fats.

The objective of this study was to evaluate the effects of including a high-fat extruded pellet in a calf starter on performance and feed intake in rearing calves. This study also aimed to evaluate the effects of hydrogenated fat sources differing in esterification and FA profile (palm and rapeseed origin) on feed intake and digestibility. In experiment 1, we hypothesized that increasing dietary fat in calf starter feed could mitigate growth loss during weaning and the postweaning period. In experiment 2, we aimed to measure differences in digestibility between the 2 sources of hydrogenated fats and evaluate their eventual relevance on energy supply and growth in postweaning calves.

MATERIALS AND METHODS

This study was conducted in 2 experiments, the first between October 2017 and January 2018 and the second between June 2020 and August 2020 at the Calf & Beef Research Facility of Trouw Nutrition Research and Development (Sint Anthonis, the Netherlands). All procedures described in this article complied with the Dutch Law on Experimental Animals, which complies with ETS123 (Council of Europe 1985 and the 86/609/ EEC Directive) and were approved by the animal welfare authority (DEC Utrecht, the Netherlands).

Experiment 1

Animals and Experimental Design. A total of 60 Holstein bull calves (45.7 ± 0.87 kg of BW, 2.5 ± 0.5 d of age) were collected from local dairy farms at a maximum distance of 14 km from the calf research facility. At the farm of origin, a standardized protocol for colostrum management was followed, wherein calves were fed 3 L of colostrum within the first 3 h after birth followed by 2 feedings of 2 L for a total of 3 feedings of colostrum in the first 24 h of life. Upon arrival at the facility, successful application of this protocol was monitored by assessment of blood IgG within 48 to 72 h after birth of the calf. Calves were assigned to 1 of 20 blocks (3 calves per block) based on IgG category (low: 1,000–2,000 mg/dL; high: >2,000 mg/ dL; NAHMS, 2007), day of arrival, and BW. Within each block, calves were randomized using the RAND function in Excel (Microsoft Corp.), to 1 of 3 solid feed treatments: (1) calf starter control pellet (**CON**, 3.1% fat); (2) control pellet mixed with an extruded pellet containing hydrogenated palm free fatty acids (**PFA**, 7.1% fat in pellet mix; Bewi-Spray 99FA, Bewital agri GmbH & Co); and (3) control pellet mixed with an extruded pellet containing hydrogenated rapeseed tri glycerides (**RFT**, 6.7% fat in pellet mix; Bewi-Spray RS 70, Bewital agri GmbH & Co). Mean serum IgG concentration was 2.429 ± 176 mg/dL for CON, 2.420 ± 179 mg/dL for PFA, and 2.498 ± 176 mg/dL for RFT.

Housing and Feeding. Calves were housed indoors in individual pens (1.22 × 2.13 m) separated by galvanized bar fences and equipped with 50% rubber-slatted floors in the front and 50% lying area, including a mattress covered with flax straw in the back until weaning (wk 7); thereafter, calves were moved to a pen that was twice the initial size (2.44 × 4.26 m) with the same characteristics until the end of the study period (wk 12). From arrival until 14 d of age, calves were fed a total of 6 L of MR per day (Sprayfo Excellent, Trouw Nutrition) containing 22.9% CP and 18.4% fat (on a DM basis) provided in 2 equally sized meals of 3 L each at 0700 and 1700 h. Meal size was then increased to 3.5 L from 15 d until 42 d of age, after which milk meal size was decreased to 2.0 L until 49 d, when calves were fully weaned. Milk replacer was reconstituted with water at 150 g/L of MR powder and supplied in a teat bucket at 40°C. Calves had *ad libitum* access to water, starter feed pellets, and chopped wheat straw (4.2% CP, 71.8% NDF, and 50.6% ADF on a DM basis; 3–7 cm chop length), all provided in separate buckets.

Experimental Diets. The CON treatment was designed to be representative of commonly used calf starter pellets. Experimental starter feeds were composed of a mixture of 90% CON pellets and 10% either PFA or RFT pellets. The 90:10 mixing ratio resulted in diets with fat concentrations of 7.1 and 6.7% for PFA and RFT, respectively. Table 6.1 describes the composition of the 3 pelleted feeds, and Table 6.2 shows the nutrient composition of the final starter feeds. The feed manufacturing processes

followed those described by Berends et al. (2018). Although the treatments could be identified by visual assessment of the feeds, treatments were randomly coded and treatment identity was blinded at the research farm.

Measurements and Chemical Analysis. Milk replacer was sampled (200 g per MR) for analysis immediately after production in the factory. Starter feed (200 g) and straw (100 g) were sampled upon arrival on the farm. Individual intakes of MR, water, concentrate, and straw were weighed and recorded daily during the entire experiment. Feed refusals were collected daily and sampled 3 times per week to monitor for potential feed sorting. The feeds and pooled refusals were analyzed for DM content by drying to a constant weight in a 103°C stove for 4h (EC 152/2009; EC, 2009). Crude protein was analyzed by combustion, according to the Dumas method (Etheridge et al., 1998). Crude fat content was determined after acid hydrolysis according to ISO Standard 6496 (ISO, 1999). Starch content was determined enzymatically as described by Rijnen et al. (2001). Crude ash content was determined by incineration in a muffle furnace by combustion at 550°C (EC, 1971; 71/250/EEG 1971). Body weight and body measurements [hip height, chest girth, withers height, and body barrel (circumference around the abdominal area of the animal)] were measured on the day of arrival and weekly thereafter. Blood samples were obtained at 4, 6, 7, 8, 10, and 16 wk at 1300 h. Blood samples were taken into one 10-mL EDTA tube for serum, and one 6-mL sodium fluoride (NaF) evacuated tube containing a glycolysis inhibitor for plasma glucose (Becton Dickinson). All blood samples were centrifuged immediately at 2,800 × g for 30 min at room temperature, and 1.5 mL of plasma was pipetted into 2-mL cryotubes and stored immediately at −20°C. Blood samples were analyzed for BHB, glucose, and IGF-1 at Synlab.vet (Leipzig, Germany). Plasma concentrations of BHB were determined using a kinetic UV test, glucose was determined using the enzymatic UV test-hexokinase method, and IGF-1 by RIA. Health was monitored daily by caretakers, and a standard veterinary protocol was followed in case of disease. A veterinarian was consulted if the symptoms were not described in the standard protocol. Photos of feces were taken daily for all calves, and fecal scores were evaluated at the end of the study by one single observer blinded to treatments. The scoring system included 4 categories, where 0 was considered normal and 3 extremely loose. Fecal scores ≥2 were considered abnormal. Medical treatments were recorded daily.

Table 6.1 Formulated ingredients and analyzed nutrient composition of the 3 pelleted solid feeds used to compose the 3 solid feed treatments

	Pellet feed		
Item	**Calf Starter**	**Extruded hydrogenated rapeseed triglyceride**	**Extruded hydrogenated palm free fatty acid**
Ingredient, % of DM			
Soya bean meal	25.0	-	-
Corn	21.0	21.3	21.3
Wheat	-	38.7	38.7
Hydrogenated palm fatty acids	-	-	40
Hydrogenated rapeseed fatty acids	-	40	-
Soya hulls	15.0	-	-
Alfalfa	10.5	-	-
Beet pulp	10	-	-
Barley	10	-	-
Sugar cane molasses	5	-	-
Limestone	1	-	-
Monocalcium phosphate	1	-	-
Mineral and vitamin premix[1]	1.5	-	-
Nutrient composition, DM basis			
CP, %	19.3	5.8	5.5
Ether extract, %	3.1	39.5	43.1
Ash, %	7.4	1.2	1.2
NDF, %	18.8	6.3	6.3
ADF, %	11.4	1.8	1.6
Starch, %	21.0	37.6	43.7
ME, Mcal/kg[2]	2.47	5.07	5.43
Part of fatty acids (%)			
Free fatty acids (FFA)	-	15	85
≤C14:0	-	1	1
C16:0 (palmitic acid)	-	4	44
C18:0 (stearic acid)	-	92	54
≥C20:0	-	3	1
Melting point °C		70	58

[1]Premix (provided per kg of concentrate): 24,000 IU/kg vitamin A, 5,000 IU/kg vitamin D3, 90 mg/kg vitamin E, 1.5 mg/kg Co ($CoCO_3$), 377 mg/kg Fe ($FeSO_4$), 88 mg/kg Mn (MnO), 87.7 mg/kg Zn (ZnO), 31.8 mg/kg Cu ($CuSO_4$), and 0.30 mg/kg Se (NaSe). [2]Calculated following NRC (2001).

Table 6.2 Analyzed and estimated nutrient composition of starter feed treatments used in the study

	Starter treatment[1]		
Item	**CON**	**RFT**	**PFA**
Ingredient, % of total starter feed			
Calf starter	100	90	90
High-fat extruded pellet	-	10	10
Nutrient, DM basis			
CP, %	19.3	18.0	17.9
Ether extract, %	3.1	6.7	7.1
Starch, %	21.0	22.7	23.3
Ash, %	7.4	6.8	6.8
GE, Mcal/Kg of DM[2]	3.93	4.04	4.21
ME, Mcal/kg[3]	2.61	2.51	2.90

[1]CON = calf starter; RFT = control pellet mixed with an extruded hydrogenated rapeseed triglyceride; PFA = control pellet mixed with an extruded hydrogenated palm free fatty acid. [2]Gross energy = CP content (g/kg) × 0.0057 Mcal/g + crude fat content (g/kg) × 0.0095 Mcal/g + (starch + sugars + fiber) × 0.0042 Mcal/g. [3]Calculated following the Quigley et al. (2019) formula for ME with the calculated digestible energy coefficient as determined in experiment 2.

Experiment 2

Animal and Experimental Design. A total of 24 weaned Holstein bull calves at 3 mo of age (different calves from those in experiment 1) were used in this study. Calves were assigned to 1 of 8 blocks based on arrival BW and age. Within each block, calves were randomly assigned to 1 of the 3 treatments described in experiment 1 (n = 8 calves/treatment). Similar to experiment 1, animals had *ad libitum* access to water, straw, and treatment feed.

Housing. During the adaptation period (14 d) to the experimental diets, calves were housed in individual pens (5.4 m2). Pens were equipped with 50% rubber slatted and 50% mattress covered with flax. After the adaptation period, calves were moved into metabolic cages with rubber-slatted floors, where they stayed for a 72-h total collection period. In the metabolic cages, calves were tethered to the front of the pen with enough space to lie down. Immediately after the end of the 72-h collection period, calves were moved back to their individual pens.

Measurements and Chemical Analysis. Individual intakes of concentrate, water, and straw were weighed and recorded daily during the entire experiment. Feces were collected for a total of 72 h using fecal bags glued to the hindquarters of the animal.

Once the fecal bags were full, the bags were removed, weighed, frozen, and then replaced with a new bag. After 3 d of total collection, feces were thawed and homogenized. Two samples of 450 g were taken from the pooled feces and frozen for later analysis. Diets and feces were analyzed for DM content by drying to a constant weight in a 103°C stove for 4 h (EC, 2009; EC 152/2009). Crude protein was analyzed by combustion, according to the Dumas method (Etheridge et al., 1998). Crude fat content was determined after acid hydrolysis according to ISO Standard 6496 (ISO, 1999). Starch content was determined enzymatically as described by Rijnen et al. (2001). Crude ash content was determined by incineration in a muffle furnace by combustion at 550°C (EC, 1971; 71/250/EEG 1971). Fecal VFA content was determined by HPLC, using the method described in Canale et al. (1984), and a pH meter was used to measure fecal pH.

Calculations and Statistical Analysis. The ME content of the MR was calculated as follows: ME (Mcal/kg) = [0.057 × CP (%) + 0.092 × crude fat (%) + 0.0395 × lactose (%)] × 0.93 (NRC, 2001). The ME content of starter feed was calculated based on NRC (2001) ME values for the raw materials and applying the equation for calf starter. The ME content of the starter treatments was estimated based on the energy digestibility coefficient calculated in experiment 2, using gross energy intake from feed and fecal energy output. Gross energy intake (Mcal/kg) was calculated as feed intake (feed supply minus refusals) multiplied by the calculated gross energy content of each feed component [CP content (g/kg) × 0.0057 Mcal/g + CF content (g/kg) × 0.0095 Mcal/g + (starch + sugars + fiber) × 0.0042 Mcal/g]. Fiber was estimated as (DM – CP – ash – crude fat – starch – sugars). Fecal energy output (Mcal/g) was calculated as [fecal DM output (g of DM/d) × fecal energy content in DM (Mcal/g of DM)]. Feed conversion was calculated by dividing the total ME (Mcal/kg) by ADG (kg). A classical power analysis was conducted to determine the number of experimental units needed. The power (1 − β) was chosen to be equal to 80%, and the α-level was 0.05. Growth performance was considered the most reliable parameter to determine power. Based on a previous study conducted at the same research facility (Calf & Beef Research, Sint Anthonis, the Netherlands) for 57 d after arrival at 0 to 3 d of age, a standard deviation (SD) of 100 g/d was assumed. The minimal meaningful difference in ADG was considered to be 90 g/d. The minimal sample size was 20 calves per treatment, accounting for a maximum mortality rate of 10%. All statistical analyses were performed using SAS Studio (version 3.2, SAS Institute Inc.). Data on growth, intakes, blood parameters, fecal pH, and fecal contents were analyzed with a mixed-effects model procedure (PROC MIXED). Proportional data were analyzed (apparent total-tract digestibility) with a β distribution using a generalized linear mixed model (PROC GLIMMIX). In all analyses, the individual calf was considered the experimental unit, with treatment and block as fixed and random effects, respectively. Time

entered the model as a repeated statement in case of repeated measurements, and then the interaction between time and treatment and the SLICE command from SAS Studio (version 3.2, SAS Institute Inc.) to control type I error were included. For both experiments, treatment averages were presented as least squares means and standard errors of the mean. The normal distribution of the residuals was checked to verify model assumptions. Significance was declared when $P \leq 0.05$, and trends were declared when $P < 0.10$.

RESULTS

Experiment 1

In total, 5 calves were removed from the study based on pre-established exclusion criteria for health or feed refusals. Data collected from these calves before removal were excluded from the analyses; therefore, the data set included 55 successful observations (CON = 19, PFA = 18, and RFT = 18 calves). Intake and Growth Performance. Treatment effects on feed intake and performance are presented in Tables 6.3 and 6.4, respectively. No differences in MR intake were observed during the preweaning period ($P = 0.66$). Similarly, no significant differences were detected for solid feed intake and straw intake during the preweaning and postweaning periods among treatments (Table 6.3); however, after weaning, PFA calves tended to consume more starter feed ($P = 0.08$) than CON and RFT calves (Table 6.3). During the entire experimental period (112 d), PFA calves consumed 146 g/d more solid feed than CON calves, and 40 g/d more than RFT calves d ($P = 0.03$; Table 6.3). Intakes of ME ($P = 0.61$) and CP ($P = 0.38$) did not differ between the 3 treatments during the preweaning period. However, as a result of the high intake of the PFA treatment and the low digestibility of the RFT diet, calves in the PFA group had the highest ME intake after weaning ($P < 0.001$) compared with CON and RFT calves (Table 6.3 and Figure 6.1). Initial BW and body measurements were similar among all animals. No differences among treatments were observed for BW ($P = 0.41$; Table 6.4) or ADG ($P = 0.77$; Table 6.4) during the preweaning period. Calves consuming the PFA treatment had greater ADG than CON and RFT calves during the weaning period ($P = 0.05$). After weaning and until the end of the study (112 d), calves in the PFA treatment had greater BW ($P = 0.01$), being 8.8 and 9.3 kg heavier than CON and RFT calves, respectively (Table 6.4). During the postweaning period, PFA calves had greater ADG than the other groups, with this difference being significant between d 50 and 84 ($P = 0.05$), and showing a tendency for a treatment effect thereafter ($P = 0.07$). Over the total period, the ADG of PFA calves was the greatest compared with that in the CON and RFT calves ($P = 0.03$). Furthermore, no differences were observed for withers height, hip height, chest girth, or body barrel for the main effect of treatment (Table 6.4).

Table 6.3 Effect of dietary treatments on intakes of young calves before (0–49 d of age) and in 2 periods after weaning (50–84 d of age) and (85–112 d of age) (experiment 1)

	Treatment[1]				*P*-value[2]	
Item	**CON**	**RFT**	**PFA**	**SEM**	**T**	**T x t**
MR intake, g/d						
0-49 d	913.5	918.7	911.6	5.7	0.66	0.87
Starter intake, g/d						
0-49 d	324	378	351	46.4	0.76	0.81
50-84 d	2,534	2,615	2,903	125.9	0.08	0.75
85-112 d	3,966	4,047	4,322	173.1	0.31	0.91
Total period	1,892[b]	1,998[a]	2,038[a]	39.7	0.03	0.99
Straw intake, g/d						
0-49 d	12	16	16	4.6	0.78	0.11
50-84 d	82	88	127	16.2	0.11	0.02
85-112 d	162	177	241	28.7	0.12	0.99
Total period	73.0[b]	79.7[a]	107.2[a]	9.4	0.03	0.99
Water intake, kg						
0-49 d	1.4	1.6	1.7	0.1	0.44	0.79
50-84 d	7.9	8.1	8.8	0.2	0.38	0.76
85-112 d	12.1	12.2	12.8	0.3	0.78	0.97
Total period	6.1	6.2	6.5	0.3	0.53	0.93
ME intake, Mcal/d[3]						
0-49 d	4.47	4.59	4.63	0.13	0.61	0.87
50-84 d	6.71[b]	6.63[b]	8.07[a]	0.30	0.01	0.41
85-112 d	10.35[b]	10.19[b]	12.29[a]	0.52	<0.01	0.92
Total period	6.62[b]	6.60[b]	7.56[a]	0.10	0.01	0.52
CP intake, g/d						
0-49 d	262	272	266	5.2	0.38	0.95
50-84 d	404	419	437	21.3	0.54	0.62
85-112 d	624	644	670	29.5	0.55	0.84
Total period	397	411	420	14.2	0.31	0.92
Feed Conversion (kg of gain/ Mcal)						
0-49 d	0.145	0.145	0.139	0.045	0.24	0.80
50-84 d	0.150	0.150	0.140	0.005	0.27	0.56
85-112 d	0.127	0.125	0.119	0.005	0.31	0.09
Total period	0.145	0.145	0.139	0.003	0.24	0.79

[a,b]Means that do not share a common letter differ (P < 0.05). [1]CON = calf starter; RFT = CON mixed with an extruded hydrogenated rapeseed triglyceride; PFA = CON mixed with an extruded hydrogenated palm free fatty acid. [2]T = effect of treatment; T × t = interaction between treatment and time. [3]Calculated following the Quigley et al. (2019) formula for ME with the calculated digestible energy coefficient as determined in experiment 2.

Table 6.4 Effect of dietary treatments on performance and body measurements of young calves until 112 days of age (Experiment 1)

	Treatment[1]				*P*-value[2]	
Item	**CON**	**RFT**	**PFA**	**SEM**	**T**	**T x t**
Bodyweight, kg						
Initial	45.4	46.2	45.5	1.1	0.84	-
49 d	78.8	81.6	81.3	2.3	0.62	-
84 d	114.2	116.6	121.1	2.4	0.12	-
112 d	153.3^{b}	152.8^{b}	162.1^{a}	2.5	0.01	-
Total period gain	93.5	95.2	97.6	2.2	0.41	0.29
ADG, kg/d						
49 d	0.66	0.69	0.68	0.04	0.77	-
84 d	0.99^{b}	0.98^{b}	1.13^{a}	0.06	0.05	-
112 d	1.28	1.23	1.40	0.06	0.07	-
Total period	0.88^{b}	0.90^{b}	0.98^{a}	0.03	0.03	0.48
Body measurements, cm						
Height at the withers						
Initial	78.5	79.4	78.7	0.57	0.49	-
Final	102.7	102.9	102.4	1.17	0.97	-
Hip height						
Initial	80.8	81.6	80.6	0.63	0.51	-
Final	106.9	107.2	107.3	1.81	0.96	-
Chest girth						
Initial	82.5	82.8	81.8	0.68	0.52	-
Final	118.2	118.1	119.2	1.98	0.92	-
Body barrel						
Initial	83.2	83.7	82.5	0.71	0.47	-
Final	148.1	147.0	149.5	3.28	0.86	-

^{abc}Means that do not share a common letter differ ($P < 0.05$). [1]CON = calf starter; RFT= CON mixed with an extruded hydrogenated rapeseed triglyceride; PFA = CON mixed with an extruded hydrogenated palm free fatty acid. [2]T = effect of treatment; T × t = interaction between treatment and time. Arrival BW is used as a covariable in the model.

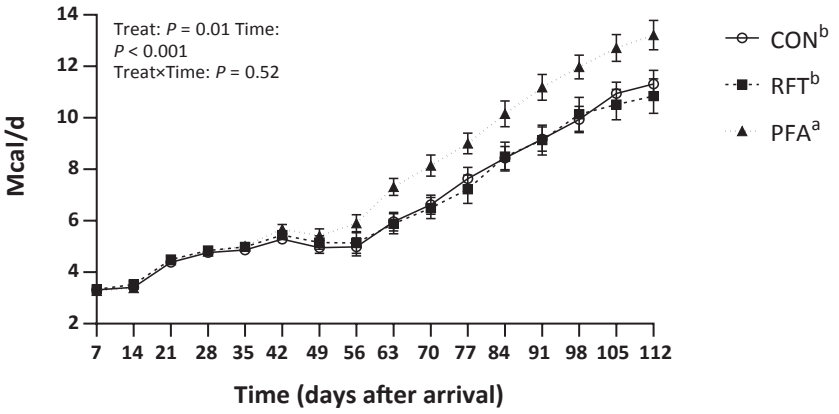

Figure 6.1 Intake of ME (Mcal/d) corrected for the digestible energy coefficient determined in experiment 2 as affected by treatment. CON = calf starter; RFT = CON mixed with an extruded hydrogenated rapeseed triglyceride; PFA = CON mixed with an extruded hydrogenated palm free fatty acid. Error bars indicate SE. Treatments that do not share a common letter (a–c) differ ($P < 0.05$). Treat = treatment

Blood Parameters. Results for blood parameters are summarized in Table 6.5. No differences in blood BHB and glucose concentrations were observed across treatments in any period (Table 6.5). Nevertheless, IGF-1 concentration differed during the preweaning period ($P = 0.02$), with RFT calves having greater concentrations than CON and PFA calves. No differences in fecal scores were observed during the experimental period (data not shown).

Experiment 2

Data collected during the digestibility assessment period are shown in Tables 6.6 and 6.7. Calves on PFA consumed more starter, straw, and water ($P = 0.01$) than those in the CON and RFT groups. The greatest total-tract apparent DM digestibility was observed in calves fed the CON diet, and the lowest was reported for calves fed the RFT diet ($P = 0.01$). Differences in total-tract apparent digestibility of crude fat were observed among all treatments. Fat digestibility was 31.1 and 6.8 percentage points higher in PFA calves ($P = 0.01$) than in RFT and CON calves, respectively (Table 6.6). The RFT treatment resulted in an overall lower nutrient digestibility than the CON treatment, even though intakes of nutrients in RFT were higher ($P = 0.01$). Dietary treatment also affected fecal pH, with PFA calves having the lowest pH ($P = 0.01$) compared with CON and RFT calves (Table 6.7). No differences were observed in total fecal VFA between treatments; however, the molar proportion of fecal butyrate was

Table 6.5 Effect of dietary treatments on plasma concentrations of BHB, glucose, and IGF-1 in growing calves preweaning (4, 6, and 7 wk) and postweaning (8, 10, and 16 wk; experiment 1)

	Treatment			SEM	*P*-value[2]
Item	**CON**	**RFT**	**PFA**		**T**
BHB (mmol/L)					
Pre-weaning	0.09	0.09	0.10	0.01	0.96
Post-weaning	0.34	0.33	0.36	0.02	0.64
Glucose (mmol/L)					
Pre-weaning	5.46	5.89	5.30	0.15	0.29
Post-weaning	4.91	5.23	4.97	0.15	0.30
IGF-1 (ng/mL)					
Pre-weaning	121.3^{ab}	139.6^{a}	106.9^{b}	7.92	0.02
Post-weaning	190.6	191.3	174.0	28.59	0.89

^{ab}Means that do not share a common letter differ (P < 0.05). [1] CON = calf starter; RFT= CON mixed with an extruded hydrogenated rapeseed triglyceride; PFA = CON mixed with an extruded hydrogenated palm free fatty acid. [2]T = effect of treatment.

Table 6.6 Effect of dietary treatments on intakes and apparent total-tract digestibility of nutrients from solid feed of 12-wk-old calves (experiment 2)

	Treatment[1]				*P*-value[2]
Item	**CON**	**RFT**	**PFA**	**SEM**	**T**
Intakes					
Starter Intake, kg/d					
Collection Period (72h)	4.07^{c}	4.39^{b}	4.76^{a}	0.20	0.01
Total period (2 weeks)	3.44^{c}	3.83^{b}	4.03^{a}	0.14	0.01
Straw Intake, g/d					
Collection Period (72h)	148	168	215	46.9	0.12
Total period (2 weeks)	115.7^{c}	162.3^{b}	178.1^{a}	38.9	0.01
Water Intake, kg					
Collection Period (72h)	15.5	16.9	17.6	0.84	0.05
Total period (2 weeks)	14.6^{b}	16.3^{a}	16.8^{a}	0.75	0.01
Apparent total tract digestibility, %					
Dry matter	79.4^{a}	75.1^{b}	76.0^{b}	1.36	0.01
Organic matter	81.1^{a}	76.4^{b}	77.1^{b}	1.27	0.03
Crude protein	78.3	75.6	75.4	1.39	0.27
Crude fat	81.0^{b}	56.8^{c}	87.9^{a}	1.55	0.01

^{abc}Means that do not share a common letter differ (P < 0.05) [1] CON = calf starter; RFT= CON mixed with an extruded hydrogenated rapeseed triglyceride; PFA = CON mixed with an extruded hydrogenated palm free fatty acid. [2]T = effect of treatment.

Table 6.7 Effect of dietary treatments on fecal parameters of 12-wk-old calves (experiment 2)

	Treatment				*P*-value[2]
Item	**CON**	**RFT**	**PFA**	**SEM**	***T***
pH	6.2[a]	6.1[b]	6.0[b]	0.06	0.01
VFA, molar proportions (%)					
Acetate	69.9	68.8	67.3	0.98	0.17
Propionate	21.9	21.7	22.9	0.87	0.56
Butyrate	8.1[b]	9.8[a]	9.5[a]	0.43	0.03
Fecal content (g/kg of DM feces)					
CP	702.7	777.2	833.2	44.32	0.15
Crude fat	98.7[c]	510.4[a]	157.3[b]	20.00	0.01
Ash	5.2	5.6	5.6	0.33	0.67

[a–c]Means that do not share a common letter differ (P < 0.05). [1]CON = calf starter; RFT = CON mixed with an extruded hydrogenated rapeseed triglyceride; PFA = CON mixed with an extruded hydrogenated palm free fatty acid. [2]T = effect of treatment.

lower in the CON treatment (*P* = 0.02) but comparable between RFT and PFA calves. Total fecal fat was affected by dietary treatment (*P* = 0.01): total fecal fat in RFT calves was 3-fold higher than in PFA calves and 5-fold higher than in the CON group (Table 6.7).

DISCUSSION

The objective of this study was to evaluate the effects of increasing the energy levels of calf starter by increasing the fat content (from different sources) on feed intake, performance, and digestibility in rearing calves during the weaning transition. In the current study, during the preweaning period, the same MR formulation was fed in similar amounts to calves in all treatments, resulting in similar daily consumption of liquid feed. It has been suggested that the main disadvantage when feeding high amounts of milk or MR is the reduction of starter intake during the preweaning period (Jasper and Weary, 2002; Terré et al., 2006; Khan et al., 2007; Kristensen et al., 2007; Borderas et al., 2009). However, in more recent studies, it has been demonstrated that, under adequate weaning practices, feeding high-energy MR at high levels (7 L, 150 g/L; Amado et al., 2019) or even *ad libitum* (Echeverry-Munera et al., 2021) is possible without negative effects on solid feed consumption or growth postweaning. During the preweaning period of the current study, solid feed intake was similar among the 3 treatments, with calves consuming, on average, 351 g of calf starter per

day. These solid feed intakes could be attributed to the high supply of MR and are comparable to those reported in recent studies (de Carvalho et al., 2021). In the study conducted by de Carvalho et al. (2021), calves were fed a high plane of MR (50 kg of MR during the preweaning phase) and were observed to consume 450 g of calf starter per day until wk 7. Earlier studies evaluating the inclusion of fat in starter feeds have associated high-fat single pellets with reduced intake and depressed weight gain (Doppenberg and Palmquist, 1991). In contrast, a later study by Araujo et al. (2014) showed that when calves were offered either restricted or moderate amounts of MR (4 or 6 L/d) along with either a low-fat (4.1% fat inclusion) or a high-fat (11.2%) starter feed, calves fed the high-fat calf starter had higher BW gain postweaning, mainly due to higher energy intakes from the solid feed.

In the current study, which aimed to resolve the conflict between energy delivery and rumen function and development, fat inclusion in starters was considered an important factor, following encouraging results in an earlier study by our group (Berends et al., 2018), in which calves were fed a traditional pellet mixed with an extruded high-fat pellet, but this study also evaluated an alternative rumen-inert fat source, hydrogenated rapeseed triglycerides. Confirming our earlier results, PFA calves tended to eat more calf starter during the weaning period and throughout the experiment compared with CON calves. Calves in the PFA and RFT groups consumed, on average, 146 and 106 g more, respectively, than CON calves during the study. Furthermore, calves across all treatments had comparable plasma BHB levels, both before and after weaning. According to Coverdale et al. (2004), BHB could be a good indicator of a shift in nutrient source from liquid feed to solid and an indicator of rumen development and fermentation around the time of weaning (Quigley et al., 1991, 1992). When using BHB as a proxy for rumen fermentation, it is possible that calves were able to access the fermentable pellets despite the high fat content in experimental diets. Similarly, Berends et al. (2018) reported no differences in the concentrations of BHB in starter diets with different inclusion levels of high-fat extruded pellets.

The increased solid feed intake observed in PFA and RFT calves, together with the high level of ME in the respective dietary treatments, resulted in greater ME intakes of PFA calves. The increased consumption of energy through solid feed potentially helped to compensate for the shortage of energy provided by the MR and the lower starter intake during the weaning period. We observed that calves in the PFA treatment required 2 wk, on average, to consume the same level of ME they were consuming before the weaning period (wk 6). In contrast, the CON and the RFT groups required 3 and 4 wk, respectively, to match their preweaning ME intake. Notwithstanding, the effect of high-fat extruded pellets (PFA or RFT) on BW and ADG began

after the weaning period and was maintained during the next month of observation (112 d), when the PFA calves had gained 8.8 kg more than the CON animals. These results add to those reported by Berends et al. (2018), in which delivering dietary fat (~7% of DM) by mixing an extruded high-fat pellet with a conventional calf starter improved solid feed intake, energy intake, and rate of BW gain compared with a traditional low-fat pellet (~3% of DM).

Usually, rumen-inert fats have a high degree of fatty acid saturation, resulting in high melting points, or are saponified with Ca. These characteristics make rumen inert fats less likely to interfere with rumen fermentation (Elliott et al., 1999). Additionally, TG forms have previously been shown to have a lower digestibility compared with FFA in dairy cows (Pantoja et al., 1995; Elliott et al., 1999; Weiss and Wyatt, 2004). In a literature review, Naik (2013) reported a range of 82.05 to 89.0% for fat digestibility of a dairy ration supplemented with rumen-inert fats. Similarly, in the current study, fat digestibility of PFA and CON was 87.9 and 81.0%, respectively, but in calves consuming the RFT diet, substantially lower total-tract fat digestibility was observed (56.8%). Elliot et al. (1999) reported that steers fed diets containing hydrogenated TG as supplemented fat showed lower digestibility when the saturated fat source contained more TG (57.7%) than FFA (64.7%). Their data showed that hydrogenated TG are resistant to lipolysis in the rumen and small intestine when TG consist of mostly SFA. The fat digestibility results in the current study might be influenced not only by the degree of esterification, but also by the FA profile of the supplement fed. This has been reported in earlier studies in mature dairy cows, where it was observed that palmitic acid is more digestible and stearic acid less digestible than the average FA mixture in ruminants (Palmquist, 1994). As a result of the reduced digestibility, a larger portion of fat was found in the feces of animals consuming the RFT diet. The poor digestibility of the fat presents a simple explanation for the decreased growth performance observed in calves consuming the RFT diet despite comparable feed intakes.

The principal VFA that result from carbohydrate fermentation in the large bowel are acetate, propionate, and butyrate (Sato and Koiwa, 2008). However, changes in diet alter both substrate flow and disappearance in the bovine large intestine (Karr et al., 1966); therefore, it is likely that fermentation patterns and VFA production rates change accordingly. Results on fecal acetate and propionate in the present study did not differ among treatments, indicating similar fermentation and an adequate transit of digesta and dilution of feces, as stated by Sato and Koiwa (2008). Nevertheless, we found that calves in the RFT (9.8%) and PFA (9.5%) treatments had higher butyrate in feces compared with CON calves (8.1%). Increased fecal butyrate might have been the result of increased hindgut fermentation. High fat concentrations in the intestine

might interfere with starch digestion or absorption, increasing the carbohydrate load in the hindgut (Jenkins and Jenny, 1989).

Plasma glucose concentration was also observed to decrease around weaning, which is consistent with previous studies (Khan et al., 2011; Echeverry-Munera et al., 2021). This may be attributed to the physiological shift in the primary energy source from glucose to VFA when the rumen in young calves becomes functional (Hammon et al., 2002). Nevertheless, in the study of Jenkins and Jenny (1989), cows fed with fat supplements tended to have higher plasma glucose. Similarly, Berends et al. (2018) reported that when young calves are provided with high dietary fat levels up to 13.4% of crude fat on a dry basis in the starter, glucose metabolism can be compromised, as suggested by an accumulation of glucose in blood following a glucose tolerance test in the calves fed the high-fat diet. However, in the current study, blood glucose concentration was similar among all groups and within the normal range for plasma glucose previously stated by Egli and Blum (1998). Moreover, calves consuming the RFT diet had the highest MR intake and levels of plasma IGF-1 during the preweaning period. Interestingly, in recent studies, it has been shown that increased nutrient intake from MR stimulates IGF-1 secretion and increases IGF-1 blood concentration in calves (Bartlett et al., 2006; Daniels et al., 2008; Schaff et al., 2016).

CONCLUSIONS

Energy intake is the main factor limiting health and performance during the weaning process of young dairy calves. In this study, we fed calves a high-fat extruded pellet to sustain energy intake and growth around the weaning period. Our results confirmed that the high-fat supplement was consumed in addition to basal starter feed intake, substantially increasing energy intake. However, this effect was better translated to growth when the fat source was hydrogenated palm free fatty acids (PFA) because of the wide differences in total tract digestibility found between PFA and RFT, which likely derived from their different esterification and fatty acid composition. Supplementing fat in the solid feed as a high-fat extruded pellet mixed with a calf starter feed was shown to supply additional energy to dairy calves around and after weaning.

ACKNOWLEDGMENTS

The authors thank Natasja Boots, Akke ten Berge, and the staff of the Trouw Nutrition Calf and Beef Research facility (Sint Anthonis, the Netherlands) for their skilled technical assistance. This study was funded and several of the authors are employed by Trouw Nutrition (Amersfoort, the Netherlands), a company with commercial interests in starter feed for calves. Trouw Nutrition R&D is committed to high standards of research integrity and adheres to the principles of the European Code of Conduct for Research Integrity (Drenth, 2011). The authors have not stated any other conflicts of interest.

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CHAPTER 7

GENERAL DISCUSSION

INTRODUCTION

The understanding of the contributions made by various macronutrients present in animal feed, particularly for livestock, requires further refinement. For example, in dairy calves, the fat content in both liquid and solid feeds falls well below the levels found in cow's milk, whereas carbohydrates tend to be higher. Based on the potential effects of early life nutrition on metabolic programming and the homeorhetic adaptations necessary for milk production as the animal develops into an adult capable of lactation, changes in feed composition might impact long-term performance. The main objective of previous chapters was to establish fundamental knowledge for formulating elevated feeding strategies that improve growth performance and health while minimizing weaning-related adverse effects. To this end, a study was performed in calves up to 21 days old to quantify responses to the incremental supply of protein, fat, and lactose in a milk replacer (**MR**) on energy and protein deposition (Chapter 2) and within-day nutrient oxidation (Chapter 3). Chapter 2 revealed that protein utilization for growth in young calves is efficient but less than expected based on body weight (**BW**) extrapolation. Increasing energy intake through lactose and fat does not necessarily enhance protein efficiency. Increased fat or lactose intake leads to notable body fat deposition, with incremental lactose promoting higher fat accumulation by reducing fatty acid (**FA**) oxidation. On the contrary, protein intake does not contribute to increased fat deposition. Chapter 3 indicated that around 3 weeks of age, dietary lactose oxidation is nearly complete, regardless of the intake of other nutrients. The thermic effect of feeding responds to feeding level and digesta outflow from the abomasum, while fat oxidation gradually increases, and carbohydrate oxidation decreases as time progresses towards 24 hours of fasting. Two experiments examined exchanging lactose for fat in MR and its effects on performance, digestibility, gut permeability (Chapter 4), and blood metabolites (Chapter 5) in male dairy calves up to 70 days old at high liquid feed supply levels. Although growth rate and BW were similar despite energy intake differences, body composition was not assessed. High fat MR correlated with increased permeability, higher NEFA and blood glucose concentrations, and improved fecal scores. Addressing the topic of insufficient energy intake during and after weaning, Chapter 6 studied the effect of a high-fat extruded pellet with a traditional calf starter, reporting improved starter and energy intake leading to greater BW gains. Moreover, this was conditioned by fat source, which influenced total tract digestibility, intake, and growth response in calves up to 112 days old.

In the following section, the chapters of this thesis are further examined. The discussion navigates through the various developmental stages of a calf, including preweaning, weaning, and post-weaning, placing specific emphasis on energy supply during these periods. Moreover, this section dives into how dietary components contribute to

protein and body fat deposition in young calves. Following, the role of fat in the health and development of heifer calves is discussed. Additionally, this detailed chapter analysis covers an exploration of feeding strategies and their direct influence on calf growth performance, including an examination of the impact of restoring fat intake levels closer to biological reference of whole milk (**WM**) and time span of milk supply, which is achieved by increasing fat content in MR and starter feed during the initial 120 days of age. General conclusions and implications conclude the chapter.

ENERGY INTAKE DURING THE PREWEANING PHASE

Optimal feeding strategies for calves depend on the farmer's objectives. They are shaped by several factors such as feed quality, quantity, and weaning age. Importantly, these objectives should not only meet productive objectives, but also be in harmony with the health and general wellbeing of the calf. While high MR programs may align well with the goals to minimize feeding costs, maximize calf growth and development, and mitigate environmental pollution, as well as enhance long-term productivity and overall welfare, it is important to recognize that other farmers may prioritize feed cost considerations or be constrained by other practical limitations. Recognizing the diversity of goals among farmers is essential in calf rearing, as it opens the possibility to of a range of diverse feeding strategies to meet these goals, which should reflect the multifaceted nature of calf rearing and the interplay between farmers' objectives and the well-being of the calves themselves.

Traditionally, intensively reared dairy calves are typically separated from the dam within 24 hours of birth and restricted to milk supplies below their potential voluntary intake. Calves are fed twice daily with a total quantity of milk corresponding to approximately 10% of their BW (NRC, 2021). In contrast, a calf left with its dam can suckle multiple times daily, averaging 7 to 10 feedings and consuming a much larger quantity of milk in smaller meals throughout the day (Albright and Arave, 1997). In *ad libitum* systems, where calves have unrestricted access to milk, reports indicate an average consumption of 10 liters of milk per day, distributed over approximately 10 meals before 4 weeks of age (Appleby et al., 2001). They maintain this intake until 3 months of age, at which point they begin consuming more solid feed (Webb et al., 2014). Despite significant advancements in the industry's transition from traditional restrictive feeding schedules, further efforts are required to lift restrictions of milk supply to calves to align feeding levels with the nutritional needs of calves in their first weeks of life.

Over the past decade, a significant trend has emerged, advocating for elevated levels of milk supply for calves, with the primary objective of increasing their average daily gain (**ADG**) and enhancing first-lactation and lifetime health and productivity. Although the relationship between increased early life dry matter intake (**DMI**) and later milk production was not particularly strong, a potential outcome of this trend is that an increase of 100 g/d in liquid DMI among calves with an ADG exceeding 500 g/d could result in an additional 66 kg of milk production during their first lactation. This effect has the potential to double when paired with increased starter feed intake (Soberon et al., 2012; Gelsinger et al., 2016). This approach challenges the traditional recommendations of restricting liquid feed to around 10% of BW by volume (Davis and Drackley, 1998). It advocates the advantages of increasing DMI, amounting to approximately 20% of BW by volume, or even offering milk *ad libitum* during the early stages of calf rearing. With this approach, calf rearing objectives have shifted towards promoting rapid growth, aiming for an ADG exceeding 700 g/d over the entire rearing period. This increased nutrient supply supports organ development (Soberon and Van Amburgh, 2017) and ultimately improves overall animal health and welfare (Khan et al., 2011).

However, one of the challenges that can negatively affect calf growth and health is the differences in the macronutrient composition of WM and common MR formulations for calves. Compared with WM, traditional MR formulations deliver relatively high lactose and low-fat levels, while protein levels are comparable. Consequently, current MR formulations provide a lower dietary energy content (approx. 15% less) than WM, and most importantly, a dietary energy is delivered in a different form. These differences gain even greater relevance when the feeding levels of MR are increased, either in concentration or daily volume. Without making any adjustments to the nutritional composition of MR, all nutrients are increased in the same proportion. This may be just the practical consequence of increasing milk supply, but it may not be optimal for a well-balanced nutrient supply.

NUTRIENT PARTITIONING

Protein metabolism

In Chapters 2 and 3, the responses to the incremental supply of protein, fat, and lactose in a MR were quantified in calves up to 21 d of age. Figure 7.1 summarizes the responses of nutrient partitioning to incremental nutrient supply. For instance, it was observed that at 21 d of age, the rate of protein deposition in response to increased protein intake was 57% of the additional ingested protein, with 44% attributed to heat production and 2% to fat deposition. Regarding fat intake, for every increase,

67% was retained as fat, 22% dissipated as heat, and with no effect on protein deposition. Similarly, for every increase in lactose intake, 49% was stored as fat, 38% lost as heat, and none as protein.

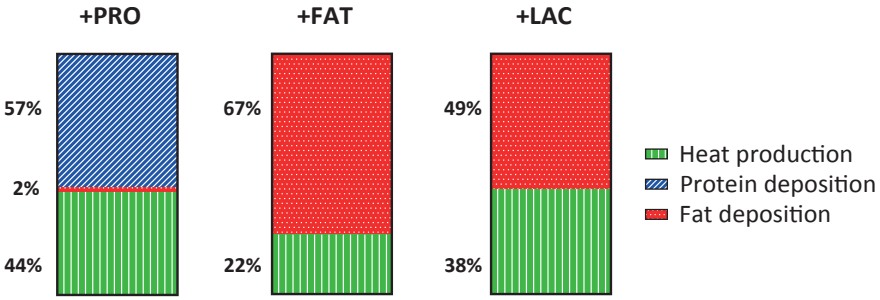

Figure 7.1 Incremental energy efficiencies to the extra supply of protein (+PRO), fat (+FAT), and lactose (+LAC) in a MR in calves up to 21 d of age (Chapter 2, this thesis).

The effects of protein intake on the growth rate and composition of young (Roy et al., 1970; Donelly and Hutton, 1976; Tolman and Beelen, 1996; Diaz et al., 2001; Blome et al., 2003; Labussière et al., 2008; Chapter 2, this thesis) and older pre-ruminant calves (Gerrits et al., 1996a; van den Borne et al., 2006a; Labussière et al., 2008) have been previously reported. Figure 7.2A summarizes this information also including the results of Chapter 2. The results suggest that calves weighing between 50 and 70 kg exhibited incremental N efficiencies ranging from 45% to 69%, while those with body weights above 100 kg, between 20% and 40%. Therefore, these studies indicate that protein deposition efficiency diminishes in pre-ruminant calves as they age and gain weight.

To further illustrate protein efficiency in young calves, the gross efficiency of digestible protein utilization for protein gain during the first 4 months of age is displayed in Figure 7.2B. While it is recognized that gross efficiencies may exhibit a higher inter-experimental variation, the values presented in the figure demonstrate that the utilization of digested nitrogen for N retention is approximately 60%. However, as BW increases above 150 kg, the gross efficiency drops below 50%. These results confirm the diminishing efficiency of digested protein retention as calves become older and increase their BW. Furthermore, when considering the 25% protein efficiency observed in growing beef steers (with a BW of 450 kg, as reported by Vandaele et al., 2019), these findings illustrate that, in addition of the total ruminal degradation of dietary protein, amino acid oxidation becomes a predominant fate of ingested proteins.

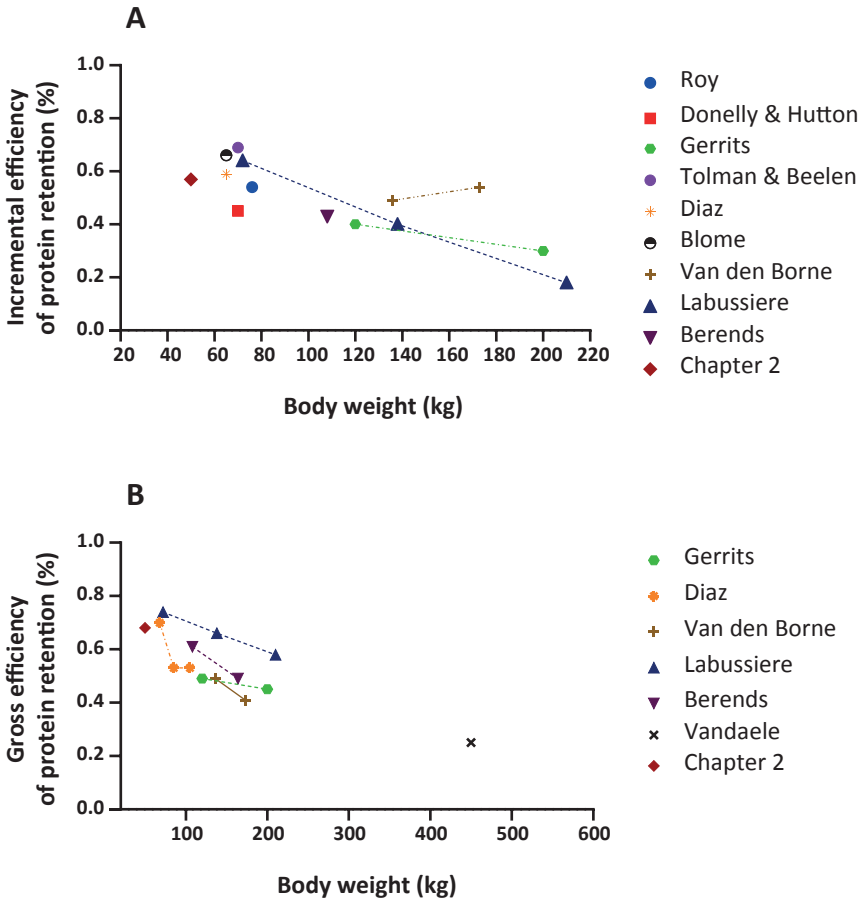

Figure 7.2 Incremental efficiency of protein retention (panel A; Roy et al., 1970; Donelly & Hutton et al., 1976; Gerrits et al., 1996a; Tolman & Beelen et al., 1996; Diaz et al., 2001; Blome et al., 2003; Van den Borne et al., 2006a; Labussiere et al., 2008; Berends et al., 2012; Chapter 2, in this thesis) and gross efficiency of protein retention (panel B; Gerrits et al., 1996a; Diaz et al., 2001; Van den Borne et al., 2006a; Labussiere et al., 2008; Berends et al., 2012; Vandaele et al., 2019; Chapter 2, in this thesis) with increasing BW in milk-fed calves.

This raises the question of why a significant portion of the absorbed amino acids were not effectively utilized for protein deposition. Compared to monogastric species, calves exhibit a low marginal efficiency of protein retention. For example, in growing pigs, incremental efficiencies between 60 and 80 % were reported (reviewed by Van den Borne, 2006b). The inefficient utilization of protein in calves is believed to have a

multifactorial origin. Various potential mechanisms have been discussed to explain this (Gerrits, 1996b; Van den Borne, 2006b): (i) ruminal drinking, (ii) insulin resistance, (iii) a lack of nutrient synchrony, and (iv) protein-to-energy imbalance. A brief and updated overview of these mechanisms is presented below.

(i) Ruminal drinking:
It refers to the leakage of MR into the rumen, which has been observed to be substantial in veal calves (~20%; Berends, 2014). Among newborn dairy calves, there is evidence that the incomplete closure of the esophageal groove can lead to milk entering the reticulo-rumen, resulting in ruminal drinking. While our study did not specifically investigate this phenomenon, it is important to bear in mind its potential contribution to reduced protein efficiency. The consequence of milk leakage into the rumen is ruminal nutrient fermentation, which radically change the process of nutrient utilization when microbes utilize energy and break amino acids down into ammonia. Consequently, energy and protein are not directly absorbed in the small intestine, leading to reduced utilization of nutrients for growth purposes (Herrli-Gygi et al., 2006). Insights from previous research suggest that ruminal drinking can introduce variability in nutrient utilization, thereby contributing to the observed inefficiencies in protein deposition among heavily pre-ruminant calves (Van den Borne, 2006b; Berends, 2014).

(ii) Insulin sensitivity:
As an anabolic hormone, insulin has been suggested to play an important role in efficiency of protein deposition in growing animals (Burrin and Mersmann, 2005). Changes in insulin sensitivity, particularly the low insulin sensitivity observed in older preruminant calves, have been suggested to be partly responsible for low protein efficiency observed in growing calves (Van den Borne, 2006b). Insulin sensitivity plays a crucial role in the metabolic health of young calves, and several studies have investigated its changes during early life. Previous studies have examined how different nutrient components influence insulin sensitivity in heavy pre-ruminant calves (Van den Borne, 2006b). These studies observed that insulin resistance increased with additional lactose intake and decreased with extra protein intake, while the impact of fat intake on insulin sensitivity remained unclear. Another study by Stanley et al. (2002) measured insulin sensitivity in neonatal dairy calves and found that it decreases from week 3 to 6 as calves are gradually weaned. Moreover, Bach et al. (2013) conducted research comparing MR allowances and their effects on insulin sensitivity in young calves (1-8 weeks of age) with *ad libitum* access to starter feed. The study found that a higher level of MR allowance (8 L/d) negatively affected the development of insulin sensitivity.

However, the results of this study were contradicted by MacPherson et al. (2016), who demonstrated that calves fed a high plane of MR from birth had similar insulin sensitivity to those fed a low plane of MR. Other research, including a study by Pantophlet et al. (2016), revealed that whole-body insulin sensitivity in calves decreases by 70% between weeks 3 and 6 of age, independent of the feeding and weaning strategy. This age-related decline in insulin sensitivity may lead to potential changes in nutrient utilization and metabolic processes during early calf development. Although insulin metabolism was not a focus of our study (Chapters 2 and 3), we hypothesize that considering the age of these calves, their insulin sensitivity was likely high, questioning the role of insulin in the observed low incremental efficiency in Chapter 2.

(iii) Nutrient synchrony:
Representing the imbalance between nutrient availability and requirements, lack of nutrient synchrony has been hypothesized as a mechanism responsible for reduced protein efficiency, as described extensively by Van den Borne (2006b). Type 1 nutrient synchrony refers to the timely supply of nutrients to meet growth and maintenance requirements within a day. Type 1 asynchrony occurs when animals are fed infrequently, leading to a temporal oversupply of nutrients, especially for those with limited deposition capacity, like amino acids. This can result in oxidative losses and lower nutrient utilization efficiency. Type 2 synchrony is specific to time pattern of availability of protein (amino acids) and glucose.

Type 1 asynchrony may have contributed to the observed N efficiencies in the experiment outlined in Chapters 2 and 3. The feeding regimen for the calves in this experiment, involving twice-daily feedings, gave rise to two distinct peaks of metabolically available nutrients within 24 hours. As a result, type 1 asynchrony likely occurred due to the low-frequency feeding, resulting in a nutrient absorption pattern that closely followed the intake schedule. Indeed, in the context of heavy pre-ruminant calves, research has demonstrated that increasing the feeding frequency from 2 to 4 meals per day yielded a 5% improvement in protein efficiency (Van den Borne, 2006b).

Type 2 nutrient synchrony may affect protein utilization when there is an asynchronous availability of protein (amino acids) and glucose. Therefore, type 2 asynchrony can occur when there is a disconnection in the absorption pattern of macronutrients resulting from differences in the timing of intake, digestion, or absorption kinetics. This can affect nutrient partitioning in growing animals if one macronutrient utilization depends on the availability of the other. In theory, achieving synchrony between energy and N supply should facilitate more efficient nutrient utilization. In preruminant

calves, providing up to 85% of the daily protein supply in one meal did not affect protein utilization (Van den Borne et al., 2006b). Conversely, in growing pigs, the asynchrony of nutrient supply decreased the efficiency of utilizing digestible protein for protein gain by 10% (Van den Borne et al., 2007a). This synchrony could increase the availability of nutrients in the small intestine, potentially enhancing animal performance and reducing nutrient excretion into the environment.

In Chapter 3, nutrient asynchrony potentially occurred, even though all macronutrients were provided in the same meal, thus simultaneously, to the calves. This asynchrony could arise from the post-absorptive availability of individual nutrients at varying times. For instance, the milk proteins provided to the calves, predominantly composed of casein (constituting around 80% of milk protein), are likely to clot in the abomasum. Consequently, the release of protein into the intestinal tract is delayed (Houlier et al., 1991), leading to distinct circadian fluctuations in heat production (**HP**) and resting metabolic rate (**RMR**), reflecting the gradual absorption of FA and amino acids (**AAs**), and subsequently resulting in elevated levels of HP and RMR (as discussed in Chapter 3). On the other hand, lactose lacks clotting properties and is rapidly absorbed compared to dietary protein. This difference in the timing of the availability of glucose (from lactose) and amino acids (from casein) throughout the day can give rise to an asynchrony in nutrient availability. Considering these observations, the asynchronous post-absorptive availability of glucose and amino acids in our study may have influenced protein efficiency.

(iv) Protein-to-energy imbalance: this mechanism will be discussed in more detail in the next section on energy metabolism.

Does nutrient partitioning of young calves fit the theory of protein and energy-dependent phases?

In monogastric animals, the relationship between energy intake and the deposition of protein and fat can be well-described by a linear plateau model (Figure 7.3A). According to this model, protein deposition (**Pd**) increases as protein intake increases up to the point at which energy intake limits protein deposition. Beyond this point, increases in energy intake will increase Pd. Any additional energy intake is directed towards fat deposition (**Fd**) when the energy intake meets the requirements for achieving maximum Pd and essential Fd. The phase leading up to the upper limit of protein deposition is known as the protein-dependent phase for Pd, followed by the energy-independent phase for Pd. However, unlike Pd, which reaches a plateau when energy intake exceeds the requirements for maximum Pd, lipid deposition does not display a similar plateau. Instead, Fd continues to rise with higher energy intake, indicating no saturation point for energy retention in the form of fat (Whittemore and Fawcett, 1976; Bikker, 1994).

Contrary to the pattern observed in monogastric animals, studies conducted with milk-fed calves (Gerrits et al., 1996a), growing heifers (Ortigues et al., 1990), growing steers (Schroeder et al., 2006), and mature dairy cows (Daniel et al., 2016) indicate that the concept of protein and energy-dependent phases does not seem to apply in the same way in bovines. Instead, both energy and protein seem to simultaneously limit protein deposition. Figure 7.3B visually illustrates that for every 100 grams of extra protein intake, only 40 gr were deposited. When calves receive a higher energy intake and lower protein intake, the efficiency of protein utilization improves, but Fd does not decrease. Consequently, energy and protein are always simultaneously limiting factors for growth in these scenarios.

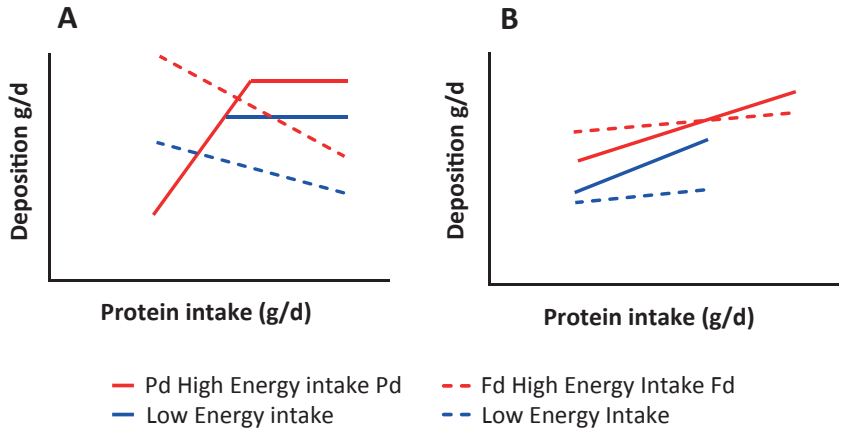

Figure 7.3 Representation of energy-dependent phase to protein deposition (Pd) and fat deposition (Fd) in pigs (panel A; based on Bikker et al., 1994) and protein and fat deposition rates to energy intake levels in milk-fed calves (panel B; based on Gerrits et al., 1996a).

The findings from Chapter 2 provide intriguing insights into the nutrient utilization patterns of calves at 21 days of age. Unlike the traditional model observed in monogastric animals, where an increase in protein intake in the energy dependent phase typically leads to higher fat deposition, the calves in this study did not increase their rate of fat deposition in response to increased protein intake. Moreover, the extra lactose and fat supply increased fat deposition as expected, but did not increase protein deposition. The absence of a response in protein deposition with increasing energy intake in Chapter 3 contradicts observations done in older (pre) ruminants (Gerrits et al., 1996a; van den Borne et al., 2006a; Labussière et al., 2008). Instead, it suggests the presence of protein- and energy dependent phases, where the calves in

Chapter 3 would then be in the protein-dependent phase. This, however, is not completely consistent with the absence of a response of fat deposition to increased protein intake, which would suggest these calves were in the energy dependent phase. In conclusion, our observations suggest that the responses of protein and fat deposition to increased nutrient intakes show some elements of, but do not completely fit into, the conventional concept of protein and energy-dependent phases. At the same time, their response to nutrient supply challenges the notion that energy and protein are simultaneously limiting factors for growth in young calves.

How important is body fat in young calves?

The significance of fat deposition in newborn calves becomes evident for several reasons; for example, adipose tissue plays a crucial role in energy metabolism, reproduction, and immune function. At birth, the body fat content of calves is low and varies based on breed, sex, and individual characteristics. Newborn calves typically have around 4% body fat. For instance, Bascom et al. (2007) demonstrated that newborn Jersey calves possess approximately 2.8% body fat as a percentage of their empty body weight at birth, which increased to 8.2% after 4 weeks of being fed with WM. Thus, proper nutrition is crucial to increase fat stores in young animals, especially since nearly 50% of body fat can be mobilized as energy should the animal need it. It is also important that not all fat is deposited in the same form, as different type of fat cells exist. For example, a significant portion of fat deposition happens in the form of brown adipose tissue, accounting for approximately 2% of the BW of newborn calves and that brown adipocytes are present in the omental, mesenteric, intestinal, abdominal, cervical, pericardial, cardiac, groove, perirenal, prescapular, popliteal, and orbital regions (Alexander et al., 1975). White adipose cells are specialized for the storage of energy in the form of triglycerides, but research in the last few decades has shown that fat cells also play a critical role in sensing and responding to changes in systemic energy balance.

Brown adipose tissue and white adipose tissue serve different biological functions to the animal. Brown adipose tissue specialized function is thermogenesis, facilitating temperature regulation shortly after birth and ensuring survival in colder environments (Bienboire-Frosini et al., 2023). This metabolic interplay, guided by adipogenesis and lipogenesis processes, is greatly influenced by early-life nutrient supply (Leal et al., 2018). Enhanced nutrient supply, particularly in brown adipocytes, increases Uncoupling Protein 1 (UCP1) gene expression. Uncoupling Protein 1 plays a crucial role in thermogenesis by 'uncoupling' energy production from adenosine triphosphate (ATP) synthesis, thus generating heat. This mechanism aids in maintaining optimal body temperature, particularly in brown adipose tissue. Studies have confirmed that increased UCP1 activity contributes to regulating body temperature and overall

energy expenditure (Ricquier, 2006). White adipose tissue, on the other hand, primarily serves as a storage site for excess energy. Furthermore, it provides insulation for temperature regulation and produces vital signaling molecules like adipokines and adiponectin, influencing metabolism and various physiological processes (Hoevenaars, 2014). Although white adipose tissue is distributed throughout the body, its anatomical location can dictate its composition and functional roles (Luo and Liu, 2016).

Adipose tissue serves as more than just energy storage and thermoregulation. Beyond brown and white adipose tissue, this tissue type is also found in vital organs like the liver and heart. In the liver, adipose tissue plays a pivotal role in overall metabolic regulation, contributing to the synthesis and storage of lipids, which are essential for maintaining energy balance (Birsoy et al., 2013). Hepatic adipose tissue is intricately involved in processes such as glucose homeostasis, lipid metabolism, and regulating hormones such as insulin (Heyde et al., 2021). Moreover, from infancy onwards, the mobilization of fat stores during infection has been acknowledged, potentially elucidating the connection between nutritional status and mortality in children (Kuzawa, 1998). Research by Urie et al. (2018) has even established a notable link between increased fat intake via milk and a subsequent reduction in mortality among pre-weaned calves on US dairy farms. This is confirmed in the findings of a study by Berends et al. (2020), where offering pre-weaned calves a high-fat milk replacer (23% DM; 150 g/L) *ad libitum* led to a decreased requirement for therapeutic interventions from birth up to 77 days of age. These findings collectively highlight the diverse and essential roles of adipose tissue in various physiological processes, encompassing energy regulation, immune function, and early-life survival.

In young calves, there is a linear relationship between fat intake and the percentage of fat in empty body weight (Bascom et al., 2007; Tikofsky et al., 2001). Bascom et al. (2007) showed that calves fed a low-fat MR (26% CP-19% fat or 20% CP-20% fat) did not exhibit increased body fat deposition after four weeks of feeding, indicating no significant increase in body fat reserves. Chapters 2 and 3 further demonstrated that additional fat and lactose in the diet served as a fuel source for body fat deposition rather than contributing to additional protein retention. This was also observed in a study conducted by Tikofsky et al. (2001), where the effects of three isonitrogenous and isocaloric treatments containing varying fat levels in MR (15%, 22%, and 31% fat DM) on body composition were determined. Some researchers have advocated for incorporating lower fat levels in milk replacer formulations (Tikofsky et al., 2001; Bartlett et al., 2006; Hill et al., 2008) due to concerns that higher fat content might lead to increased fat deposition and negative impacts on mammary gland development (Sejrsen and Purup, 1998). Nevertheless, there is currently no evidence suggesting that fat deposition in the mammary gland during the pre-weaning period results in

detrimental effects on subsequent milk production (Brown et al., 2005; Meyer et al., 2006; Daniels et al., 2009). Therefore, the question of whether fat deposition is advantageous or harmful for young animals requires further examination.

The rapid surge in adipose tissue accumulation during early life, reflecting both feeding strategies and essential developmental needs, underscores brown and white adipose tissue pivotal role in promoting healthy growth and optimal physiological functioning. Striking a balance between fat and lean tissue gain is vital to support their growth, immune function, and overall metabolic health. While achieving optimal lean growth in young calves is desirable as it leads to faster growth rates, it is equally important to acknowledge that fat deposition serves critical metabolic needs and contributes significantly to overall development and well-being. By understanding the intricate relationship between fat and lean growth, we can better design calf nutrition strategies that promote healthy development while maximizing their growth potential and long-term productivity. These results raise the question: What would be the ideal fat percentage in a young calf?

Converting dietary nutrients to body fat

Understanding how energy is partitioned between lipid and protein deposition in the body is crucial, as these factors represent the body composition of the calf. In growing calves, maintenance requirements account for approximately 40% of the total energy requirements (Labussière et al., 2011). Calves will only deposit fat when energy intake exceeds their energy requirements for maintenance. Therefore, accurately determining maintenance requirements is essential when assessing the extent of lipid and protein deposition in the body. Fasting heat production (**FHP**) is often used as a proxy for maintenance requirements. FHP represents the amount of heat produced by physiological processes upon prolonged fasting when the animal is at rest. It indicates the basal metabolic rate, reflecting the minimum energy needed to sustain essential life functions. In Chapter 3, no significant differences of previous feeding were observed in the estimated FHP among the different treatments, suggesting that the additional supply of fat, lactose, or protein did not impact the overall metabolic rate of the calves.

Lactose

The capacity of young calves to absorb lactose from the digestive tract has been demonstrated to be high (Huber et al., 1984; Gilbert et al., 2015). After absorption, carbohydrates are almost totally oxidized, even when feed intake increases (Van den Borne et al., 2007b). In Chapter 3, we found that also in very young calves, dietary lactose was almost completely oxidized, regardless of other substrates provided. Surprisingly, *de novo* FA synthesis from glucose was negligible in these young calves.

Instead, increased fat deposition rates observed at higher levels of lactose intake were mainly caused by reduced oxidation of FA. This confirms earlier findings for older calves that dietary FA is the predominant precursor of lipid synthesis in young calves (Van den Borne et al., 2007b). Only modest amounts of glucose are utilized for FA synthesis in heavy milk-fed calves with low insulin sensitivity. In calves, the lactate pathway has been suggested to play an important role in oxidative disposal of high levels of lactose intake (Gerrits, 2019), and it was suggested that the rapid decline in insulin sensitivity after birth, as found by Pantophlet et al. (2016) plays a role in this process. The calves in Chapter 3, measured at 3 weeks of age, were likely more insulin sensitive than those studied by Gerrits (2019). Hence, the role of insulin sensitivity in the oxidative disposal of glucose can be questioned. In Chapter 2, while the additional supply of lactose did not enhance protein utilization efficiency, it did increase fat deposition. Based on the differences in energy intake and growth rates observed, clear distinctions in body composition were expected between the treatments.

Fat

In young calves, the role of fat deposition takes on significant importance. Fat supply contributes to approximately 40% of the digestible energy intake in young calves, given their high digestive capacity. However, as feed intake increases, research indicates a reduction in the proportion of dietary FA oxidized, dropping from 80% to around 30% (Van den Borne et al., 2007b). In Chapters 2 and 3, we observed that providing calves with more dietary fat led to increased fat deposition, as anticipated. Interestingly, the extra fat supply did not come with a corresponding increase in protein deposition. This leads us to consider that the increase in fat deposition in response to dietary fat could be a natural mechanism in young calves, designed to efficiently manage energy for storage and foster the development of adipose tissue, aligning with the advantages outlined in the previous section.

Protein

Altering the protein to energy ratio in the diet influences the body composition of preweaned calves. As previously discussed, the protein requirements for pre-ruminant calves depend on their intake and energy levels. In Chapters 2 and 3, we found that extra protein supply did not increase fat deposition in young calves. Conversely, in older calves, increased protein consumption has been linked to increased deposition of both protein and fat, as supported by studies conducted by Lodge and Lister (1973), Donnelly and Hutton (1976), and Gerrits et al. (1996a). Increased lean gain has been observed with increased MR protein content, while greater fat deposition in calves has been associated with high-fat inclusion in diets (Diaz et al., 2001; Tikofsky et al., 2001; Bartlett et al., 2006). Bartlett et al. (2006) elucidated that inadequate provision of protein levels necessary for lean tissue growth may lead to the storage of surplus

dietary energy as fat. Furthermore, Tikofsky et al. (2001) reported that increasing the ratio of carbohydrates to fats in isonitrogenous and isocaloric diets reduced calf body fat deposition. Nonetheless, the potential implications of elevated protein intake during early life, leading to accelerated weight gain and intensified lean tissue growth, on long-term health outcomes in dairy calves remain ambiguous. While the association between protein intake and rapid weight gain during infancy has been acknowledged as a risk factor for later obesity in infant nutrition (Koletzko et al., 2013), its analogous implications in dairy calf nutrition warrant further investigation.

FEEDING STRATEGIES AND CALF DEVELOPMENT

A well-designed calf-feeding system should aim for balanced growth and development while addressing health problems and reducing mortality rates. The choice of an "ideal" system depends on different goals, such as minimizing feed costs, maximizing welfare, lowering emissions, or maximizing future milk production. Thus, an effective calf-feeding approach requires a careful balance among these objectives. In the past years, there has been a preference for using MR due to its consistent nutrient supply compared to WM (Hill et al., 2009). Commercial MR formulations typically have high lactose content (40-50% DM), high protein content (24-26% DM), and lower fat content than WM (16-20%) (Hof, 1980; Park, 2009, Berends et al., 2020). The emphasis on leaner growth and reduced fat deposition in calves has been motivated by the belief that it supports optimal development. However, research has provided insights that challenge the notion of restricting fat deposition during the early months of a calf's life (Brown et al., 2005; Van Niekerk et al., 2021), as adequate fat deposition contributes to crucial physiological functions. Fat serves as an energy reservoir and is essential to some metabolic processes and immune function.

Energy intake during the preweaning phase is defined by MR intake and by the macronutrient composition of MR, primarily fat content, which affects diet energy content. Commercial MR products provide lower metabolizable energy (**ME**) than WM, with MR ranging between 4.6 and 4.7 Mcal of ME/kg DM compared to WM's 5.4 Mcal of ME/kg DM (Drackley, 2008). In Chapters 4 and 5, we examined two MR compositions: high-lactose MR (**HL**), similar to commercial MR formulation, and high-fat MR (**HF**), resembling WM composition. Calves were fed these diets restrictively (~ 8 L of MR/d, 140 g/L) provided in two meals per day, following a step-up/step-down protocol. These studies yielded insights into the effects of MR compositions on calf starter intake, growth performance, intestinal permeability, and blood metabolites, discussed in this section.

Figure 7.4A illustrates the total ME intake from both HF and HL milk replacers (Chapter 4) to examine the variations in ME intake from the perspective of two distinct feeding regimens: *ad libitum* and restricted. This figure includes ME intake from starter consumption in all the systems represented. The total ME intake observed in Chapter 3 reached comparable levels to the voluntary consumption seen in the *ad libitum* system, distinctly higher than the restrictive system. Differences in energy content between the HF and HL milk replacers were compensated for in the HL calf group, primarily through an increased intake of starter feed. Despite the HF calves exhibiting a lower starter intake, both treatment groups managed to achieve similar energy consumption by the end of the study period. In both studies (Chapters 4 and 5), calves fed HF MR exhibited numerical differences in growth performance compared to those fed HL MR, with lower growth rates observed. Although we did not directly measure body composition, drawing insights from the findings of Chapters 2 and 3 and the lack of significant differences in BW and frame size, we hypothesize that HF and HL calves displayed a greater tendency toward increased fat deposition without a corresponding rise in protein deposition. Moreover, comparable results were obtained in Chapter 5, where high levels of lactose or fat in the diet did not affect calf growth, performance, or overall starter intake.

Expanding on these findings, Chapter 4 evaluated the intestinal permeability through the implementation of lactulose-D-mannitol and Cr-EDTA permeability tests, which aimed to assess the mucosal integrity of the small intestine. Interestingly, calves fed an HF MR exhibited higher marker recoveries, indicating a potentially increased gastrointestinal permeability. This outcome raises interesting questions regarding the impact of dietary fat intake on intestinal function. For instance, recent research has demonstrated that calves fed WM exhibited increased urinary recovery of Cr-EDTA and lactulose within the first 6 hours after marker administration. This suggests a higher paracellular permeability in calves receiving WM than those given a MR with a similar macronutrient composition (Mellors et al., 2023). Furthermore, the influence of both WM and MR on intestinal permeability appeared to occur without significant alterations in the gene expression of tight junction proteins, which are critical for maintaining intestinal barrier integrity (Mellors et al., 2023). While we observed higher marker recoveries in response to HF MR feeding, it seems that changes in urinary recoveries of permeability markers such as Cr- or Co-EDTA do not necessarily indicate compromised intestinal barrier function. Despite these differences in recovery, the biological relevance and potential effects on health and performance in young calves require further investigation. Moreover, in contrast to expectations, the HF treatment, with its higher permeability, resulted in calves showing a lower frequency of abnormal fecal scores, particularly in the first weeks and around the weaning period (Chapter 4). This observation suggests that increased recovery of

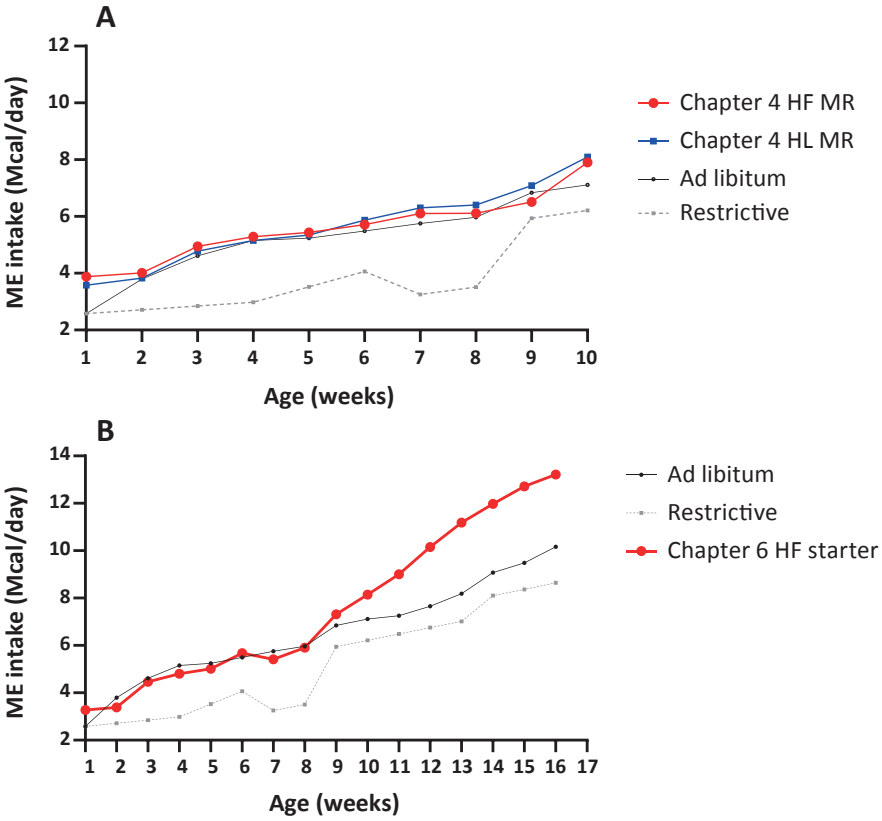

Figure 7.4 Total metabolizable energy (ME) intake (Mcal/day) in: Panel A: *Ad libitum* milk replacer (MR) and starter intake (● solid line; data adapted from Webb et al., 2014) and restrictive, ME from MR and *ad libitum* starter feed (●dotted line; data adapted from Leal et al., 2021) and Chapter 4 High fat (-●- HF) and High lactose (-■- HL) MR and *ad libitum* starter feed. Panel B: Two milk feeding systems and Chapter 6 Restrictive MR and *ad libitum* High fat (-●-HF) starter. *Ad libitum* milk replacer (MR) and starter intake (● solid line; data adapted from Webb et al., 2014) and restrictive, ME from MR and *ad libitum* starter feed (●dotted line; data adapted from Leal et al., 2021) over a period of 4 months of life. The ME values for MR and starter feeds were assumed as 4.07 Mcal/day from MR and 2.7 Mcal/day from the starter feed.

permeability markers is not necessarily associated with diarrhea or indicative of a damaged epithelium. However, it's important to note that nutritional diarrhea, associated with excessive lactose intake, can occur in young calves during their early weeks of life (Hof, 1980). In Chapter 2, the higher concentration of lactose (60% in DM) and the lowest fecal dry matter content (14%) in the extra lactose supply treatment could

potentially surpass the absorptive capacity of calves, leading to adverse effects on nutrient digestibility (Lodge and Lister, 1973). This could result in osmotic diarrhea, which may contribute to increased permeability.

ENERGY INTAKE DURING THE WEANING AND POST-WEANING

Under natural conditions, the weaning process typically involves a gradual reduction in milk intake and an increase in solid food consumption. When the cow and calf are kept together, nursing frequency gradually declines after the first few weeks of life as calves begin to graze and ruminate. However, calves will typically continue to receive milk from the cow until 6 to 9 months of age (Flower and Weary, 2003). In contrast to natural weaning, farmed calves are usually weaned abruptly by separating the newborn from their dam (and thus, the milk supply). Furthermore, farm calves are often weaned much earlier than in nature. They may face additional stressors, such as social changes and moving to unfamiliar pens or barns. It is well-documented that during the weaning process, due to the decreased milk supply and slow increase in starter intake, total energy intake decreases (See Figure 7.3B), and the calves experience a reduction in growth rates, often losing BW for several days during and after weaning (growth check). Some studies have been done to decrease this growth check and improve the weaning methods. These studies have shown that step-down weaning allows the calves to adapt to the low milk supply by gradually increasing starter intake, enough to maintain growth during the weaning transition (Khan et al., 2007; Steele et al., 2017; Welboren et al., 2019).

When larger volumes of milk are offered, weaning strategies play a crucial role in ensuring optimal growth and gut development in young calves. This is particularly significant because these elevated MR programs delay solid feed intake, consequently delaying rumen development (Khan et al., 2007; Terré et al., 2007; Weary et al., 2008). In Chapters 4 and 5, a gradual weaning approach was implemented for the calves, utilizing a stepwise method that ultimately resulted in comparable feed intake levels and growth outcomes. This strategy enabled the calves to counterbalance the decline in nutrient intake from milk by increasing their consumption of starter feed, thereby preserving the growth advantage that had been established prior to weaning. Similar trends of solid feed intake during the weaning phase have been previously recorded in other research studies involving the provision of high milk volumes and the implementation of gradual weaning protocols (Khan et al., 2007; Sweeney et al., 2010; Rosenberger et al., 2017).

With the high energy intake derived from substantial volumes of WM or MR, coupled with relatively lower ME intake through starter feed, the weaning gap becomes a significant concern. The term 'weaning gap' refers to the phase of the weaning process where calves encounter a decline in energy intake (approximately 40% lower ME intake; Leal et al.2021), potentially leading to growth setbacks. Ecker et al. (2015) have illustrated the substantial influence of weaning age and duration on both growth and gut development in calves. For instance, calves weaned at 8 weeks of age exhibited enhanced growth and improved gut development compared to those weaned at 6 weeks. The calves weaned at 8 weeks demonstrated higher starter intake (2.5 kg vs. 1.3 kg/d), ME consumption (6.5 vs. 3.5 Mcal/d), and ADG (1.06 vs. 0.35 kg/d) during the weaning period, in contrast to the 6-week-old counterparts. To address the weaning gap and support calf health and development, it is essential to carefully plan the weaning strategy, considering the energy content of both liquid feed and starter feed. Gradual weaning processes that allow calves to adapt to reduced liquid feed while increasing starter intake gradually can effectively minimize growth setbacks and ensure a smooth transition to solid food. Additionally, providing nutritionally balanced starter feeds with adequate energy levels is vital to support calf growth and development during the weaning phase.

In an attempt to increase the energy density of the starter feed, fat addition has been considered, and it has generally shown different effects before and after weaning. Table 7.1 summarizes various studies demonstrating the opportunities and limitations of including fat in starters. Depending on the type and percentage of fat inclusion, we can observe diverse responses in the DMI of the starter feed and growth performance. In some studies, increasing the fat content in the pellet negatively affected DMI and growth performance (Miller et al., 1982; Kuehn et al., 1994; Hill et al., 2015; Ghasemi et al., 2017), regardless of the fat source. Conversely, other studies reported that DMI and ADG were not significantly affected by the inclusion of fat (Johnson et al., 1956; Miller, 1962; Doolatabad et al., 2020; Ghorbani et al., 2020). However, recent research has demonstrated the positive effects of supplemental fat on both DMI and ADG in calves, particularly when specific fat sources and compositions are employed during and after the weaning period (Berends et al., 2018; Panahiha et al., 2022; Chapter 6, this thesis).

Overall, the responses on DMI and ADG to fat inclusion in the studies (Table 1) can be summarized based on two main factors: (i) levels of fat inclusion, and (ii) fat source and composition.

Table 7.1 Summary of research studies evaluating the effect of fat addition in starter feeds on calf performance.

Study	Fat % in Control	Fat Inclusion	Fat Source	Age (days)	DMI (kg/d)	BW (kg)	DMI response	ADG response
Johnson et al., 1956	3.6	10%	Tallow	91	1.45	98	=	=
Miller et al., 1959	4.0	10%	Brown grease or hydrogenated cottonseed oil	49	0.70	~68	↓	↓
Miller 1962	3.7	10%	Tallow, lard, butter, and hydrogenated cottonseed oil	60	0.64	~77	=	=
Fallon et al., 1986	2.5	5-20%	Ca-soaps	77	1.22	96	↓	↓
Kuehn et al., 1994	3.7	7%	Soybeans	56	2.26	68	↓	↓
Araujo et al., 2014	4.1	11%	Full-fat soybeans	60	1.00	61	=	↑
Hill et al., 2015	4.1	2%	Tallow or soybean oil	56	0.92	76	↓	↓
Ghasemi et al., 2017	3.1	3%	Tallow, soybean oil, hydrogenated palm fat, or a mixture of palm, soybean oil and fish oil	70	1.30	87	↓	↓
Berends et al., 2018	3.3	10%	Hydrogenated palm FA	84	1.50	117	↑	↑
Doolatabad et al., 2020	3.5	7.50%	Full-fat soybeans and Hydrogenated palm fat	70	1.05	85	=	=
Ghorbani et al., 2020	2.8	2%	Soybean oil or extruded or heated soybeans	73	1.20	88	=	=
Chapter 6, this thesis, 2022	3.1	10%	Hydrogenated palm FA	112	2.00	162	↑	↑
Panahiha et al., 2022	3.0	3%	Hydrogenated palm FA	83	1.00	99	↑	↑

Different signs denote: no differences (-), increase (↑) or reduction (↓) of DMI or ADG as the response of fat addition in starter feeds when compared with Control.

(i) Levels of fat inclusion

Increasing the levels of fat in the starter pellet has been found to have certain disadvantages. Higher fat inclusion can negatively impact rumen function, particularly carbohydrate degradation (Doreau, 1997), and may affect pellet hardness and durability (Thomas et al., 1997). Some studies have reported that the inclusion of fat in the starter feed resulted in lower apparent total tract digestibility of DM and CP (Johnson et al., 1956; Miller, 1962; Fallon et al., 1986; Hill et al., 2015; Ghorbani et al., 2020; Panahiha et al., 2022). However, in Chapter 6 of this thesis, the inclusion of 10% of hydrogenated palm free fatty acids (**PFA**) did not impair rumen function or apparent total tract digestibility, which is consistent with the findings of other studies by Berends et al. (2018) and Araujo et al. (2014) with 11% of full-fat soybeans inclusion. The use of specific fat sources, such as PFA, can help overcome some of the limitations associated with higher fat inclusion, providing a solution to optimize calf performance during the weaning period (Chapter 6).

(ii) Fat source and composition

The choice of fat source incorporated into the diet can significantly influence the outcomes of including fat in starter feeds due to the distinct attributes of different sources. Dairy cattle have been exposed to a diverse range of supplementary fats, encompassing varied combinations of individual FA (NRC, 2021). The fat composition refers to the proportion of various fatty acids within a given fat source. Two key factors, esterification, and FA profile, significantly influence the digestibility and subsequent effects of dietary fats. Fatty acids with variations in chain lengths, levels of saturation, or chemical structure (such as rumen inert and unprotected FA) can impact calf performance. The process of esterification, where FA is bound to glycerol to form triglycerides, directly influences how fats are digested in the calf's gastrointestinal tract. For instance, unsaturated fatty acids, particularly those not effectively protected from interacting with the rumen, can exert significant effects, such as modulating the ruminal protozoa population and altering the fermentation processes in the rumen environment. In contrast, rumen-inert fats, characterized by a high degree of FA saturation and elevated melting points, or saponification, exhibit distinct properties that set them apart. These properties make rumen-inert fats less susceptible to interference with rumen fermentation processes (Elliott et al., 1999). Interestingly, the diversity in findings across various studies could be attributed to the variations in fat composition utilized. Ghasemi et al. (2017) noted that distinct fat sources with varying FA profiles led to decreased DMI and ADG compared to a control starter. This decline in performance could be linked to the low apparent tract digestibility of these fat sources (ranging from 68% to 70%). This impact also becomes evident in Chapter 6, where calves fed a starter mixed with an extruded pellet coated with hydrogenated rapeseed triglyceride exhibited low apparent total tract

digestibility of crude fat, showing a decrease in ADG and BW when compared to PFA calves. It is important to note that the outcomes observed in Chapter 6 may be shaped by the extent of esterification and the specific FA profile present in the fat supplement fed.

Figure 7.4B illustrates the total ME intake of the PFA treatment, providing insights into how the energy gap between an *ad libitum* system and the increased energy density of the starter can help ease the weaning transition for calves. Although Chapter 6 involved calves not fed with larger amounts of MR and weaned at 42 days, including a high-fat extruded pellet with hydrogenated free palm fatty acids can shorten the period of low energy intake typically observed in elevated milk-feeding programs with abrupt weaning strategies. The higher ME consumption through solid feed helped compensate for the energy shortage caused by MR withdrawal and the still low starter intake during the weaning period. This addition increased the diet's energy density, leading to improved energy intake and, consequently, a greater growth response in the calves. This could have significant implications for calf growth and development, allowing smoother transitions during this crucial phase of their lives and maintaining the growth advantage during the next rearing phase.

CONCLUSIONS AND IMPLICATIONS

Main conclusions from this thesis

At about 3 weeks of age:

- Protein utilization for growth (gross efficiency ~70%; marginal ~60%) is efficient, although not as efficient as anticipated from body weight extrapolation.
- Only about 31-36% of the extra energy intake from protein, lactose, or fat provided in the MR was effectively retained by the calves as energy.
- Increasing fat or lactose supply leads to an increase (67% and 49%, respectively) in body fat deposition.
- Additional lactose supply promotes higher fat deposition by reducing fatty acid oxidation.
- Protein intake does not contribute to increased fat deposition.
- Extra energy in the form of lactose and fat does not enhance protein efficiency in calves, suggesting that increased energy intake does not necessarily translate into improved protein utilization.
- Extra lactose intake increases physical activity levels in calves, indicating a potential impact on calf behavior.
- Oxidation of dietary lactose is virtually complete, and unaffected by the intake of other nutrients.

- The thermic effect of feeding responded to the feeding level and outflow of digesta from the abomasum.
- When measuring FHP in calves, allowing a time span of over 24 hours is advisable, as this duration ensures that the respiratory quotient (RQ) approaches 0.7.

At 8 weeks of age:
- Exchanging lactose by fat in the MR does not affect calf growth, performance, total starter intake, or apparent total digestion of macronutrients.
- High fat milk replacers can lead to increased appearance of permeability markers in urine, but this does not necessarily reflect compromised barrier function.

At 12 weeks of age:
- Increasing the fat content in the starter feed does not negatively affect starter intake; instead, it contributes to increased ME intakes and BW gain around and after weaning. This partially compensates for the energy intake shortage during the weaning period.

Implications

This thesis provides valuable insights into the effects of protein, fat, and lactose inclusion in milk replacers and starter feeds on calf growth, metabolism, and overall health. The findings suggest optimizing nutrient composition, particularly fat content, in calf diets can contribute to better growth rates, and nutrient utilization in young calves. The knowledge gained from this research improves our understanding of nutrient partitioning in the body of young calves, aiding the development of more efficient feeding strategies. By tailoring diets to meet the specific requirements of calves at various developmental stages, calf production efficiency can be improved, and the environmental impact of the ruminant food production can be reduced.
An accurate definition of protein requirements for dairy calves is important for an appropriate supply of AA for growth and lean tissue accretion while minimizing cost and excess N excretion, especially in the first month of life. This may involve reevaluating the use of high-protein milk replacers. Additionally, recognizing the importance of fat in calf metabolism and the significance of balanced body composition, managing fat deposition in calves may involve using high-fat milk replacers when increased rates of fat deposition are desired. Aligning current calf rearing practices with biological reference points, such as whole milk, energy intake, and fat content, may be an adequate way to set rearing goals.

As the calf rearing industry shifts towards higher planes of nutrition, preventing the loss of pre-weaning gain in later stages due to poor solid feed intake and nutrient digestibility around weaning is essential. This challenge can be addressed through the

strategic implementation of high-fat starter feeds to do not interfere with rumen development, which may increase energy intake and support calf growth before and after weaning.

In the broader context of calf nutrition, this thesis aligns with existing literature and offers insights into the development of a comprehensive calf rearing program (Figure 7.5). This program includes key elements, such as weaning between 8 to 10 weeks of age, employing a milk volume equivalent to 20% of body weight, or adopting *ad libitum* feeding approach. A smooth transition from liquid to solid feeds over a minimum 4-week period, utilizing a step-down method, ensures adequate energy supply, contributing to optimal growth and development.

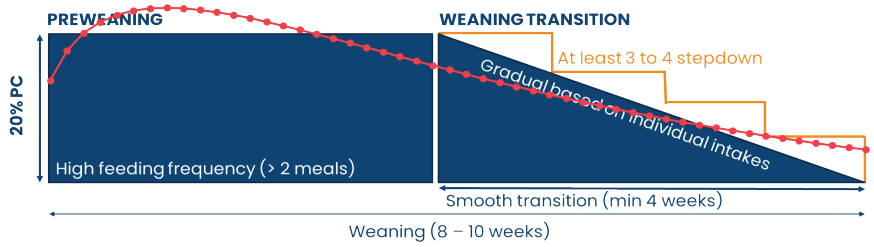

Figure 7.5 Schematic representation of calf rearing during the preweaning and weaning phases

In conclusion, this research sheds light on specific factors such as protein efficiency, fat deposition, and energy intake, all of which play critical roles in shaping effective calf rearing strategies. By aligning the findings displayed in this thesis with established best practices, we can contribute to formulating well-rounded and successful calf rearing programs to support healthy calf development and long-term productivity in the dairy industry.

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SUMMARY

Historically, the amount of milk or calf milk replacer (**MR**) fed to dairy calves has been restricted compared to their natural intake capacity. Due to the higher cost of liquid feeding, this strategy aimed to stimulate starter intake and rumen development and allow for early weaning. However, research over the past decade has demonstrated that restoring milk supply closer to ad libitum levels improves calf's growth and health, and even long-term health and productivity. While the benefits of increased nutrient supply to dairy calves are well accepted, the contribution of the different macronutrients is poorly understood. Particularly, fat content of both liquid and solid dairy calf feed is well below the levels present in cow milk, while carbohydrates are typically higher.

The aim of this thesis was to expand fundamental knowledge that can be used for developing feeding strategies and to provide insights into the effects of protein, fat, and lactose inclusion in MR on the metabolism of calves younger than 21 days of age. In addition, to investigate the short-term impact of restoring fat intakes in early weaned calves, closer to the biological reference of suckling calves, by combining higher inclusions of fat in MR and a starter feed during the first 120 d of life that promotes calf growth and development while minimizing adverse effects related to the shortage of energy intake during and after weaning.

Nutrient requirements of calves younger than 21 days of age, specifically those of calves fed with high levels of MR, are insufficiently understood. The utilization efficiency of macronutrients, especially protein, decreases as calves age, but there is a lack of data for the early weeks of life. It is unclear whether, like older animals, young calves might also face simultaneous limitations in protein and energy for growth. Therefore, In **Chapter 2**, thirty-two groups of 3 mixed-sex Holstein-Friesian calves up to 21 days of age were included to study the incremental responses of an extra supply of protein, fat, and lactose on energy and protein deposition. Over 19 days, calves were fed a basal MR (23.3 % CP, 21.2% CF, and 48.8% lactose of DM), provided at 550 kJ/kg $BW^{0.85}$ per day (**CON**), or the basal MR incrementally supplied with 126 kJ of DE/$BW^{0.85}$ per day as milk fat (**+FAT**), lactose (**+LAC**) or milk protein (**+PRO**). The calves were given two daily meals of the MR and had unlimited access to water, but they were not given calf starter or other solid feed. After two weeks of getting acclimated to their assigned diets, groups of calves were placed for 1 week in an open-circuit respiration chamber for nitrogen and energy balance measurements (5 d). At 21 days of age, calves showed an efficiency of 57% in retaining extra protein for growth, while 44% contributed to heat production. Incremental fat supply exhibited the largest effect on body composition, with 67% of the added fat intake

being retained as body fat, with 22% being released as heat. Likewise, extra lactose supply resulted in 49% being stored as fat with 38% being released as heat. Additional protein supply did not trigger fat deposition. Neither extra lactose nor fat supply increased protein efficiency. In the same experiment (**Chapter 3**), detailed diurnal patterns of energy expenditure, respiratory quotient (**RQ**), fasting heat production (**FHP**), and net rates of carbohydrate and fat oxidation were described. Increasing lactose supply can lead to higher fat retention due to a reduction in fatty acid oxidation. This effect is distinct from the impact of protein and fat supply, which consistently raise resting metabolic rate (**RMR**) throughout the day, while lactose supply primarily affecting RMR after the meal. Glucose oxidation remains relatively unaffected by the extra supply of fat or protein. Moreover, higher protein supply contributes to an increase in heat production (**HP**) compared to similar intakes of fat and lactose. This increase in HP appears to be partly attributed to the oxidation of fatty acids, as indicated by higher fatty acid oxidation rates with extra protein supply. Lastly, the FHP of young, group-housed calves is comparable to literature values and remains unaffected by energy intake. Fasting RQ around 24 hours after a meal was approximately 0.8, indicative of the increasing fat oxidation and declining carbohydrate oxidation patterns as the 24-hour mark approached.

Chapters 4 and 5 investigated the effects of increasing fat inclusion in MR on growth, feed intake, digestibility, gut permeability, and blood metabolites in male Holstein calves up to 70 days of age. In Chapter 4, 60 male Holstein-Friesian calves were fed with either a high-fat (**HF**; 23% fat; 37% lactose) or high-lactose (**HL**; 17% fat; 44% lactose) MR. In Chapter 5, 68 Holstein calves (40 females and 28 males) were assigned to the same treatments as described in Chapter 4. In both experiments, lactose was exchanged by fat on a weight for weight basis, resulting in a 6% difference in metabolizable energy density per kilogram of MR. Throughout the studies, the MR was provided twice daily until 49 d of age, followed by a gradual weaning period of 14 d. Calves had unrestricted access to starter feed, straw, and water. The energy source did not affect gain, total energy, protein intakes, or apparent digestibility. Higher fat inclusion in the MR increased gut permeability but lowered abnormal fecal scores during the initial weeks and weaning phases. Calves consuming the high-fat diet had greater blood NEFA and glucose concentrations than those fed the high-lactose MR. These findings suggest that increasing fat at the expense of lactose provides a viable alternative in MR formulations, aligning more closely with the biological reference while maintaining comparable growth performance and development in young calves.

The weaning process poses a substantial challenge to health and growth performance in young dairy calves, primarily limited by energy intake. However, fat inclusion in solid feeds may limit rumen fermentability and development. The aim of **Chapter 6**

was to evaluate the effects of increasing the energy supply of solids feeds, by including a high-fat extruded pellet into a calf starter feed. To this end, sixty Holstein bull calves were assigned to 1 of 3 treatments: a standard control calf starter (**CON**; 3.1% fat) or two mixtures of CON with 10% inclusion of two different high-fat extruded pellets containing 85% of either hydrogenated free palm fatty acids (**PFA**, 7.1% fat) or hydrogenated rapeseed triglycerides (**RFT**, 6.7% fat) from birth until 112 days of age. Calves were offered the same amount of MR until 42 d of age, followed by a gradual weaning period of 7 d. Calves had *ad libitum* access to the starter diets, straw, and water. A second experiment was conducted to measure differences in digestibility between the two sources of hydrogenated fats and evaluate their eventual relevance on energy supply and growth in postweaning calves. Increasing dietary fat mitigated growth loss during both the weaning and post-weaning phases. Including dietary fat in calf starter feed, characterized by specific esterification and fatty acid composition, can enhance growth, feed intake, and digestibility in rearing calves.

Chapter 7 discusses the energy provision during distinct stages of calf development, namely preweaning, weaning, and post-weaning. The primary focus encompassed the investigation of the mechanisms governing nutrient utilization, along with protein and energy metabolism. The section covers also how dietary elements contribute to protein and body fat accretion in young calves. It particularly highlights the significance of body fat in the health and growth of heifer calves. Additionally, the chapter discusses feeding approaches and the effects of increasing fat intake in early-weaned calves by adjusting fat content in MR and starter feed during their initial 120 days.

Overall, the work of this thesis offers valuable insights into how the inclusion of protein, fat, and lactose in milk replacers, and supplementation of fats in starter feeds, influences calf growth, development, and general health in young calves.

ACKNOWLEDGEMENTS

Success is not final; failure is not fatal: it is the courage to continue that counts. (Winston Churchill).

The most challenging part is to begin, and that applies to projects, life, and writing. However, when it's almost finished, the satisfaction and gratitude are immense. And like everything in life, it's not possible to do it alone. In these few words, I would like to thank all the people who accompanied me in completing this thesis and were present during my Ph.D. journey.

I don't have enough words of gratitude for my supervisors, Javier Martín-Tereso, Leonel Leal, and Walter Gerrits, for all the support, teachings, and patience over these years. Javier (or should I say, sí Señor), thank you so much for giving me the opportunity and the trust to be part of your team. Undoubtedly, working under your leadership has been one of the best experiences of my professional life. I won't forget our long conversations filled with positivity (which I still find hard to fully grasp) that often left me without clear conclusions but with more questions. Nevertheless, they helped me grow both professionally and personally.

Leonel, I still remember our trip to Brazil where we first met, perhaps a year before I came to the Netherlands. I told you about my ambition to continue studying, and if I've reached this point today, it's partly because of your encouraging words, urging me to give it a try. Little did you know that you'd end up supervising me, and it has been quite a journey. Thank you very much for being an excellent supervisor, manager, and colleague. Besides, I greatly appreciate all the hours you've served as my unofficial psychologist – my mental health owes you a tremendous debt of gratitude.

Professor Gerrits (just joking), Walter, thank you for assisting me with your valuable insights and contributions to complete this thesis. I appreciate all the time you dedicated to explaining and, to be honest, teaching me mathematics when we worked on the data analysis for two of the most interesting chapters of this thesis. I'm truly glad that I had the opportunity to meet you. You are one of the most excellent, approachable, friendly, and intelligent person I've had the pleasure of knowing.

I couldn't have embarked on this journey without the support of Trouw Nutrition Mexico, particularly Roberto Tellez and Luis Lauro Gonzalez. Words cannot adequately express my gratitude. First and foremost, thank you for giving me the opportunity to go to Mexico and receive excellent training in the service of livestock farmers. Your enthusiasm for nutrition and animal well-being truly inspired me and played a

significant role in the important decisions I've made in my life. Secondly, I'm thankful for your ongoing support and guidance, which have allowed me to continue learning and pursuing my passion in this field. Roberto, I sincerely appreciate all the knowledge, support, and friendship you've provided since the very beginning.

I had the pleasure of working with remarkable individuals such as John Doelman, Jean-Baptiste Daniel, Kelly Nichols, Juliette Wilms, Dave Seymour, Harma Berends, and Harmen van Laar. You stand out as one of the most diversely interested and scientifically experienced people I've had the privilege of knowing. I want to extend my best wishes to you on your journey to make a significant contribution to creating a better world. This project would not have been possible without the collaboration and constant support from farm technicians and research assistants at the Calf & Beef Research Centre in Boxmeer, the Netherlands. A very special thank you to Natasja, Hanneke, Ada, Mieke, Chantal, and Akke for your help in planning and executing experiments, and for the hard work you put in day by day to ensure excellence in all the results.

To my close friends, Juanita, and Victoria, for their editing help, feedback sessions, moral support, and love, I won't forget your kindness. Cristina, Wendy, Sonia, and Nataly, your unwavering support, and moments of laughter during the stress have made this journey not just bearable but also enjoyable. Your friendship is a treasure I hold dear.

To my many PhD colleagues and friends, especially Ilaria, Sebastian, Marcela, Corentine, Evelien, Gonzalo, and Tom, thanks for the friendship, for letting me crash on your couch so many times after a good night of beers in Wageningen, and for making me feel like part of your family. I liked sharing our complaints about PhD life, science, and the world in general with you. To Sofia, Aracely, Esther, Diana, and Amalia, thank you for all the good times, the friendship and for always being there. I would also like to express my gratitude to Fabian for your constant encouragement, love, and support.

Lastly, I would like to extend my sincere gratitude to my family, especially my mother Mercedes and my sister Maritza. Thank you for your boundless love and for believing in me even when I doubted myself. Your unwavering faith in my abilities has been a driving force in my success. Your presence during both the triumphs and the challenging moments has been an endless source of strength and motivation.

This achievement wouldn't have been possible without you all. It was a great, inspiring, and exhausting time at the same time, with highs and lows. However, when I look back, all I see now is a successful, happy, and fulfilling journey that I was able to experience together with you. I felt I was always in the right place with you, and I am happy to be able to incorporate these positive experiences into my future and continue to grow.

Thank you for sharing this journey with me.

Liliana

CURRICULUM VITAE

Liliana Amado is originally from Bogotá, Colombia. In 2009, she graduated with a BSc in Veterinary and Animal Sciences from the San Martin University. After working as a veterinarian in dairy and beef farms in Colombia, she relocated to Mexico to work for Trouw Nutrition as a technical advisor in calf rearing in one of the largest dairy regions in the country. There, Liliana was responsible for the development of nutritional and management programs for farms with over 3000 lactating cows. After experiencing first-hand the challenges of dairy producers on calf rearing, she decided to continue her education through a doctoral program in animal nutrition at Wageningen University in the Netherlands, to fill the knowledge gaps she had experience in practice. During her Ph.D., Liliana has conducted research on the developmental, health, and metabolic effects of high-energy diets (milk replacers and starters) in calves from birth to 3 months of age, culminating in her thesis. Liliana is set to continue her work with Trouw Nutrition, focusing on calf nutrition.

LIST OF PUBLICATIONS

Amado, L., Berends, H., Leal, L. N., Wilms, J., Van Laar, H., Gerrits, W. J. J., and Martín-Tereso, J. (2019). Effect of energy source in calf milk replacer on performance, digestibility, and gut permeability in rearing calves. Journal of dairy science, 102(5), 3994-4001.

Amado, L., Leal, L. N., van Laar, H., Berends, H., Gerrits, W. J. J., and Martín-Tereso, J. (2022). Effects of mixing a high-fat extruded pellet with a dairy calf starter on performance, feed intake, and digestibility. Journal of Dairy Science, 105(10), 8087-8098.

Echeverry-Munera, J., **Amado, L.**, Berends, H., Leal, L. N., Steele, M. A., and Martín-Tereso, J. (2023). Effect of partial exchange of lactose with fat in milk replacer on performance and blood metabolites of Holstein calves. JDS communications, 4(1), 19-24.

Amado, L., Leal L.N., Berends H., van Keulen P., Martín-Tereso J., and Gerrits W.J.J. Responses to incremental nutrient supply on energy and protein metabolism in pre-weaning dairy calves. Submitted to Journal of Dairy Science.

Amado, L., Leal L.N., Berends H., van Keulen P., Martín-Tereso J., and Gerrits W.J.J. Incremental supply of lactose, protein or fat influences the diurnal pattern of heat production and substrate oxidation in pre-weaning dairy calves. Submitted to Journal of Dairy Science.

TRAINING AND SUPERVISION PLAN

Completed in the fulfilment of the requirements for the education certificate of Wageningen Institute of Animal Science (WIAS), total 32 ECTS[1] as accredited by WIAS.

	Year
Basic package (3 ECTS)	
WIAS Introduction Course on Personal Effectiveness for your PhD	2021
WIAS Introduction Day	2022
WGS Scientific Integrity course	2023
WGS Ethics and Animal Sciences course	2023
Disciplinary Competences (14 ECTS)	
Introduction to Laboratory Animal Science	2018
Species-Specific Laboratory Animals Course: Ruminants	2018
Indirect Calorimetry (course, Brazil)	2019
Advanced Statistics Course Design of Experiments	2020
Advances in Feed Evaluation Science	2021
Writing research proposal	2021
Healthy and Sustainable Diets: Synergies and Trade-Offs	2023
Professional Competences (4 ECTS)	
Project and time management	2021
Critical thinking and argumentation	2022
WIAS course the Final Touch: writing the General Introduction and Discussion	2022
Intensive Writing Week	2022
Supervising BSc and MSc thesis students	2022
WGS Reviewing a scientific manuscript	2023
WGS PhD workshop carousel	2023
Societal Relevance (1.5 ECTS)	
WIAS course Societal Impact of your Research	2021

[1] One ECTS equals a study load of 28 hours

	Year
Presentation Skills (4 ECTS)	
"Effects of inclusion of a high-fat extruded pellet mixed with a conventional pellet in rearing calf", ADSA, Florida, USA, oral presentation	2020
"The effect of incremental nutrient responses on energy and protein metabolism in pre-weaning dairy calves", ISEP, Granada, Spain, oral presentation	2022
"Effects of inclusion of a high fat extruded pellet in starter feeds on digestibility for rearing calves", EAAP, Portugal, poster presentation	2022
Jornada internacional de rumiantes TN, Spain, oral presentation	2022
"The effect of incremental nutrient intake in the diurnal pattern of heat production and substrate oxidation in pre-weaning Holstein calves", European Buiatrics Congress, Berlin, Germany, poster presentation	2023
Teaching competences (5.6 ECTS)	
Supervision BSc thesis students	2021
Supervision MSc thesis student	2022
Supervision Ruminal evacuation practical (course Biology of Domestic Animals, WUR)	2022-2023

LIST OF ABBREVIATIONS

AAs	amino acids
ADF	acid detergent fiber
ADG	average daily gain
ATTD	apparent total-tract digestibility
BHB	β-hydroxybutyrate
$BW^{0.85}$	metabolic body weight
CF	crude fat
CMR	calf milk replacer
CON	control
COX	carbohydrate oxidation
CP	crude protein
DE	digestible energy
DM	dry matter
DMI	dry matter intake
ER	energy retention
FA	fatty acids
FFA	free fatty acids
FHP	fasting heat production
FOX	fat oxidation
GE	gross energy
H_{act}	activity related heat production
HF	high-fat
HL	high-lactose
HP	heat production
ME	metabolizable energy
MR	milk replacer
N	nitrogen
NDF	neutral detergent fiber
NEFA	nonesterified fatty acids
NR	nitrogen retention
PFA	hydrogenated free palm fatty acids
RFT	hydrogenated rapeseed triglycerides
RMR	resting metabolic rate
RMR_{max}	maximum resting metabolic rate
RQ	respiratory quotient
SE	standard error
SEM	standard error of the mean
TG	triglycerides
VFA	volatile fatty acids
WM	whole milk
+FAT	basal MR incrementally supplied with milk fat
+LAC	basal MR incrementally supplied with lactose
+PRO	basal MR incrementally supplied with milk protein

COLOPHON

The research described in this thesis was financially supported by Trouw Nutrition Research & Development. Financial support from Trouw Nutrition Research & Development for printing this thesis is gratefully acknowledged.

Cover concept: Liliana Amado Barrantes

Design and Layout: Promotie In Zicht (www.promotie-inzicht.nl)

Printing: Promotie In Zicht