Effects of milk replacer feeding strategies on performance, ruminal development, and metabolism of dairy calves

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ABSTRACT

Performance, rumen development, and metabolism of male Holstein calves ($n = 54$) were evaluated according to 1 of 3 liquid feeding strategies: 4 L of milk replacer (MR)/d until 60 d old (4L-60d), 6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d (6L-29d/4L-60d), or 6 L of MR/day until 60 d old (60d-6L). Water and starter were provided ad libitum. Intakes of MR and starter were monitored daily and body weight (BW) weekly. Blood samples for glucose and insulin concentrations and ruminal content samples for volatile fatty acids and ruminal ammonia concentrations were collected at 15, 30, 45, 60, 75, and 90 d of age. Six calves on each treatment were euthanized at 30, 60, and 90 d of age. Empty weights of forestomach and abomasum, papillae length, and mitotic index were measured. Average MR intake/d (expressed as a percent of BW) was greater for calves receiving 6 L/d than for those receiving 4 L/d until 30 d of age. Calves on the 6L-29d/4L-60d had the smallest MR intake from 30 to 60 d old, followed by the 4L-60d and 6L-60d treatments. Starter intake (kg of dry matter/day) did not differ between groups. It increased from 0.065 kg/d in the first month to 0.386 kg/d in the second month, and to 2.065 kg/d after weaning. Weight gain was greater for calves fed more MR in the first month, but no difference was observed during the second month. After weaning, 6L-60d calves had greater rate of weight gain than others and were heavier at 30, 60, and 90 d of age. Weight of empty forestomachs, ruminal pH, and ammoniac nitrogen concentration were not different among groups. Propionate concentration was lower for 6L-60d calves, but acetate and butyrate concentrations were not influenced by MR feeding strategy. Calves fed more MR until d 30 had greater ruminal epithelium mitotic index. The different MR feeding strategies did not influence papillae length or ruminal epithelium thickness. Lesions such as rumi-

nal parakeratosis or hyperkeratosis were not observed. The MR feeding strategy did not affect glucose concentration, but insulin was higher in 6L-60d calves than in the other groups. Glucose concentration increased with age, whereas insulin decreased until 45 d old, and then started to increase until 90 d. In conclusion, MR feeding strategy did not influence ruminal development. Feeding calves 6 L of MR/d over 60 d resulted in greater rate of weight gain without negative effects on starter intake or forestomach development. The weight advantage that 6L-60d calves obtained preweaning was maintained until 90 d of age.

Key words: glucose, growth, preweaning, starter intake

INTRODUCTION

Dairy calves in Brazilian farms are usually fed 4 L of milk or milk replacer (MR) in fixed amounts until they are 60 d old. Calves are reared outside in individual shelters on tropical grass and exposed to climatic variations. The tropical climate imposes a large range of environmental temperatures during the day. Low rates of weight gain are usually observed, mostly because of restricted milk feeding and nutritional needs for thermoregulation. According to Davis and Drackley (1998), feeding 4 L/d (equivalent to approximately 10% of birth weight) during the whole milk-feeding period results in decreased alimentary efficiency and thus leads to suboptimal performance.

Calves fed milk ad libitum gained 0.9 kg/d between 14 and 18 d of with an intake of 9.3 kg of milk/d (Sweeney et al., 2010). Diaz et al. (2001) showed that growing calves can have DMI of milk solids of 2.5% of BW, which is higher than amounts usually fed. These authors also stated that if nutrient supply does not limit growth, feed efficiency can be as high as that reported for piglets and lambs. However, farmers and technicians in Brazil are still reluctant to increase the amount of milk or MR fed to calves because of possible reductions in starter intake and ruminal development, in addition to the belief that this practice would increase diarrhea

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incidence. Increase in incidence of scours due to greater milk intake has not been shown (Jasper and Weary, 2002; Khan et al., 2007b).

Khan et al. (2007b) related that feeding dairy calves at 20% of BW for the first 25 d with subsequent gradual reduction to 10% of BW until weaning (step-down method) reduced starter intake in the pre-step phase, but resulted in increased starter intake post-step and postweaning. Calves fed in the step-down program had greater weight gain and better forestomach development than calves conventionally fed. Feeding more milk or MR can also be beneficial for reproductive and productive adult life traits. Shamay et al. (2005) related reduction in age at puberty and higher production of 3.5% FCM when milk was fed ad libitum compared with 450 g of MR/d.

Adequate growth relies on nutritional status, which is affected by conditions such as disease and nutrient intake. The effect of feed intake on hormonal regulation should be understood to ensure proper development. Insulin's primary function is to stimulate anabolic processes. Adequate plasma concentration of insulin is essential for protein synthesis and high rates of weight gain (Breier et al., 1988; Guyton and Hall, 2006). Glucose is the primary regulator of insulin release and synthesis, but other molecules such as mannose, leucine, fatty acids, and ketone bodies also affect insulin release (Kaneko, 1997).

Concentrations of glucose and insulin in the blood of preweaned calves are similar to values observed for monogastric animals. As calves grow older and achieve significant intake of solid feed, ruminal fermentation is established and lower blood glucose and insulin concentrations are observed. This is due to hepatic gluconeogenesis, with propionate as the main substrate (Owens et al., 1986; Davis and Drackley, 1998; Daniels et al., 2008).

Recent research has focused on alternatives to increase the amount of milk fed to dairy calves to improve weight gain and health without negative effects on starter intake or ruminal development. The objectives of the present study were to evaluate weight gain, starter intake, ruminal development, and blood glucose and insulin concentrations of dairy calves fed MR in 3 different strategies during 60 d of liquid feeding and an additional 30 d after weaning, in rearing conditions representative of those observed in dairy farms of Minas Gerais state, Brazil.

MATERIALS AND METHODS

The Federal University of Minas Gerais (UFMG) ethical committee (CETEA n. 44/2009) approved all the procedures of this research.

Male Holstein calves ($n = 54$) were taken from commercial dairy farms to the experimental facility at 1 to 3 d old. Calves were reared in individual shelters over tropical grass pasture (*Paspalum notatum*) from September 2010 to March 2011 in the Veterinary School of UFMG (Minas Gerais, Brazil). This trial was conducted during the warmest and rainiest months of the year. Mean minimum and maximum environmental temperatures were 20 and 32°C. Rainfall during the experimental period was 1,059 mm (15.8 ± 15.3 mm/d), resulting in the calves being wet for a significant amount of time.

General procedures such as navel dipping with iodine solution (10%) and colostrum feeding at the source farm (previously frozen colostrum) until 6 h after birth were adopted for all calves. After arrival at the experimental facility, calves were weighed, ear tagged, and fed 4 L/d of MR divided in 2 equal feedings until 3 d old. Feeding according to treatments started at 3 d old (18 calves per treatment).

Milk replacer feeding strategies were as follows: (1) 4 L of MR/d until 60 d old (**4L-60d**); (2) 6 L of MR/d until 29 d old and 4 L of MR/d from 30 to 60 d old (**6L-29d/4L-60d**); or (3) 6 L of MR/d until 60 d old (**6L-60d**). All calves were fed the same milk replacer (Lacthor, Nutrifed, Veghel, the Netherlands), which was diluted to 12.5% total solids. Starter and water were provided ad libitum (Table 1). Treatments were divided in 3 groups by age of planned euthanasia (30, 60, or 90 d old). Calves reared until 90 d old were abruptly weaned at 60 d old. After weaning, calves were fed the same starter as fed in the preweaning period and water ad libitum. We also observed that the calves grazed (i.e., grass was ingested, not just used for bedding), especially after weaning.

Milk replacer was fed first by bottle and then from a bucket as soon as the calves were able to drink from it. Milk replacer was offered twice daily in 2 equal portions at 0800 and 1600 h. Refusals were measured at each feeding.

Health status was assessed daily according to Diaz et al. (2001). Calves with scours received 4 L/d of oral electrolyte solution (2 L, twice a day; 20 g of glucose, 5 g of NaCl, 4 g of NaHCO₃, and 1 g of KCl for each liter of water). All disease episodes and medical treatments were recorded. Maximum and minimum environmental temperatures and the amount of rain were measured daily.

Calves were weighed upon arrival at the experimental facility, weekly before the morning MR feeding, and immediately before euthanasia. Starter intake (kg of DM/d) was calculated daily by measurement of amount offered and leftovers in the next morning. Starter was sampled weekly and pooled by month for chemical analysis. Starter, grass, and milk replacer were ana-

Table 1. Chemical composition (% of DM unless noted otherwise) of starter, grass and milk replacer

Composition	Starter ¹	Grass	Milk replacer ²
DM (%)	96.5	22.0	94.6
CP	20.4	15.9	20.7
Ether extract	6.9	4.9	17.0
NDF	12.5	49.0	—
ADF	4.0	25.3	—
Ash	16.0	12.8	9.7
Ca	2.95	0.32	2.88
P	1.26	0.24	0.76
ME ³ (Mcal/kg)	—	—	4.16

¹Ground starter ingredients: pantothenic acid, extruded soybean meal, dicalcium phosphate, butyric acid, vanilla flavor, sodium bicarbonate, carbon-amino-phospho-chelate sulfur, carbon-amino-phospho-chelate manganese, carbon-amino-phospho-chelate selenium, carbon-amino-phospho-chelate zinc, potassium chloride, sodium chloride, elemental sulfur, carbon-amino-phospho-chelate copper, calcium carbonate, monensin (21.05 mg/kg), riboflavin, niacin, *Saccharomyces cerevisiae*, magnesium oxide, zinc sulfate, vitamin A, sodium selenite, sulfate, cobalt sulfate copper, manganese sulfate, vitamin D₃, vitamin E, kaolin, calcium iodate, extruded whole soybeans, whey powder.

²Milk replacer ingredients: whey (70%), coconut oil, soy protein concentrate, wheat protein concentrate, vitamin premix.

³ME of milk replacer estimated as follows: ME = 0.9 [(9.21 × % fat) + (5.86 × % CP) + (3.95 × % lactose)] (Drackley, 2008).

lyzed for DM, CP, ether extract, Ca, and P according to AOAC International (1995); NDF and ADF were analyzed by the sequential method of Van Soest et al. (1991).

Blood samples were collected via venojugular puncture after local antisepsis. At 5 d old, one sample was collected in 10-mL Vacutainer tubes (Becton Dickinson, São Paulo, Brazil) without anticoagulants for evaluation of serum IgG concentration by radial immunodiffusion (Fleenor and Stott, 1981). Samples for glucose and insulin concentration were collected in 10-mL Vacutainer tubes with EDTA and sodium fluoride and without anticoagulants, respectively. Sampling was performed before the morning MR feeding (16 h after previous feeding), at 15, 30, 45, 60, 75, and 90 d old. For group 6L-29d/4L-60d, the 30-d sample was obtained on the day the amount of MR offered was reduced, and the 60-d sample was collected on the weaning day, after the last MR feeding in the previous afternoon.

Samples were centrifuged at 800 × *g* for 10 min and plasma or serum was harvested and stored frozen at −20°C until analysis. Plasma glucose concentration was analyzed by colorimetric method (Bioclin Glucose Monoreagent, Bioclin, Belo Horizonte, Brazil). Readings were done in a semi-automatic spectrophotometer (TP Analyzer, Varginha, Brazil). Serum insulin concentration was determined by RIA (Porcine Insulin RIA kit PI-12K, Millipore, Billerica, MA) and read in a gamma counter. Analyses were performed at the Veterinary School and the Biological Sciences Institute of UFMG.

Ruminal contents were sampled by oral tube at the ages of 15, 30, 45, 60, 75, and 90 d old, 4 h after the

morning feeding. Approximately 150 mL was obtained and filtered, and a 5-mL sample was added to 1 mL of 20% metaphosphoric acid for VFA analysis (Coverdale et al., 2004). The VFA were quantified in a gas chromatograph (GC-17 A, Shimadzu, Tokyo, Japan), with a Supelco Nukol silica capillary column (0.53 mm i.d., 30 m long, 0.5-μm film thickness; Supelco Inc., Bellefonte, PA). Another sample of ruminal content (50 mL added to 5 mL of 50% sulfuric acid) was used for ammonia concentration analysis by AOAC International (1995; method 941-04) without addition of urease.

Six calves in each treatment were euthanized at 30, 60, and 90 d old. Ruminal pH was measured by using a portable meter directly from the rumen during the necropsy. Forestomachs were then removed, emptied, washed with water, dried, and weighed. Ruminal epithelium was sampled from the ventral sac for determination of morphological description, papillae length, and mitotic index. Duodenum, jejunum, and ileum were also sampled for histopathology. Eventual necropsy findings were recorded. After being fixed in formaldehyde during 24 h, rumen and small intestine fragments were processed according to Luna (1968).

Ruminal epithelium was classified according to Coelho (1999). Papillae morphometric analysis was performed in 3 different ventral sac fragments (24 ± 10 papillae/calf; Lesmeister et al., 2004). Nonkeratinized ruminal epithelium and keratin layer thickness were measured in 5 papillae/calf. Total thickness of ruminal epithelium was obtained by addition of both measurements (Melo, 2007). Mitotic index was evaluated in 3,771 ± 503 nuclei/calf on the ruminal epithelium basal layer (Melo, 2007).

Table 2. Milk replacer intake (in kg/d and L/d, relative to BW) and starter intake, BW, and BW gain of Holstein calves fed milk replacer (MR) in different strategies

Parameter and period (mo)	MR feeding strategy ¹		
	4L-60d	6L-29d/4L-60d	6L-60d
MR intake (kg/d)			
1	0.464 ± 0.019	0.669 ± 0.035	0.661 ± 0.045
2	0.473 ± 0.001	0.473 ± 0.001	0.710 ± 0.001
MR intake (kg/d as % of BW)			
1	1.270 ± 0.001 ^{a,A}	1.824 ± 0.002 ^{b,A}	1.744 ± 0.002 ^{b,A}
2	1.126 ± 0.001 ^{a,B}	1.097 ± 0.002 ^{b,B}	1.471 ± 0.001 ^{c,B}
MR intake (L/d as % of BW)			
1	10.16 ± 0.01 ^{a,A}	14.59 ± 0.01 ^{b,A}	13.95 ± 0.02 ^{b,A}
2	9.01 ± 0.01 ^{a,B}	8.77 ± 0.02 ^{b,B}	11.76 ± 0.01 ^{c,B}
Starter intake (kg/d)			
1	0.070 ± 0.045 ^A	0.067 ± 0.036 ^A	0.059 ± 0.031 ^A
2	0.362 ± 0.279 ^B	0.413 ± 0.215 ^B	0.385 ± 0.166 ^B
3	1.905 ± 0.553 ^C	2.019 ± 0.388 ^C	2.272 ± 0.307 ^C
BW ² (kg)			
Initial	38.5 ± 5.4 ^{a,A}	37.1 ± 4.4 ^{a,A}	38.5 ± 4.8 ^{a,A}
1	40.2 ± 4.4 ^{a,A}	44.5 ± 4.9 ^{b,B}	44.0 ± 4.9 ^{b,B}
2	52.2 ± 5.8 ^{a,B}	55.8 ± 6.2 ^{a,C}	60.2 ± 6.7 ^{b,C}
3	75.0 ± 8.3 ^{a,C}	76.8 ± 8.5 ^{a,D}	86.7 ± 9.6 ^{b,D}
BW gain (kg/d)			
1	0.065 ± 0.131 ^{a,A}	0.235 ± 0.153 ^{b,A}	0.208 ± 0.156 ^{b,A}
2	0.429 ± 0.170 ^{a,B}	0.402 ± 0.121 ^{a,B}	0.516 ± 0.116 ^{a,B}
3	0.734 ± 0.175 ^{a,C}	0.757 ± 0.193 ^{a,C}	0.995 ± 0.150 ^{b,C}

^{a-c,A-D}Means with different lowercase letters (in rows) or uppercase letters (in columns) for the same trait differ by Student-Newman-Keuls test ($P < 0.05$).

¹4L-60d = 4 L of MR/d until 60 d old; 6L-29d/4L-60d = 6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d; 6L-60d = 6 L of MR/d until 60 d old.

²Values for each month are those obtained in the last weighing of the respective month.

Statistical analysis was done in a completely randomized split-plot design, with animals as random and age as the repeated measure. Variables were analyzed in 3×3 and 3×6 (3 MR feeding strategies and 3 or 6 ages at sampling) factorials. Birth weight was used a covariate. Descriptive analysis was used for rumen and small intestine histopathology results. Homogeneity of variance and standard normal probability distribution were assessed for all the variables. Means were tested by Student-Newman-Keuls test for parametric variables ($P = 0.05$). Nonparametric variables were tested with the Kruskal-Wallis test, with 95% confidence for treatment comparisons. Analyses were performed using the SAS software package (version 9.1; SAS Institute Inc., Cary, NC).

RESULTS

Passive immunity transfer was similar among groups ($P > 0.05$). Mean IgG serum concentrations (mg/mL) were 16.0 ± 7.3 , 17.0 ± 4.2 , and 14.4 ± 5.3 for treatments 4L-60d, 6L-29d/4L-60d, and 6L-60d, respectively. Average initial weights were not different among treatments ($P > 0.05$). Calves in treatments 4L-60d, 6L-29d/4L-60d, and 6L-60d weighed 38.5 ± 5.4 , 37.1 ± 4.4 , and 38.5 ± 4.8 kg, respectively (Table 2).

Intake of MR in the first month was inferior to the amount offered to the calves. During this period, calves in treatment 4L-60d ingested an average of 3.9 L/d, whereas those receiving 6 L/d ingested 5.6 L/d. Monthly averages of MR intake (in kg/d and L/d, relative to BW) are shown in Table 2. Intake of MR was higher for treatments 6L-29d/4L-60d and 6L-60d than for 4L-60d in the first month ($P < 0.05$). From 30 to 60 d old, MR intake as a proportion of BW was lesser ($P < 0.05$) for calves in treatment 6L-29d/4L-60d, even though they were offered the same amount as group 4L-60d during this period.

Average starter DMI increased with age ($P < 0.05$), but was not different among groups before or after weaning ($P > 0.05$; Table 2, Figure 1). Feeding calves 6 L of MR/d resulted in a greater rate of weight gain during the first month ($P < 0.05$) (Table 2; Figure 2). However, from 30 to 60 d of age, rates of weight gain were not different between groups ($P > 0.05$). Feeding calves more MR during the whole 60-d period resulted in a greater rate of weight gain after weaning ($P < 0.05$), even with similar starter intakes ($P > 0.05$). Calves fed 6 L/d for the full 60-d period were heavier at 30, 60, and 90 d of age.

Milk replacer feeding strategy did not influence ruminal pH, ammonia concentration (N-NH₃), or acetate

Figure 1. Starter DMI (kg/d) of Holstein calves fed milk replacer (MR) in different strategies and weaned abruptly at 60 d old [4L-60d (4 L of MR/d until 60 d old); 6L-29d/4L-60d (6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d), and 6L-60d (6 L of MR/d until 60 d old)].

and butyrate concentrations on the ruminal fluid ($P > 0.05$; Table 3). Propionate concentration was lower for 6L-60d calves ($P < 0.05$). We observed an effect of age on ruminal pH, which was lower in all groups at 30 and 60 d old ($P < 0.05$).

Acetate, butyrate, and propionate concentrations in ruminal content increased with age ($P < 0.001$). Proportion of each VFA changed as the calves got older. The proportion of acetate increased, whereas that of butyrate decreased from 30 to 90 d of age ($P < 0.001$; Table 4).

Milk replacer feeding strategies did not influence rumen-reticulum empty weights ($P > 0.05$; Table 5). The empty omasum was heavier at 90 d for group 6L-60d, and the abomasum was heavier for calves that were offered 6 L of MR/d during the first month of life ($P < 0.05$).

Ruminal ventral sac papillae size was not affected by MR feeding strategy ($P > 0.05$; Table 6), even though propionate concentration (both in mmol/L and as a proportion of total VFA) differed among groups ($P < 0.05$). Propionate concentration was higher for 4L-60d and 6L-29d/4L-60d (2.71 and 2.67 mmol/100 mL) than for 6L-60d (2.04 mmol/100 mL; $P = 0.03$). Ruminal papillae, nonkeratinized epithelium, and keratin layer thickness increased with age ($P < 0.001$) and were not

Table 3. Ruminal pH and N-NH₃ concentration of Holstein calves fed milk replacer under different strategies and weaned abruptly at 60 d old

Age (d)	pH	N-NH ₃ (mg/100 mL)
30	6.6 ± 0.8 ^a	11.39 ± 8.12
60	6.2 ± 0.4 ^a	11.36 ± 7.54
90	6.9 ± 0.5 ^b	10.76 ± 6.61

^{a,b}Means with different letters (in columns) for the same trait differ by Student-Newman-Keuls test ($P < 0.05$).

Figure 2. Weight gain (kg/d) of calves fed milk replacer (MR) in different strategies and abruptly weaned at 60 d old [4L-60d (4 L of MR/d until 60 d old); 6L-29d/4L-60d (6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d), and 6L-60d (6 L of MR/d until 60 d old)].

affected by MR feeding strategy ($P > 0.05$; Table 6). Calves that were fed 6 L of MR during the first month had a higher mitotic index than those fed 4 L during the same period ($P = 0.003$; Table 6).

No difference was observed in glucose plasma concentrations between groups ($P > 0.05$; Figure 3). Glucose levels varied little during the preweaning period. Postweaning plasma glucose was higher than during preweaning period ($P < 0.05$; Figure 3).

Milk replacer feeding strategies had an effect on serum insulin concentration ($P < 0.05$; Figure 4). Calves fed 6 L/d during the whole preweaning period had greater insulin concentrations than others ($P < 0.05$). Calves fed 4L-60d presented intermediate insulin concentrations, and those in group 6L-29d/4L-60d had the lowest values ($P < 0.05$). As observed for glucose concentration, serum insulin concentration was different between evaluated ages ($P < 0.05$; Figure 4); the concentration decreased from 15 to 45 d, but increased from 45 to 90 d of age.

Table 4. Proportion of acetate (C₂), propionate (C₃), and butyrate (C₄) to the total VFA in ruminal content of calves fed milk replacer in different strategies and abruptly weaned at 60 d old

Age (d)	C ₂	C ₃	C ₄
15	0.59 ± 0.11 ^A	0.34 ± 0.10 ^A	0.07 ± 0.04
30	0.57 ± 0.11 ^{AB}	0.37 ± 0.11 ^{AB}	0.06 ± 0.02
45	0.53 ± 0.09 ^{ABC}	0.38 ± 0.08 ^{ABC}	0.08 ± 0.03
60	0.50 ± 0.11 ^{BC}	0.42 ± 0.08 ^{BCD}	0.08 ± 0.07
75	0.49 ± 0.04 ^C	0.44 ± 0.04 ^{CD}	0.07 ± 0.02
90	0.46 ± 0.07 ^C	0.47 ± 0.07 ^D	0.07 ± 0.02

^{A-D}Means with different letters (in columns) for the same trait differ by Student-Newman-Keuls test ($P < 0.05$).

Table 5. Weight of rumen-reticulum, omasum and abomasum and total compartments of calves fed milk replacer (MR) in different strategies and abruptly weaned at 60 d old

Weight (kg)	MR feeding strategy ¹			Mean
	4L-60d	6L-29d/4L-60d	6L-60d	
Rumen-reticulum				
30 d	0.350 ± 0.088	0.394 ± 0.068	0.313 ± 0.054	0.353 ± 0.075 ^A
60 d	0.848 ± 0.272	1.058 ± 0.404	1.078 ± 0.231	0.995 ± 0.311 ^B
90 d	2.359 ± 0.571	2.571 ± 0.605	2.664 ± 0.499	2.531 ± 0.543 ^C
Mean	1.186 ± 0.944	1.341 ± 1.018	1.352 ± 1.051	
Omasum				
30 d	0.084 ± 0.017 ^{a,A}	0.095 ± 0.027 ^{a,A}	0.072 ± 0.017 ^{a,A}	
60 d	0.174 ± 0.049 ^{a,B}	0.195 ± 0.050 ^{a,B}	0.228 ± 0.080 ^{a,B}	
90 d	0.473 ± 0.115 ^{a,C}	0.439 ± 0.146 ^{a,C}	0.640 ± 0.137 ^{b,C}	
Mean	0.244 ± 0.184	0.243 ± 0.171	0.313 ± 0.261	
Abomasum				
30 d	0.267 ± 0.029	0.326 ± 0.021	0.305 ± 0.038	0.299 ± 0.038 ^A
60 d	0.300 ± 0.057	0.363 ± 0.076	0.390 ± 0.038	0.351 ± 0.068 ^B
90 d	0.460 ± 0.079	0.473 ± 0.058	0.568 ± 0.067	0.500 ± 0.081 ^C
Mean	0.342 ± 0.103 ^a	0.387 ± 0.083 ^b	0.420 ± 0.122 ^b	

^{a,b,A-C}Means with different lowercase letters (in rows) or uppercase letters (in columns) for the same trait differ by Student-Newman-Keuls test ($P < 0.05$).

¹4L-60d = 4 L of MR/d until 60 d old; 6L-29d/4L-60d = 6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d; 6L-60d = 6 L of MR/d until 60 d old.

DISCUSSION

Temperature range and rainfall observed during the trial resulted in adverse environmental conditions for the calves. The lower critical temperature of a calf is 13.4°C, and the thermoneutral zone upper limit is 26°C

for the first week of life (Gonzalez-Jimenez and Blaxter, 1962; Spain and Spiers, 1996). When temperatures are above or below the thermoneutral zone, animals waste energy to dissipate or generate heat, increasing maintenance requirements for metabolizable energy (Holmes and McLean, 1975; Singh and Newton, 1978). As the

Table 6. Mitotic index, papillae length, thickness of nonkeratinized epithelium and keratin layer of the basal layer of ruminal epithelium of the ventral ruminal sac of calves fed milk replacer (MR) in different strategies and abruptly weaned at 60 d old

Trait and age (d)	MR feeding strategy ¹			Mean
	4L-60d	6L-29d/4L-60d	6L-60d	
Mitotic index (%)				
30 d	0.39 ± 0.17	0.49 ± 0.10	0.53 ± 0.30	0.47 ± 0.21
60 d	0.51 ± 0.20	0.67 ± 0.14	0.68 ± 0.12	0.62 ± 0.18
90 d	0.39 ± 0.12	0.76 ± 0.18	0.61 ± 0.23	0.59 ± 0.23
Mean	0.43 ± 0.17 ^a	0.64 ± 0.18 ^b	0.61 ± 0.23 ^b	
Papillae length (mm)				
30 d	0.46 ± 0.14	0.41 ± 0.13	0.34 ± 0.05	0.40 ± 0.12 ^A
60 d	0.47 ± 0.08	0.60 ± 0.29	0.57 ± 0.17	0.55 ± 0.19 ^A
90 d	1.75 ± 0.09	2.36 ± 0.60	2.39 ± 0.60	2.17 ± 0.55 ^B
Mean	0.89 ± 0.63	1.12 ± 0.97	1.10 ± 1.00	
Nonkeratinized epithelium (mm)				
30 d	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01 ^A
60 d	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01 ^B
90 d	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.02 ^B
Mean	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.01	
Keratin layer (mm)				
30 d	0.015 ± 0.003	0.017 ± 0.004	0.013 ± 0.004	0.015 ± 0.004 ^A
60 d	0.018 ± 0.004	0.017 ± 0.004	0.019 ± 0.006	0.018 ± 0.005 ^{AB}
90 d	0.018 ± 0.006	0.019 ± 0.006	0.022 ± 0.008	0.019 ± 0.007 ^B
Mean	0.017 ± 0.004	0.017 ± 0.005	0.018 ± 0.007	

^{a,b}Different lowercase letters (in rows) for the same trait differ by Kruskal-Wallis test ($P < 0.05$).

^{A,B}Different uppercase letters (in columns) for the same trait differ by Student-Newman-Keuls test ($P < 0.05$).

¹4L-60d = 4 L of MR/d until 60 d old; 6L-29d/4L-60d = 6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d; 6L-60d = 6 L of MR/d until 60 d old.

Figure 3. Mean ($\pm$ SD) concentration of plasma glucose (mg/dL) in calves fed milk replacer (MR) in different strategies and abruptly weaned at 60 d old [4L-60d (4 L of MR/d until 60 d old); 6L-29d/4L-60d (6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d), and 6L-60d (6 L of MR/d until 60 d old)].

Figure 4. Mean ($\pm$ SD) concentration of serum insulin (μ IU/mL) in calves fed milk replacer (MR) in different strategies and abruptly weaned at 60 d old [4L-60d (4 L of MR/d until 60 d old); 6L-29d/4L-60d (6 L of MR/d until 29 d old and 4 L/d from 30 to 60 d), and 6L-60d (6 L of MR/d until 60 d old)].

environmental conditions were extreme in the current study, weight gain and alimentary efficiency were likely to be reduced.

Average IgG serum concentration indicated adequate passive immunity transfer through colostrum feeding in the current study. Even though IgG concentrations were above the desirable threshold of 10 mg/mL (Davis and Drackley, 1998), the most common pathologies that affect dairy calves occurred frequently. Diarrhea was first observed on average at 6 d of age for 6L-29d/4L-60d and 6L-60d calves, and at 8 d of age for 4L-60d calves, with equal incidence between treatments. Incidence of respiratory diseases and omphalophlebitis was 13 and 15%, respectively. According to McGuirk (2008), diarrhea is the most important disease in calves <30 d old, but for calves >30 d old, pneumonia causes up to 21% of deaths. Feeding calves 6 L of MR/d was not enough to reduce diarrhea incidence, but allowed faster recovery as evidenced by the rate of weight gain (Table 2).

Milk replacer intake was inferior to the recommendations of Drackley (2008), which specify 1.5% of BW of solids in the first week of life and 2% of BW from the second week to weaning. Calves fed 6 L/d were offered an amount of solids equivalent to 1.6% of BW from the second week on (Table 2), but intake of the full amount was not achieved until 4 wk of age. The incidence of diarrhea and the challenging environmental conditions might have influenced milk replacer intake. Jasper and Weary (2002) reported a decrease in voluntary intake of milk from 7 to 14 d of age, which coincides with the period of diarrhea incidence. Loss of appetite is part of the behavioral response to infections and is related to proinflammatory cytokines (Borderas et al., 2007).

Milk replacer was diluted to 12.5% of solids, a concentration similar to that of raw milk. An increase in

the amount of solids in the milk replacer could guarantee ingestion of the desired amount of nutrients, thus increasing nutritional status, particularly in the first weeks of life when calves have lesser thermoregulatory ability and the disease incidence is higher.

Differences observed in MR intake in the first month were mainly related to treatments (i.e., fixed amounts of MR), being greater for calves offered 6 L/d. However, in the second month, differences in BW resulted in different relative MR intakes between groups (Table 2). Because 6L-29d/4L-60d calves gained more weight than 4L-60d calves during the first month, intake as a proportion of BW was less ($P < 0.05$) for 6L-29d/4L-60d calves during the second month compared with 4L-60d calves.

Khan et al. (2007a) observed a hypophagic response to increased milk intake in dairy calves. Because calves in the current trial were fed a smaller amount of MR solids, the amount of MR consumed did not affect starter DMI, even though a 50% difference in MR availability occurred between treatments. Because of the greater rate of weight gain in the first month ($P < 0.05$), calves fed 6 L/d during this period were heavier at 30 d and would have higher nutrient requirements. During the second month, we expected that the 6L-29d/4L-60d group would have a greater starter DMI than the others, given that calves in this group were heavier and were receiving a smaller amount of MR during this period. However, starter intake did not differ among groups ($P > 0.05$; Table 2).

Starter DMI during the second month might not have been greater due to the small intake observed in the first month, which probably did not induce sufficient rumen development and establishment of ruminal fermentation. The failure of 6L-29d/4L-60d calves

to increase starter intake at a greater rate than the other groups resulted in loss of the weight advantage this group had at 30 d old compared with the 4L-60d group. After 60 d of age, no more weight difference was observed among 6L-29d/4L-60d and 4L-60d groups ($P > 0.05$; Table 2, Figure 2).

During the first month, feeding more MR likely increased feed efficiency, as an allowance of 50% more MR resulted in a rate of weight gain more than double that observed for 4L-60d calves (Table 2). Starter intake was irrelevant in quantity and was not different among groups during this period. A 40-kg calf with an ADG of 0.4 kg/d requires 2.63 Mcal of ME/d and 129 g of CP/d (NRC, 2001). Content of ME and CP in 500 g of this MR (amount diluted to 4 L) would be 2.08 Mcal and 103.5 g, respectively. From this, it is evident that the energy and protein content of 4 L of the MR used here were insufficient to sustain a growth rate of 0.4 g/d, which is lower than ideal in the first month of life. Six liters of MR contained 3.12 Mcal of ME and 155.25 g of CP, representing a better source of nutrients to achieve the desired growth rates. According to Bartlett et al. (2006), higher feed efficiency observed due to increased milk feeding is related to provision of nutrients above maintenance requirements. During the second month, feeding calves 6 or 4 L of MR/d did not result in different rates of weight gain (Table 2).

As a consequence of MR feeding strategies, weight at weaning and at 90 d was higher for group 6L-60d ($P < 0.05$) and equal among the 4L-60d and 6L-29d/4L-60d groups. Calves that were fed 6 L/d during the whole period had also a greater rate of weight gain after weaning ($P < 0.05$). One of the greatest challenges of accelerated growth feeding strategies is to maintain the weight advantage obtained during the milk or MR feeding period after weaning. The amount of MR fed was enough to promote better development of calves and did not show negative effects in the postweaning phase. The management strategy adopted, which was to keep feeding calves in the same facility the same starter from the preweaning period, might have been favorable to the calves' postweaning performance.

We expected that ruminal content parameters would be similar because starter intake was not different between groups (Table 3). Ruminal pH values were lower at 30 and 60 d of age ($P < 0.05$), even though intake was higher at 90 d. This indicates the development of ruminal fermentation and VFA absorption over time, allowing better control of ruminal pH. Ruminal pH values were higher than reported elsewhere for calves fed milk and starter. The availability of fresh grass for ad libitum intake might have been favorable to the establishment of ruminal fermentation, rumination, and maintenance of pH values in a desirable range.

Concentrations of VFA increased with age ($P < 0.001$) because of the increase in starter intake. Proportional to total VFA, concentrations of acetate decreased and propionate increased with age ($P < 0.001$; Table 4). This pattern of change in VFA proportions is also a result of the increase in starter intake with age.

Milk replacer feeding strategies did not influence rumen-reticulum empty weights ($P > 0.05$). This result would be expected once starter intake was not different between groups (Table 5). The heavier omasum at 90 d old for group 6L-60d could be a result of the numerically higher starter intake for 6L-60d calves after weaning. The weight of the abomasum increases proportionally with BW and independently of solid feed intake (Davis and Drackley, 1998), so the higher volume of MR might have accounted for the difference in abomasum weight between groups. Abomasum weight differences due to MR feeding strategy occurred without negative effects on forestomach development.

Propionate and butyrate are indicated as the VFA that have the greatest effects on ruminal epithelium, because of increased blood flow during absorption and metabolism and a direct effect on ruminal epithelium gene expression (Baldwin et al., 2004). Ruminal ventral sac papillae size was not affected by MR feeding strategy ($P > 0.05$). Propionate concentration was different between groups ($P < 0.05$), which could not be explained by starter intake or papillae size, but might be related to passage and absorption rates.

Changes in ruminal papillae length and thickness of nonkeratinized epithelium and keratin layer with age ($P < 0.05$) were related to the increase in solid feed intake and thus increased VFA concentration in ruminal content (Table 4). Lesions such as ruminal parakeratosis or hyperkeratosis were not observed at any of the evaluated ages. Ruminal epithelium mitotic index is stimulated by VFA, growth factors, hormones, and micronutrients (Stalano-Coico et al., 1990; Suter et al., 1997), indicating epithelium proliferation. The higher mitotic index observed in calves that were fed more MR ($P = 0.003$; Table 4) was probably due to hormonal and metabolic alterations induced by nutritional status.

No macroscopic lesions were observed in the small intestine. Histopathological evaluation showed few alterations. One 30-d-old calf in group 4L-60d had villi atrophy in the duodenum and jejunum, and 2 other calves of the same age and group had crypt abscesses in the ileum. At 60 d old, no calves from 4L-60d had small intestine alterations. At 90 d old, 1 calf had discrete mural eosinophilic enteritis in the jejunum and ileum. In group 6L-29d/4L-60d at 30 d old, 2 calves had jejunum crypt abscesses and 3 had ileum crypt abscesses. At 60 d old, 2 calves had discrete mural eosinophilic and plasmocitary enteritis on duodenum. At 90 d old,

2 calves had ileum crypt abscesses. In group 6L-60d, only one 30-d calf had small intestine alterations, represented by jejunum crypt abscesses. Alterations found in the small intestine were evenly distributed among groups and were probably not related to MR feeding strategy.

We expected that plasma glucose concentrations would be greater for calves that were offered more MR, because of greater lactose intake, as related by Smith et al. (2002) and Daniels et al. (2008). However, no difference was observed between groups at any of the evaluated ages. Average glucose concentration for all the sampling ages up to 60 d old was lower than values found in the literature. These lower values might indicate that lactose contribution from the MR was insufficient to promote adequate glycemia, even though the lactose content of the MR used here (44%) was similar to that of an average MR. It can be inferred that the low glucose concentrations are related to the long interval between the evening MR feeding and the blood sampling the next morning (16 h). Low starter intake until d 30 and stressful environmental conditions can be added to this effect. Thermal stress increases the energy requirements for maintenance, resulting in faster utilization of the circulating glucose (Drackley, 2008).

Results indicated that calves presented some degree of hypoglycemia before the morning MR feeding. Glucose concentrations would probably be higher if the time interval between evening and morning MR feedings was shorter, especially during the first month of life, when starter intake was irrelevant. Stanley et al. (2002), however, did not observe different glucose concentrations before the morning feeding when milk was fed once or twice with starter intake of approximately 200 g/d in the second week of life.

Variation in glucose concentration with age was inverse to those previously reported (Quigley et al., 1991; Stanley et al., 2002), being higher after weaning compared with the preweaning period ($P < 0.05$). Low glucose concentrations at 15 and 30 d old were related to the time interval between evening and morning MR feedings and to stress from environmental conditions. Glucose concentration increased after weaning because of the greater starter intake. Associated with a better thermoregulatory ability, this would result in higher plasma glucose concentrations compared with preweaning period. Webb et al. (1969) related that when calves are fed milk in amounts restricted to 10% of BW, postweaning starter intake can account for an increase in glucose concentration.

Serum insulin concentrations differed among MR feeding strategies ($P < 0.05$; Figure 4). Insulin concentration was greater for group 6L-60d compared with the

others, indicating better energetic status of these calves during the evaluated period ($P < 0.05$). Group 4L-60d had intermediate insulin concentration values, whereas group 6L-29d/4L-60d had the lowest insulin concentration ($P < 0.05$). The lower concentration observed for this group might be related to the abrupt reduction in the amount of MR offered, which was not compensated by an increase in starter intake. This resulted in lower nutrient supply and a reduction in rate of weight gain in wk 5 for 6L-29d/4L-60d calves (Table 2).

Increase in glucose concentration as a result of increase in insulin levels can be perceived by variation of insulin concentrations with age ($P < 0.05$). Serum insulin concentrations decreased from 15 to 45 d old, when glucose concentration was also low. At 60 d, both glucose and insulin concentrations increased ($P < 0.05$). Insulin concentrations did not change from 60 to 90 d of age (Figure 4). As glycemia was stable through preweaning period and increased after weaning, we expected that insulin concentrations would also increase after weaning. The lowest insulin concentration was observed at 45 d, similar to the age associated with the lowest insulin concentration reported by Daniels et al. (2008). This might be related to the fixed amount of MR fed, which resulted in lactose contributions being lower compared with energy requirements as the calves grew older and larger. Starter intake at 45 d was significant, but proper ruminal function was still being established, especially with a meal starter (Porter et al., 2007).

CONCLUSIONS

Feeding dairy replacement calves according to the 6L-29/4L-60d strategy is not recommended because performance was similar to that of calves fed 4L-60d, even though MR intake was higher in the first month. Changes in glucose and insulin concentrations were related more to age than to MR feeding strategy. However, higher insulin concentrations for calves fed 6L-60d indicated better energy status for those animals. The 6L-60d strategy resulted in better performance, without declines in starter intake pre- and postweaning or negative effects on rumen development and establishment of ruminal fermentation.

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