Effects of fat inclusion in starter feeds for dairy calves by mixing increasing levels of a high-fat extruded pellet with a conventional highly fermentable pellet

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ABSTRACT

The objective of this study was to evaluate the effect of inclusion of an extruded high-fat pellet mixed with a conventional pelleted calf starter on energy intake and performance around weaning in calves. To this end, 75 female Holstein newborn calves (41.0 ± 4.98 kg) were randomly assigned to 1 of 5 iso-nitrogenous solid feed treatments consisting of 4 levels of fat inclusion by mixing a low-fat highly fermentable control pellet with 3 different levels of inclusion of an extruded high-fat pellet [control (100:0), 90:10, 80:20, and 70:30], and a high-fat single pellet (HFSP). The HFSP was equivalent iso-energetic and iso-nitrogenous, although it had almost 1 percentage point difference in fat relative to the 80:20 treatment, to contrast the effect of the dual-component pellet mixture. The extruded high-fat starter feed contained a high proportion of fat (38%), mainly from hydrogenated palm fatty acids. Calves were offered a milk replacer up to 900 g/d, and then pre-weaned at 49 d of age by halving milk allowance until 56 d when calves were weaned. Calves had ad libitum access to the starter diets, chopped straw, and water. Individual milk replacer and starter intakes were recorded daily and BW was determined weekly. A glucose tolerance test was performed at 49 and 84 d of age to evaluate blood glucose homeostasis. Apparent total-tract digestibility was determined from 70 to 75 d of age. Calves on the 90:10 treatment had greatest starter feed intake mainly due to a marked increase in solid feed intake around weaning. Metabolizable energy intake was increased when the extruded pellet was included in the starter. No differences were present in digestibility of ether extract among solid feed treatments. The area under the

curve of blood glucose concentration after the glucose tolerance test was greatest in 80:20; intermediate in 70:30, HFSP, and control; and lowest in 90:10 calves. No differences were observed for insulin or other parameters related to blood glucose homeostasis. Delivering dietary fat by mixing an extruded high-fat pellet with a conventional highly fermentable pellet to reach a total fat content of 7% results in increased starter intake, energy intake, and body weight gain until 84 d of age compared with a conventional low-fat pellet, or a single pellet with increased fat content.

Key words: calf, extrusion, solid feed, weaning stress

INTRODUCTION

Dairy calves are born without a fully functioning rumen, and they initially rely on milk or milk replacer (MR) to obtain the necessary nutrients to sustain growth (Khan et al., 2016). Irrespective of physical presence of the dam, ad libitum consumption of milk or MR has been reported to reach a maximum of around 12 L of milk per day (Jasper and Weary, 2002; Berends et al., 2016), distributed in >6 meals/d (Berends et al., 2016). In commercial dairy production systems, calves are transitioned from milk to solid feed as early as possible to minimize labor costs. Early weaning methods were traditionally developed based on the encouragement of solid feed intake by restricting milk feeding below 50% of ad libitum intake (Bach et al., 2017). Over the past decade, the industry has shifted toward greater milk supply in early life because of greater growth and feed efficiency in early life, increased long-term survival (Bach, 2011), and lifetime milk production (Bach, 2012; Soberon et al., 2012). However, large milk allowances delay solid feed intake, and thus rumen development (Terré et al., 2007) and growth around weaning (Cowles et al., 2006; Terré et al., 2006; Eckert et al., 2015).

Research on starter feeds has mainly focused on stimulating solid feed intake (Khan et al., 2011) to

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foster the initiation of rumen fermentation and accelerating rumen development (Baldwin et al., 2004). Only recently, Araujo et al. (2014) demonstrated benefits of increasing energy content of solid feeds by inclusion of full-fat soybean in pelleted starters when providing 750 g/d of MR. Fat inclusion in starter feeds has commonly been disregarded because it may negatively influence rumen development as it provides energy to the calf but not to rumen bacteria (or very little). Furthermore, when fat and fermentable ingredients are combined in one pellet, fat may physically impregnate fermentable components, limiting bacterial access to these particles and thus hampering potential microbial growth and rumen development. Rumen inert fat sources are available in the form of hydrogenated fat prills and calcium soaps, and are commonly used to overcome technological limitations associated with fat inclusion in pelleted feeds. First, these fat sources lose their physical form when they melt during the pelleting process and soak or coat other feed components, which in calves has been shown to compromise intake (Kazemi-Bonchenari et al., 2016). Calcium soaps also appear to be detrimental to solid feed intake (Fallon et al., 1986), and at high inclusion rates (i.e., >3%) pellet quality is impaired (Thomas et al., 1998). Feeding rumen inert fats separate from the pellets is not an option because of their poor palatability, and homogeneous mixing to prevent sorting is unfeasible because their particle size is much smaller in comparison with pellets. Alternatively, extrusion and vacuum coating feed technology allows for high inclusions (e.g., 30–60%) of fat in pellets, which is a common practice in fish and pet feed manufacturing, but not used for calf starters. The similar size of these pellets offers an opportunity to homogeneously mix them with conventional pellets, preventing sorting by calves, and in this way facilitate the inclusion of fat in solid feeds for calves.

The underlying hypothesis of the study was that supplying increasing levels of fat in starter feeds along with generous amounts of MR would improve performance of young calves. Furthermore, offering fat in a separate pellet and then mixing it together with another pellet rich in carbohydrates would increase the energy content of the TS feed offered to calves without impairing rumen fermentation and compromising feed palatability. Therefore, the objective of the current study was to evaluate the effects on performance between birth and 4 wk postweaning of different levels of fat supplementation in starter feeds by combining conventional low-fat pellets with a high-fat pellet (manufactured by extrusion and vacuum coating technology), and to contrast this with simply adding an equivalent amount of fat to a complete pellet.

MATERIALS AND METHODS

Animals and Feeding Program

A total of 75 female Holstein newborn calves (41.0 ± 4.98 kg of BW) from a single farm were enrolled in this study between March and June 2016. Calves were managed under common animal management conditions under the supervision of Institut de Recerca i Tecnologia Agroalimentàries technicians and the approval of the Animal Care Committee of Institut de Recerca i Tecnologia Agroalimentàries (Barcelona, Spain). Calves were individually housed in hutches (1.20×1.45 m) with access to an outdoor area (1.20×1.25 m) equipped with one bucket for water, one for concentrate, and one for forage. Hutches were bedded with sawdust on a daily basis and calves were bottle-fed MR. Within the first 6 h of life, calves received 3.5 L of colostrum (previously thawed) using an esophageal tube. Colostrum quality was tested for density using a colostrometer and only colostrums with >50 mg/mL of IgG were frozen and used in this study. Calves were fed 3 L of MR (Sprayfo Excellent, Trouw Nutrition, Deventer, the Netherlands) containing 23.2% CP (all of milk origin) and 18.6% fat (on a DM basis) twice a day at 12.5% concentration of MR powder (750 g/d as fed) for the first week, and then 2 meals of 3 L at 15% concentration (900 g/d as fed) until 49 d of age. Then, MR was limited to a single offer of 3 L also at 15% (450 g/d as fed) until 56 d when calves were fully weaned. For the duration of the study (84 d), calves had ad libitum access to water, pelleted starter feed, and chopped (about 3.0 cm in length) barley straw (2.6% CP, 80.5% NDF, and 51.8% ADF on a DM basis).

Solid Feed Treatments

Calves were randomly assigned to 1 of 5 iso-nitrogenous solid feed treatments consisting of 4 levels of fat inclusion by mixing a low-fat control pellet with different proportions of an extruded high-fat pellet [control (100:00), 90:10, 80:20, and 70:30] leading to target final fat concentrations of the pellet mix of 3.5, 7.0, 11.0, and 14.0% (DM basis), respectively, as well as a high-fat single pellet (**HFSP**) with a fat (11%), protein, and energy content equivalent to the 80:20 treatment, to contrast the effect of the dual-component pellet mixture against a high-fat single-pellet. Table 1 depicts the composition of the 3 pelleted feeds, and Table 2 shows the relative mixture proportions of each pellet and the analyzed nutrient composition of the 5 solid feed treatments.

Pelleted feeds were produced (Forfarmers, Lochem, the Netherlands) with a pellet die diameter of 5 mm.

Table 1. Formulated ingredient and analyzed nutrient composition of the 3 pelleted feeds used to compose the 5 solid feed treatments

Item	Pellet type		
	Low fat	High-fat extruded	High-fat single pellet
Ingredient, % of DM			
Corn	34.7	12.5	30.2
Soybean hulls	13.6		11
Wheat	9.7	10.2	10
Corn gluten meal	7.3	15.8	9
Soybean meal	8.2	10	8.5
Hydrogenated palm fatty acids		40	8
Beet pulp	7.6	6	6.7
Wheat middlings	6.6		5.3
Beet molasses	4.4	1.8	4.4
Rapeseed meal	5		4
Limestone	1.5	1.5	1.4
Magnesium oxide	0.2	0.4	0.2
Sodium chloride	0.7	0.8	0.7
Monocalcium phosphate	0.1	0.7	0.1
Mineral and vitamin premix ¹	0.4	0.4	0.4
Nutrient composition			
CP, %	17.1	17.3	17.1
NDF, %	29.5	6.9	24.4
ADF, %	14.9	3.33	12.8
Ether extract, %	3.3	37.5	11.0
Ash, %	6.2	5.3	5.9
NFC, %	43.9	33.0	41.6
ME, Mcal/kg	2.62	5.14	3.12

¹Premix (provided per kilogram of concentrate): 10,476 IU/kg of vitamin A; 2,381 IU/kg of vitamin D₃; 27 mg/kg of vitamin E; 0.3 mg/kg of Co (CoCO₃); 0.95 mg/kg of I (CaI); 129 mg/kg of Fe (FeSO₄); 19 mg/kg of Mn (MnO); 32 mg/kg of Zn (ZnO); 19 mg/kg of Cu (CuSO₄); and 0.30 mg/kg of Se (NaSe).

The extruded feed was produced at the facilities of Feed Design Lab (Venray, the Netherlands) using an extruder and vacuum coater. Before extrusion, the mixed meal (Forfarmers, Lochem, the Netherlands) was ground on a 1-mm screen, and thereafter, sieved on a 1.5-mm screen to remove oversized particles. Before extrusion, 5% of hydrogenated palm fatty acid prills were included in

the meal. The feeding rate of the extruder was adjusted to reach a target bulk density of 400 g/L. Conditioning was regulated to reach 25 to 28% of moisture (e.g., from ingredients plus water and steam), minimum temperature was 95°C, and steam pressure of the conditioner was set at 200,000 Pa. The outlet of the extruder held 6 die inserts with a conical inlet and a measured land

Table 2. Pellet mix and analyzed nutrient composition of the 5 solid feed treatments

Item	Starter feed ¹				
	Control	90:10	80:20	70:30	HFSP
Ingredient, % of total starter feed					
Low-fat pellet	100	90	80	70	—
High-fat extruded pellet	—	10	20	30	—
High-fat single pellet	—	—	—	—	100
Nutrient, DM basis					
CP, %	17.1	17.1	17.1	17.2	17.1
NDF, %	29.5	27.4	25.1	22.6	24.4
ADF, %	14.9	13.7	12.4	11.3	12.8
Ether extract, %	3.3	6.8	10.1	13.4	11.0
Ash, %	6.2	6.1	6.0	5.9	5.9
NFC, %	43.9	42.8	41.7	40.6	41.6
ME, ² Mcal/kg	2.62	2.87	3.12	3.38	3.12

¹Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20 = 80% control, 20% high-fat; 70:30 = 70% control, 30% high-fat; HFSP = high-fat single pellet.

²Calculated following NRC (2001).

length of 6.7 and 4 mm diameter. Total die open area was 75.4 mm². After extrusion, the product was dried at 110°C for about 30 min and subsequently conveyed from the dryer to the coating line pneumatically. Then, a vacuum coater was used to flood the hot pellets with approximately 35% heated hydrogenated palm fatty acids. Thereafter, the product was cooled and packed.

Measurements

Calves were weighed using an electronic weighing scale at birth and on a weekly basis thereafter. Individual MR and solid feed consumption (both pellets and straw) were determined daily by measuring leftovers. Feeds and pooled refusals were analyzed for DM (4 h at 103°C), ash (550°C calcination), and CP with and automatic distiller Kjeldahl (Kjeltec Auto 1030 Analyzer, Tecator) with copper sulfate/selenium as a catalyst instead of copper sulfate/titanium dioxide (method 988.05; AOAC International, 2000), NDF was analyzed with sodium sulfite and heat-stable α -amylase (Van Soest et al., 1991), ADF was analyzed following (Robertson and Van Soest, 1981), and ether extract following the method 920.39 from AOAC (1990) with petroleum ether used for distillation instead of diethyl ether (AOAC International, 2000) with previous acid hydrolysis (Stoldt, 1952).

At 49 and 84 d of life, 10 calves per treatment were randomly selected, blocked by initial BW, and subjected to a glucose tolerance test (**GTT**) to assess insulin sensitivity following Bach et al. (2013). Briefly, each calf received an intravenous infusion of 180 mg/kg of BW of glucose 5 h after the morning meal. The 5-h interval from last meal was chosen because it has been proposed that changes in insulin sensitivity in calves are more evident after eating, when a decrease occurs in the number of insulin receptors (Hugi et al., 1998). An indwelling catheter (Abbocath-T 16 gauge $\times$ 140 mm; Hospira Inc., Lake Forest, IL) was placed in the left jugular vein. The catheter was used to infuse glucose (500 g/L of glucose anhydrous; B. Braun Medical, Terrassa, Spain) and to collect blood samples. Blood was harvested in 4-mL evacuated tubes containing a glycolysis inhibitor (BD Vacutainer fluoride tubes, Becton Dickinson, Madrid, Spain) to determine plasma glucose concentrations, and 5-mL evacuated tubes containing EDTA (BD Vacutainer serum tubes) for plasma insulin determinations. Blood samples were obtained at -15, -5, 0, 4, 8, 12, 18, 25, 35, 45, 60, and 120 min relative to glucose infusions. Catheter patency was maintained by flushing with 5 mL of heparinized saline solution (1,000 USP units of heparin/mL).

At 21, 49, 56, 70, and 84 d of life, feeding, resting, and rumination behavior of all calves was monitored for 4 h after the morning feeding using scan sample technique as described elsewhere (Terré et al., 2013). On the same days, blood samples were obtained before the morning feeding to determine plasma concentration of β -OH-butyrate and cholecystokinin (**CCK**). Plasma concentrations of β -OH butyrate were determined using a colorimetric technique with a commercial kit (Rand-but, Randox Laboratories Limited, Crumlin, United Kingdom) and CCK plasma concentrations were measured using RIA as described by Villalba et al. (2011) with an intraassay coefficient of variation of less than 2.6%.

The kit used for CCK determination binds to CCK-8 sulfate and cross-reacts with CCK-33 sulfate.

Total-tract DM and ether extract digestibility was performed on 7 calves per treatment, which were randomly chosen between 70 and 75 d of age using indigestible NDF as a marker to estimate fecal output (Oba and Allen, 2003). Indigestible NDF was determined after 120 h of in vitro rumen incubation following Goering and Van Soest (1970).

Pellet hardness (which measures the compression force required to fragment a pellet into small particles and fines expressed in kg) was determined following the method described by Thomas and van der Poel (1996), and pellet durability (expressed as the ratio of the weight after tumbling over the weight before tumbling) was determined following the procedure described by Pfof (1963). In brief, pellet hardness was assessed using a Kahl device, and pellet durability was determined using a tumbling rotating device and quantifying the proportion of particles <2.5 mm.

Calculations and Statistical Analysis

Sorting activity for fat in the solid feed was calculated as the actual fat intake expressed as a percentage of the predicted intake, which was calculated as the difference between the amount of fat in the feed offered and that in the feed refused. The predicted fat intake was calculated as the product of DMI of the total diet times the percentage of fat in the offered feed. Values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for). Values for sorting were tested for potential difference from 100% using a *t*-test.

Calculations for insulin sensitivity followed Bach et al. (2013). In brief, blood samples collected at -15, -5, and 0 min relative to glucose infusion were averaged

to determine baseline concentrations of both glucose and insulin. Then, the area under the curve for these 2 metabolites was calculated as the increase with respect to the baseline using the trapezoidal method.

Performance and blood data were analyzed using a mixed-effects model accounting for the fixed effects of treatment, time of measurement, and their 2-way interaction, and the random effect of calf. Time entered the model as a repeated measure using an autoregressive covariance matrix. Digestibility data were analyzed using an ANOVA with treatment as the main effect. Behavior data were summarized individually as the total time (min) devoted to each monitored behavior. Then, each behavior was expressed as a percentage of total observed behaviors and the data analyzed in the same fashion as the performance data.

RESULTS AND DISCUSSION

The current study was designed to evaluate potential differences on performance between birth and 4 wk postweaning of different levels of fat supplementation in solid feeds by either combining regular pellets with a high-fat pellet in extruded form or by directly feeding a regular HFSP. Higher inclusion levels of the extruded high-fat pellet increased ME content by fat inclusion; however, the nutrient content of the 80:20 pellet combination and that of HFSP was almost identical (with the exception of slightly lower ether extract content in 80:20 than in HFSP), yet the fat was included in a different physical form. Because solid feed treatments were iso-nitrogenous across the different inclusion levels of the extruded pellet, energy:protein ratio changed (Table 2); thus, the potential effect of different energy:protein ratio on intake or other relevant output parameters among some solid feed treatments cannot be separated from the net addition of fat. This is an important aspect to take into consideration when evaluating the effects reported herein. Additionally, feed and energy intake are response variables that can influence other parameters.

All calves consumed their daily MR allowances throughout the study. Starter feed intake was greatest ($P < 0.05$) in 90:10 calves and lowest for the HFSP (Table 3). Previous studies found that increasing the fat content of starter reduced intake (Miller et al., 1959; Kuehn et al., 1994), whereas other researchers observed no negative effect (Johnson et al., 1956; Araujo et al., 2014). These differences in outcome could be explained primarily by the differences in fat sources and to a lesser extent by weaning age and management. For instance, Araujo et al. (2014) reported that fat content of starter feed had no effect on solid feed intake when providing

750 g/d of MR and using full fat soybean as a fat source in the starter feed, which is known to be highly preferred by calves (Montoro and Bach, 2012). However, a similar study (Hill et al., 2015) found reduced intake when feeding starter feeds rich in fat. The different results between Araujo et al. (2014) and Hill et al. (2015) could be linked to the type of fat (e.g., saturation) and the way it was presented to calves. Araujo et al. (2014) used whole soybeans as a source of fat, whereas Hill et al. (2015) used either tallow or soybean oil. Nevertheless, from a feed technology standpoint, the inclusion of fat in single-pellet starters is limited because it negatively affects pellet hardness and durability. In fact, hardness herein was lower in the pellets with high fat, although durability was equivalent among treatments. For low-fat control pellet, extruded high-fat pellet, and HFSP, pellet hardness was 8.1, 5.9, and 5.1 kg, respectively, and pellet durability was 97.8, 97.7, and 97.6%, respectively. Because pellet durability (associated with proportion of fines) was similar among treatments, it is unlikely that differences in intake were due to physical aspects of the calf starter feed in this study. Differences in solid feed intakes were most apparent in the period between weaning, at 8 wk of age, and at the end of the study at 12 wk (Figure 1). No differences were observed in straw intake among treatments, and as previously reported (Castells et al., 2013, 2015), calves maintained the ad libitum consumption of straw to <10% of TS feed intake.

As expected (and designed in the experiment), calves in the 70:30 treatment consumed the greatest amount of fat, followed by calves in 80:20 and HFSP that consumed the same amount of fat, and control calves consumed the lowest, with 90:10 calves consuming intermediate values (Table 3). Intake of ME was lowest for control and HFSP calves (Table 3) as a result of the lowest starter ME content in control and low starter feed intake in HFSP calves (Table 2). As occurred with starter intake, differences in ME intake were also more evident between 9 and 12 wk (Figure 2). Interestingly, control and 90:10 calves consumed the same amount (Figure 3A) of fermentable (low-fat pellet), but 90:10 calves still consumed additional energy because they also ingested high-fat pellets (Figure 3B). These results might suggest that maximum intake was limited by the fermentation capacity of the rumen because the amount of fermentable pellets (low-fat) consumed by calves was the same for control and 90:10 treatments. Thus, we hypothesize that calves have a ceiling for maximum starter intake dictated by the capacity of the rumen to ferment solid feed, and that a nonfermentable, high-fat pellet allows calves to consume more energy beyond their maximum capacity to consume fermentable feeds.

Figure 1. Solid feed intake as affected by treatment. Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20 = 80% low-fat, 20% high-fat; 70:30 = 70% low-fat, 30% high-fat; HFSP = high-fat single pellet. *Denotes differences ($P < 0.05$) between 90:10 and the rest of treatments; **Denotes differences ($P < 0.05$) between 90:10 and HFSP. †Denotes differences between 90:10 and the rest of treatments and between 80:20, 70:30, and control versus HFSP. ††Denotes differences between 90:10 and the rest of treatments and between 80:20 and control versus control and HFSP. Error bars indicate SE.

In that sense, observed effects are expected to depend on weaning age and strategy. Rumen development is considered incomplete before 8 wk of age (Meale et al., 2017). More gradual (Meale et al., 2015) or delayed weaning (Eckert et al., 2015; Wood et al., 2015) could allow more time for rumen development and reduce the contrasts among treatments. On the other hand, calves on the 80:20 or 70:30 treatments did not consume more ME than 90:10 calves, and thus it seems that in these cases feed intake was limited by total energy supply from solid feed. In fact, plasma CCK concentrations

were greatest in 80:20 calves, although did not differ from those in 90:10, 70:30, and HFSP (Table 4). Cholecystokinin is a gut peptide secreted in response to the presence of fat and protein in the upper small intestine (Liddle et al., 1985). Increased concentrations of CCK have been linked to depression of appetite and intake in both adult cattle (Allen and Piantoni, 2013) and young calves (Montoro et al., 2012), and thus it could be speculated that CCK or other hormones could be acting at a metabolic level limiting intake of solid feed when the concentration of fat in the solid feed is high. In fact, in the current study, plasma CCK concentrations followed the same pattern as fat intake. Lastly, consumption of ME with the HSFP treatment was equivalent to that of control calves, and in this case, it could be speculated that intake was limited by both a lack of fermentation activity in the rumen (and thus poor rumen development) and a high energy density of the starter feed (and metabolic inhibition of intake). However, blood β -OH-butyrate concentrations, which were similar among treatments except for HFSP, which was greatest (Table 4), a priori, do not seem to corroborate the hypothesis of limited rumen development with HFSP treatment. Blood β -OH-butyrate concentrations have been associated with rumen development because it is a product of rumen cell wall while metabolizing butyrate (Weigand et al., 1972). However, blood β -OH-butyrate concentrations could also have originated from the partial oxidation of fatty acids (from both MR and high-fat starter feeds) in the liver, which is increased in high-fat diets due to alterations in the oxidation process within the mitochondrion (Vial et al., 2011). An alternative hypothesis for the equivalent ME consumption between HSFP and control calves could be found in a decreased apparent DM digestibility with

Table 3. Performance and intake of young calves until 12 wk of age as affected by treatments

Item	Treatment ¹					SE	P-value ²	
	Control	90:10	80:20	70:30	HFSP		T	T × t
Starter feed, g/d	1,337 ^b	1,527 ^a	1,375 ^b	1,304 ^{bc}	1,224 ^c	33.9	<0.001	<0.01
Straw intake, g/d	40.6	44.3	51.0	31.2	62.2	20.4	0.25	0.96
Fat intake, g/d	14.2 ^d	20.1 ^c	23.6 ^b	27.6 ^a	23.5 ^b	0.29	<0.01	<0.01
ME intake, ³ Mcal/d	6.17 ^b	7.06 ^a	6.98 ^a	7.08 ^a	6.47 ^b	0.096	<0.01	<0.01
Initial BW, kg	41.1	40.2	40.3	42.2	41.3	1.31	0.82	—
Final BW, kg	113.7	117.1	114.8	116.1	115.1	2.45	0.89	—
BW gain, kg	73.6 ^b	75.4 ^a	74.1 ^b	74.9 ^{ab}	74.5 ^{ab}	0.67	0.02	0.99
Feed efficiency, ⁴ %	50.2	49.5	49.7	52.6	52.5	1.19	0.13	0.96

^{a-d}Values with uncommon superscripts differ at $P < 0.05$.

¹Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20 = 80% control, 20% high-fat; 70:30 = 70% control, 30% high-fat; HFSP = high-fat single pellet.

²T = effect of treatment; T × t = interaction between treatment and time.

³Calculated following NRC (2001).

⁴Calculated as [weight gain (g/d)/feed intake(g/d)] × 100.

Figure 2. Intake of ME (Mcal/d) as affected by treatment. Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20: 80% low-fat, 20% high-fat; 70:30 = 70% low-fat, 30% high-fat; HFSP = high-fat single pellet. *Denotes differences ($P < 0.05$) between 90:10, 80:20, and 70:30 versus control. **Denotes differences ($P < 0.05$) between 70:30 and 90:10, and between 90:10, 80:20, and 70:30 versus control and HFSP. †Denotes differences ($P < 0.05$) between control and HFSP. ††Denotes differences ($P < 0.05$) between 90:10 and 70:30 versus 80:20, and between 90:10, 80:20, and 70:30 versus control and HFSP, and differences between control and HFSP. Error bars indicate SE.

HFSP (Table 5). Total-tract apparent DM digestibility was greatest in calves fed 80:20 and 70:30 and lowest for calves fed HFSP, but no differences were observed among treatments on total-tract apparent digestibility of ether extract (Table 5). Interestingly, DM apparent digestibility was substantially greater in 80:20 than in HFSP calves. The fact that both treatments had similar amounts of fat clearly illustrates the negative effects on digestibility of mixing high levels of fat in a single pellet.

The observed differences in DM and ME intake were not reflected in differences in final BW. However, BW gain was greatest with the 90:10 treatment, although it did not differ from the BW gain obtained with the 70:30 and HSFP treatments (Table 3). Accretion of BW was lowest in the control and 80:20 treatments. It is in-

Figure 3. Intake (g/d) of fermentable low-fat pellet (A) and extruded high-fat pellet (B) as affected by treatment. Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20: 80% low-fat, 20% high-fat; 70:30 = 70% low-fat, 30% high-fat pellet. *Denotes differences ($P < 0.05$) between 90:10 versus 70:30; **Denotes differences ($P < 0.05$) between control and 90:10 versus 80:20 and 70:30, and between 80:20 and 70:30. †Denotes differences ($P < 0.05$) between 90:10 and 70:30. ††Denotes differences ($P < 0.05$) among all 3 fat-containing treatments. Error bars indicate SE.

teresting to note that BW gain did not follow the same pattern as ME intake, which was greater in 80:20 than in control calves. Probably variation in homeorhesis (or energy distribution for different physiological tasks or

Table 4. Plasma β -OH-butyrate and cholecystokinin (CKK) concentrations as affected by treatment

Item	Treatment ¹					SE	P-value ²		
	Control	90:10	80:20	70:30	HFSP		T	t	T $\times$ t
β -OH-butyrate, mM	0.164 ^b	0.158 ^b	0.164 ^b	0.156 ^b	0.183 ^a	0.007	<0.01	<0.001	0.10
CKK, pM	9.97 ^b	13.33 ^{ab}	16.44 ^a	13.17 ^{ab}	13.35 ^{ab}	1.31	0.02	0.02	0.17

^{a,b}Values with uncommon superscripts within a row differ at $P < 0.05$.
¹Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20 = 80% low-fat, 20% high-fat; 70:30 = 70% low-fat, 30% high-fat; HFSP = high-fat single pellet.
²T = effect of treatment; t = effect of time; T $\times$ t = interaction between treatment and time.

Table 5. Digestibility of DM and fat from solid feed of 10-wk-old calves as affected by treatment

Item	Treatment ¹					SE	P-value ²
	Control	90:10	80:20	70:30	HFSP		T
DM, %	74.6 ^b	74.2 ^b	76.7 ^a	78.3 ^a	69.4 ^c	0.80	<0.001
Fat, %	92.7	80.0	80.7	86.7	85.8	3.46	0.09

^{a-c}Values with uncommon superscripts differ at $P < 0.05$.

¹Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20 = 80% low-fat, 20% high-fat; 70:30 = 70% low-fat, 30% high-fat; HFSP = high-fat single pellet.

²T = effect of treatment.

tissues) could explain the different response in growth rate observed herein. For example, an increased intake of energy, especially in the form of fat, is expected to lead to differences in body composition. In fact, inclusion of high contents of fat in MR has been shown to increase fat deposition in young calves (Tikovsky et al., 2001; Bascom et al., 2007; Hill et al., 2008).

Inclusion of fat in ruminant diets can improve energy efficiency due to direct use of fatty acids in the metabolic pathways of fat synthesis without the need for acetate and glucose (Machmuller et al., 2000). During the weaning period, the relatively low energy intake due to poor solid feed consumption (Eckert et al., 2015) could be potentially (and partially) compensated by increasing dietary fat in the starter feed. Despite this potential advantage, the use of fat is limited in calf starters due to technological difficulties. Feeding rumen inert fats separate from the pellets is not considered an option for starter feeds because of poor palatability. Furthermore, physical mixing is unfeasible because particle size of fat prills is small in comparison with pellets. However, in the current study, mixing was plausible because extrusion and vacuum coating feed technology allows for similarly sized pellets. In the current study, sorting was negligible as shown by analyses of

feed refusals (Table 6). No differences were observed in lying, standing, ruminating, and drinking behavior among treatments, except for the daily proportion of time that calves spent eating, which increased with the 70:30 treatment (Table 6). This could be potentially attributed to a reduced pellet hardness of the high-fat extruded pellet in comparison with the low-fat pellet.

The glucose tolerance tests at both ages revealed no differences in insulin kinetics, although peak glucose concentrations tended ($P = 0.06$) to be greater in HFSP than in 80:20 and 70:30 treatments. Furthermore, AUC for plasma glucose concentration for the 120 min following a GTT was greatest in 80:20, intermediate in 70:30, HFSP, and control, and lowest in 90:10 calves (Table 7). Interestingly, control and 90:10 calves consumed the same amount of fermentable pellet, but 90:10 calves responded with a lower accumulation of glucose in plasma in the GTT. These data would suggest that the additional ME consumed in 90:10 compared with control calves had a positive effect in glucose metabolism. In a natural (cow-calf) situation, calves would feed on glucose and fat for a longer period of time because of gradual weaning. However, when dietary fat was increased (80:20, 70:30, and HFSP), glucose accumulated in blood following a GTT. Thus, it seems that feeding

Table 6. Animal behavior (% of daily observations) and fat sorting in the solid feed as affected by treatment

Item	Treatment ¹					SE	P-value ²		
	Control	90:10	80:20	70:30	HFSP		T	t	T × t
Lying, %	54.4	54.6	53.1	53.2	51.9	1.62	0.70	0.09	0.33
Standing, %	34.6	35.0	35.7	33.4	36.3	1.42	0.63	0.13	0.15
Ruminating, %	9.6	7.3	7.6	7.5	7.2	0.91	0.41	<0.01	0.96
Eating, %	7.7 ^b	7.6 ^b	7.6 ^b	10.4 ^a	7.7 ^b	0.65	<0.01	<0.01	0.25
Drinking, %	1.5	1.4	1.6	1.4	1.6	0.20	0.90	0.27	0.37
Fat sorting, ³ %	—	97.9	97.4	96.0	—	0.56	0.85	0.72	0.87

^{a,b}Values with uncommon superscripts differ at $P < 0.05$.

¹Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20 = 80% low-fat, 20% high-fat; 70:30 = 70% low-fat, 30% high-fat; HFSP = high-fat single pellet.

²T = effect of treatment; t = effect of time; T × t = interaction between treatment and time.

³Sorting (%) = $100 \times (\text{fat intake}/\text{fat intake predicted})$. Sorting values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for). Data are averaged over 7 d for each week for 15 calves per treatment.

Table 7. Parameters of the glucose tolerance test (GTT) performed at 49 and 84 d of life as affected by treatment

Item	Treatment ¹					P-value ²			
	Control	90:10	80:20	70:30	HFSP	SE	T	d	T × d
Basal glucose, ³ mg/dL	91.9	92.3	90.8	86.9	91.3	1.86	0.21	0.23	0.71
Basal insulin, ⁴ μ U/mL	0.49	0.61	0.48	0.47	0.48	0.04	0.14	0.002	0.03
Peak glucose, ⁵ mg/dL	168.6	165.0	163.1	160.0	171.2	2.94	0.06	0.22	0.48
Peak insulin, ⁶ μ U/mL	4.73	4.94	4.64	3.81	4.98	0.33	0.13	<0.001	0.83
Cr _{glucose} , ⁷ %/min	6.04	8.53	7.08	6.13	7.96	0.93	0.26	0.42	0.95
CR _{insulin} , ⁸ %/min	9.07	11.29	9.15	9.18	7.90	0.84	0.10	0.23	0.89
AUC _{120 glucose} , ⁹ mg/dL × 120 min	1,604 ^b	1,404 ^c	2,123 ^a	1,759 ^b	1,793 ^b	160.6	0.03	0.01	0.56
AUC _{120 insulin} , ¹⁰ μ U/mL × 120 min	86.2	77.6	82.8	65.8	93.8	7.66	0.12	<0.001	0.86
Time to glucose baseline, ¹¹ min	137.5	114.7	145.7	155.6	125.1	21.02	0.67	0.31	0.57
Time to insulin baseline, ¹¹ min	44.65	40.25	48.90	44.45	59.33	7.11	0.41	0.25	0.77
Insulin/glucose ratio ¹²	6.22	6.63	5.53	4.47	10.97	1.79	0.11	0.07	0.27
Si, ¹³ (pM/mL) ⁻¹ per min	6.91	6.77	6.95	6.83	6.94	0.06	0.25	0.24	0.39
Sg, ¹⁴ per min	3.86	3.60	3.92	3.65	3.89	0.17	0.59	0.92	0.43

^{a-c}Values with uncommon superscripts differ at $P < 0.05$.

¹Control = 100% low-fat pellet; 90:10 = 90% low-fat, 10% high-fat; 80:20 = 80% low-fat, 20% high-fat; 70:30 = 70% low-fat, 30% high-fat; HFSP = single high-fat pellet.

²T = effect of treatment; d = effect of day; T × d = interaction between treatment and day.

³Average glucose concentration in blood samples taken before the start of the GTT.

⁴Average insulin concentration in blood samples taken before the start of the GTT.

⁵The maximum glucose concentration measured during the GTT.

⁶The maximum insulin concentration measured during the GTT.

⁷Clearance rate of glucose determined following Pires et al. (2007).

⁸Clearance rate of insulin determined following Pires et al. (2007).

⁹Area under the curve (calculated using the trapezoidal method) of glucose during the first 120 min of the GTT.

¹⁰Area under the curve (calculated using the trapezoidal method) of insulin during the first 120 min of the GTT.

¹¹Time to glucose or insulin baseline determined following Pires et al. (2007).

¹²Area under the curve of glucose/area under the curve of insulin.

¹³Insulin sensitivity index derived using the minimal model.

¹⁴Glucose effectiveness derived using the minimal model (Christoffersen et al., 2009).

large amounts of fat compromises some aspects of glucose metabolism in young calves.

From an evolutionary perspective, calves are naturally weaned at 6 to 8 mo (Reinhardt and Reinhardt, 1981) and thus dietary fat as a source of digestible energy is part of their diet for a longer time than in common practice in the dairy industry. An advantage of solid feeding over milk feeding would be that calves receive this fat source in a gradual manner throughout the day following patterns of rumen retention and passage. Given the low feeding frequency of milk feeding generally applied in the dairy industry (typically twice daily) providing fat via the solid feed may be beneficial in terms of constancy in nutrient supply. In addition, whole milk is greater in fat content than MR; therefore, providing additional dietary fat may deliver a nutrient profile that is closer to that found under natural conditions (cow-calf). This hypothesis is further supported by the final nutrient composition consumed by calves offered free-choice in a cafeteria feeding experiment by Montoro and Bach (2012), where fat content averaged 6.3%, which is greater than fat content generally ob-

served in calf diets postweaning and is closest to the treatment with the highest intake in the current study.

CONCLUSIONS

Delivering dietary fat (to about 7% of DM) by mixing an extruded high-fat pellet with a high-carbohydrate low-fat conventional pellet, and thus maintaining the supply of fermentable carbohydrates while increasing the energy density of the solid feed results in improved solid feed intake, energy intake, and rate of BW gain when compared with a conventional low-fat pellet, or a single pellet with increased fat content.

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