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Effects of Feeding Programs Based on One or Two Milk Replacer Daily Meals on Growth, Solid Feed Intake and Rumen Fermentation and Development of Dairy Calves

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Abstract

Sixteen Holstein calves were used to study the effects of two feeding programs (FP) on growth, intake, rumen development and ruminal metabolism from birth to weaning. Two feeding programs based on milk replacer (MR) were tested: a once a day (OAD) MR (200 g/L) distribution vs. a standard twice a day (TAD) MR (125 g/L) distribution. All calves received water, wheat straw and a starter concentrate *ad libitum*. Four calves per group were slaughtered at weaning and rumen epithelium from the ventral sac was sampled for papillae (RP) density. Results showed that the FP had no effect on body weight of calves and total feed intake. From day 42 to day 56, ruminal pH was lower (P = 0.036) and ruminal oxydo-reducing potential was higher (P = 0.001) in OAD than TAD calves. Ruminal total volatile fatty acid (VFA) concentrations did not significantly differ between FP. From day 21 to day 63, butyrate ruminal concentration was significantly higher in OAD than TAD calves (5.17 vs 3.95 mmol/L). This probably explained the higher development of RP in calves fed once daily. Finally, the tested feeding system based on a once daily MR distribution affects the concentrate feeding pattern of calves.

Keywords

Dairy Calves, Milk Replacer, Feeding Frequency, Oxydo-Reducing Potential, Rumen Development

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1. Introduction

For dairy farmers, successful rearing of heifers targeting a future productive dairy cow is associated with economic and efficient management but also with labor simplification. Actually, raising heifers is an expensive and time-consuming investment for dairy farmers, specifically during the pre-weaning period. Current dairy farmers practice is to provide calves with whole milk or milk replacer (MR) twice a day and encourage starter intake to promote anatomical and functional development of the rumen before weaning. Because of the increasing size of herds, reduction of work load is one of the main criteria for dairy producers to decide the feeding strategy of dairy calves. Consequently, some farmers are choosing simplified feeding programs in order to save time without compromising digestive health and growth performance.

Early-life feeding of calves is decisive for subsequent productivity of dairy cows [1]. Studies tackling the question of quantity of MR [2]-[4] or milk feeding frequency [5]-[7] during early calf-rearing are well documented in literature. Formerly, Galton and Brakel [8] stated that feeding MR once daily reduced labor without affecting health, weight gain, or starter consumption of calves weaned at 40 days of age. However, growth performances were very low in their experiment: average daily gain (ADG) from birth to 6 weeks for calves fed once daily was 290 g/d compared to 280 g/d for those fed twice daily. Currently, in French dairy farms, objectives regarding calves in the pre-weaning phase are to double weight from birth to weaning (*i.e.* ADG of 750 g/d from birth to weaning on average) and to attain a daily consumption of 2 kg of starter concentrate just before weaning at 2 months of age on average.

Providing calves with MR once a day is already a common practice in French dairy farms for calves from 3 weeks of age to weaning but feeding calves once a day from the first week of life is unusual. A way to combine labor reduction and animal performances is to provide daily high quantity of MR once a day, *i.e.* in a limited volume ranging from 2 to 3.5 L.

Consequently, the present study aimed at evaluating two different feeding programs differing on MR composition, distribution and feeding frequency (once or twice a day) from 5 days of age to weaning and their respective effects on growth, solid feed intake, rumen development, ruminal physicochemical and fermentative parameters of calves.

2. Materials and Methods

2.1. Calves, Experimental Design and Diets

All animal housing and handling procedures were in accordance with the guidelines for animal research of the French Ministry of Agriculture [9]. Two groups of eight male Holstein calves each (mean initial body weight (BW) = 45.9 ± 5.7 kg at birth) were involved in the trial from birth (d 1) to weaning (d 63). They were immediately separated from their mother at birth (d 1) and placed in individual pens (1.05 × 2.30 m, bedded with straw) without any contact with adult animals. At birth (d 1), they received colostrum in two equal meals. Then they received 2 L of whole milk twice a day (0800 and 1600 h) until d 4 in the morning. On d 4 in the evening, they received an oral rehydratant (Vitactif sachet repas, Chasseneuil du Poitou, France) mixed in 2 L of water to manage a transition between whole milk and MR distribution beginning on d 5. Two feeding programs were tested: 1) one based on a once a day (OAD) MR distribution (200 g/L, Technique Once a Day®, Bonilait Protéines, Chasseneuil du Poitou, France) and 2) another based on a standard twice a day (TAD) MR distribution (125 g/L). Both MR were formulated by the same supplier (Bonilait-Protéines, Chasseneuil-du-Poitou, France) with the same proportion of dairy proteins but were different in terms of skim milk powder and/or whey protein contents. They had similar chemical compositions: on average 20.8% crude protein (CP) and 18.0% fat, on a dry matter (DM) basis (**Table 1**). Calves were randomly allocated to a feeding program on d 5 in the morning.

The volume of milk distributed per calf and per meal was similar among feeding programs and varied with age: 2 L/meal (d 5 to d 7), 2.5 L/meal (d 8 to d 14), 3 L/meal (d 15 to d21), 3.5 L/meal (d 22 to d 42), 3 L/meal (d 43 to d 49), 2.5 L/meal (d 50 to d 56) and 2 L/meal (d 57 to d 63). Total amounts of MR over 63 d were 33.0 and 39.7 kg of DM/calf for OAD and TAD programs, respectively. From d 5 to weaning, calves received *ad libitum* a starter concentrate and wheat straw (**Table 1**), in a hayrack. Each calf had free access to water from d 3 to the end of the experiment (d 63). Water and concentrate were distributed individually, in separate buckets, in two meals at 0800 and 1600 h.

Table 1. Chemical composition of milk replacers, starter concentrate and wheat straw fed to calves.

Items	OAD Milk[1] replacer	TAD Milk[1] replacer	Starter[2] concentrate	Wheat straw
Dry matter, %	96.9	97.0	89.0	89.4
% of dry matter				
Organic matter	92.4	92.2	91.2	94.2
Crude protein	20.7	20.9	17.5	4.5
Neutral detergent fibre	ND	ND	78.9	87.2
Acid detergent fibre	ND	ND	32.2	57.5
Acid detergent lignin	ND	ND	22.4	12.5
Starch	ND	ND	19.5	ND
Ether extract	18.0	18.0	4.3	ND

OAD = calves received MR once a day (200 g/L of MR); TAD = calves received MR (125 g/L of MR) twice a day; MR = milk replacer. [1]Contained skim milk powder, buttermilk, concentrated milk proteins, whey powder, vegetal and dairy fat, products and by products of wheat; vitamin A (25000 UI/kg), vitamin D3 (10000 UI/kg), vitamin E (60 mg/kg), vitamin B1 (10 mg/kg), vitamin K3 (2 mg/kg), vitamin C (150 mg/kg), Fe (80 mg/kg), Cu (10 mg/kg), Se (0.25 mg/kg), lysine (1.27%), methionine (0.55%); Bonilait-Protéines, Chasseneuil du Poitou, France. [2]Contained vitamin A (10080 UI/kg), vitamin D3 (3100 UI/kg), Cu (25 mg/kg), Zn (124 mg/kg), I (1.24 mg/kg), Co (0.23 mg/kg), Se (0.31 mg/kg). ND = not determined.

2.2. Growth Performances

The calves were weighed on d 1 before the first *colostrum* meal and then on a 7-day interval basis until weaning (d 63). Average daily gain was calculated between birth and weaning and also separately from d 1 to d 22 and from d 22 to d 63.

2.3. Feed Intake and Apparent Total Tract Digestibility

Amounts of MR, starter concentrate and straw offered and refused were individually and daily recorded. Chemical composition of each feedstuff was determined from samples taken at the beginning, in the middle and at the end of the experimental period.

Approximately 1 h after the morning meal, spot fecal samples were collected over 5-consecutive days during weeks 4 and 9 for each calf. They were immediately frozen for subsequent analysis. The diet organic matter (OM) digestibility was calculated from fecal CP as suggested by Lukas *et al*. [10] and already used by Lohakare *et al*. [11] for dairy calves before weaning (35 to 70 days of age):

$$Y = 79.76 - 107.7e^{(-0.01515 \times X)},$$

where X is fecal CP concentration (g/kg OM) and Y is diet OM digestibility (%).

2.4. Ruminal Sampling

Samples of whole ruminal contents were obtained from each calf during its first 24 h of life and then every 7 days over the 9 weeks of the experimental period. Ruminal content samples (approximately 200 mL) were collected via a stomach tube (internal diameter 13 mm, sterilized before use with 2% Steranios (Centravet 803910, disinfection of surgical and medical equipment) 1 h after the milk morning meal and just before the distribution of straw and starter concentrate. Ruminal pH and oxydo-reducing potential (E_h) were measured as described by Rey *et al*. [12]. Then, a representative aliquot (50 g) was strained through a metal sieve (1.6-mm mesh) and 25 g of the filtrate was acidified to pH 2 with 50% H_2SO_4. Subsamples (8 mL) were frozen at −20˚C for later determination of volatile fatty acids (VFA) and ammonia (NH_3-N) concentrations.

2.5. Rumen Development Parameters

Four calves per group were slaughtered at weaning in an industrial slaughterhouse. Carcass weight (CW) was recorded. The reticulorumen, omasum, and abomasum were separated, emptied, rinsed repeatedly with water

and weighed individually. Rumen epithelium was sampled at a standardized location (ventral sac) and fixed in a neutral 10% formaldehyde solution. Rumen papillae (RP) were counted on three 1 cm^2 areas by image analysis (Visilogue 6.5 Noesis, France). Other samples were embedded in paraffin, and serial histological sections were stained with hematoxylin and eosin for morphometry analysis under light microscope: two slides for each tissue sample were prepared. Morphometry analysis involved papillae length (L), and width at basis (Wb) and at summit (Ws). For each tissue sample, 20 to 25 measurements were done on each slide using an optical binocular microscope (Leica Wild M3C, Leica, France) coupled via a digital camera to a computer equipped with an image analysis software (Visilogue 6.5 Noesis, France). The absorption surface was calculated as [L × (Wb + Ws)/2] × RP × 2. The mucosa (epithelium, chorion and submucosa) was also observed for histopathological investigation.

2.6. Chemical Analysis

The concentrations of VFA were determined using the gas chromatographic method of Playne [13], modified by Marden *et al*. [14]. The determination of NH_3-N was based on the modified Berthelot reaction with the Skalar Method, followed by a colorimetric test [15]. Dry matter and OM content of samples were determined by oven drying at 104˚C for 24 h (48 h for feces) and by ashing at 550˚C for 12 h, respectively. Crude protein was analyzed using a RapidN Cube (Elementar, Donaustrasse 7, Hanau, Germany). Acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin fractions were determined by means of van Soest extraction protocol [16] using a fiber analyzer (Fiberbag Ankom 220, Macedon, NJ, USA): NDF was assayed without heat stable amylase and expressed inclusive of residual ash; ADF was expressed inclusive of residual ash and lignin was determined by solubilization of cellulose with sulphuric acid (72%).

2.7. Statistical Analysis

All data were analyzed using R.3.1.1 software and were reported as mean values with standard error. Data dealing with several measurements within animal over time were analyzed as a mixed model with repeated measures with calf as random effect, age and feeding program as fixed effects. Three periods were focused on: 1) d 1 to d 63 as total experimental period, 2) d 21 to d 63 as part of the experimental period (high growth period) and (3) d 42 to d 56 as part of the experimental period (last three weeks before switching to a once a day MR distribution for all the calves). Data not dealing with several measurements within animal over time were analyzed as a linear model with calf as random effect and feeding program as fixed effect. Regarding ADG, total starter concentrate and straw intakes, three periods were focused on: 1) d 1 to d 63 as total experimental period, 2) d 1 to d 21and (3) d 22 to d 63. Differences were considered significant at $P \leq 0.05$ and trends were discussed at $0.05 < P \leq 0.10$.

3. Results

The results concerning the effect of feeding program on growth, solid feed intake, fecal nitrogen concentration and organic matter digestibility in calves from birth to weaning are presented in **Table 2**. Solid feed intake, *i.e*. starter concentrate and straw, varied considerably with age but were not affected by feeding programs ($P > 0.05$). Calves ate an average of 2.9 kg DM of straw and 44.9 kg DM of concentrate from birth to weaning.

Body weight of calves did not differ between feeding programs: calves weighed 45.9 kg at d 1 and reached 96.2 kg at d 63, on average. The ADG from birth to weaning did not differ between feeding programs: 755 and 842 g/d for OAD and TAD calves, respectively. However, ADG between d 1 and d 21 was higher ($P = 0.011$) for TAD calves than OAD calves (+158 g/d) whereas ADG between d 21 and d 63 (1017 g/d, on average) was similar ($P = 0.513$) between feeding programs. From d 42 to d 56, the effect of the interaction between age and feeding programs was significant ($P = 0.047$) and OAD calves increased more rapidly their starter concentrate intake than TAD calves (**Figure 1**). Nitrogen fecal content and OM digestibility were not affected by feeding program and averaged 52.8 and 34.6 g/kg OM and 78.9% and 77.1%, respectively at week 4 and week 9, respectively.

Ruminal pH averaged 6.24 over the experimental period and was not affected by age or feeding program (**Table 3**). These factors tended to interact ($P = 0.10$). Focusing on the d 42 to d 56 period, pH values were significantly higher for TAD than OAD calves ($P = 0.036$, **Figure 2(a)**. Ruminal E_h drastically varied with age ($P < 0.001$): positive values were recorded at d 1 (+253 mV on average) and negative values were observed

Table 2. Effect of feeding program on growth, solid feed intake, fecal nitrogen concentration and organic matter digestibility in calves from birth to weaning.

Items	OAD[1] (n = 8)	TAD (n = 8)	SEM[2]	*P*-value
Body weight, kg				
d1	45.8	46.1	1.44	0.93
d7	47.9	48.6	1.28	0.78
d14	48.6	51.0	1.36	0.38
d21	52.7	56.4	1.64	0.25
d28	58.7	63.3	1.87	0.20
d35	64.5	69.3	2.12	0.24
d42	69.6	76.5	2.57	0.17
d49	77.0	84.0	2.60	0.16
d56	84.9	91.4	2.68	0.21
d63	93.4	99.1	2.86	0.31
ADG, g/day				
d1 - d21	313	471	36.2	0.011
d22 - d63	992	1042	37.5	0.51
d1 - d63	755	842	32.1	0.15
Straw intake, g DM				
d1 - d21	385	349	23.4	0.45
d22 - d63	2634	2459	319.0	0.79
d1 - d63	3019	2808	331.5	0.75
Starter concentrate intake, g DM				
d1 - d21	2293	3102	460.1	0.38
d22 - d63	43011	41339	3585.5	0.82
d1 - d63	45305	44441	3964.0	0.91
N in feces, g/kg MO				
Week 4	50.4	55.2	6.40	0.15
Week 9	38.2	31.0	3.67	0.58
OM digestibility[3], %				
Week 4	79.1	78.7	0.29	0.49
Week 9	76.9	77.3	0.38	0.58
FCR d1 - d63	1.70	1.63	0.03	0.35

[1]OAD = calves received MR once a day (200 g/L of MR); TAD = calves received MR (125 g/L of MR) twice a day; MR = milk replacer. [2]SEM = standard error of the mean. [3]Calculated by the equation of Lukas *et al*. [10].

Table 3. Effect of feeding program on ruminal physicochemical (n = 10 per calf) and fermentation parameters (n = 10 per calf) during pre-weaning period.

Items					*P*-value[3]	
	OAD[1]	TAD	SEM[2]	FP	A	FP × A
Physicochemical parameters						
E_h[4], mV	−81	−115	10	0.059	<0.001	0.88
pH	6.13	6.36	0.06	0.97	0.37	0.10
Fermentation parameters						
Volatile fatty acids						
Total, mmol/L	73.2	69.1	3.0	0.92	<0.001	0.65
Acetic acid, mmol/L	44.9	41.9	1.7	0.79	<0.001	0.20
Propionic acid, mmol/L	21.9	21.6	1.2	0.86	<0.001	0.75
Butyric acid, mmol/L	4.2	3.6	0.2	0.16	0.003	0.84
Acetic acid, %	65.1	63.8	0.8	0.35	<0.001	0.56
Propionic acid, %	26.3	28.4	0.7	0.15	<0.001	0.87
Butyric acid, %	6.0	5.1	0.4	0.26	0.83	0.80
Ammonia nitrogen, mg/L	71.5	48.6	4.5	0.072	0.36	0.75

[1]OAD = calves received MR once a day (200 g/L of MR); TAD = calves received MR (125 g/L of MR) twice a day; MR = milk replacer. [2]SEM = standard error of the mean. [3]FP = feeding program; A = age effect; FP×A = interaction effect between feeding program and age. [4]E_h, = oxydo-reducing potential.

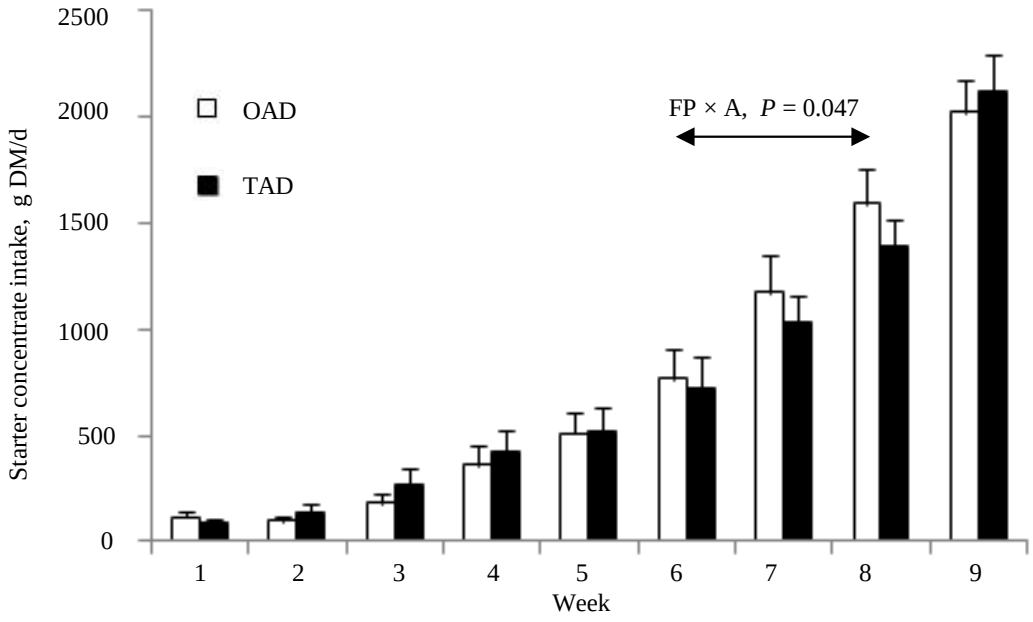

Figure 1. Effect of feeding program on starter concentrate intake pattern with the age of calves by week (w) during the pre-weaning period. Vertical bars show standard errors. OAD = calves received MR once a day (200 g/L of MR); TAD = calves received MR (125 g/L of MR) twice a day; MR = milk replacer; FP = feeding program effect; A = age effect; FP × A = interaction effect between feeding program and age.

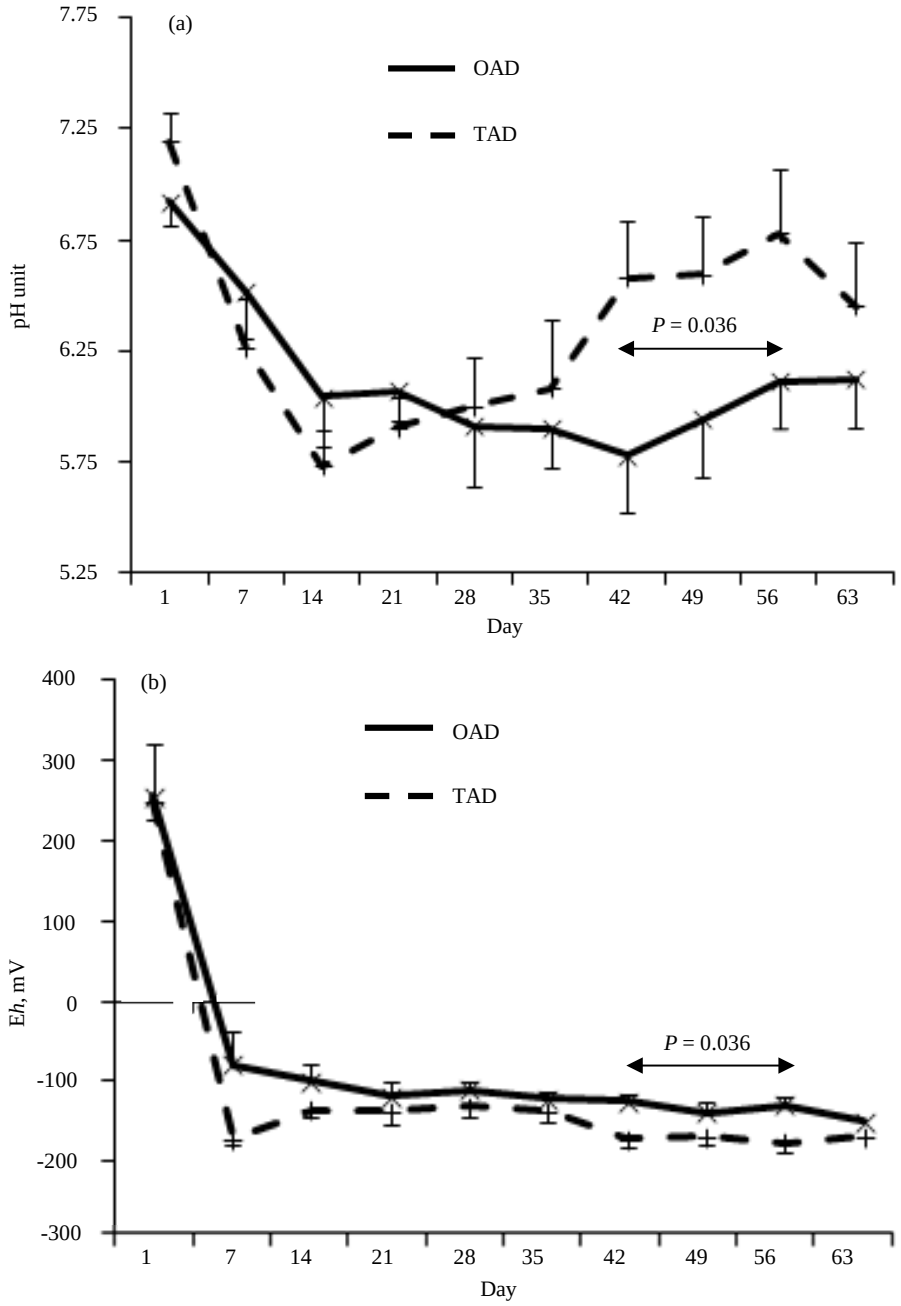

Figure 2. Effect of feeding program on evolution of pH (a) and oxydo - reducing potential (E_h; b) with the age of calves during the pre-weaning period. Vertical bars show standard errors. OAD = calves received MR once a day (200 g/L of MR); TAD = calves received MR (125 g/L of MR) twice a day; MR = milk replacer.

from d 7 to d 63 (**Figure 2(b)**). Values tended to differ with feeding program over the pre-weaning period (P = 0.059): −81 and −115 mV for OAD and TAD calves, respectively. A significant effect of feeding program was observed for E*h* values recorded between d 42 and d 56 (P = 0.001).

Total and individual VFA ruminal contents varied with age of calves from birth to weaning but were not significantly affected by feeding program: total VFA concentration averaged 71.2 mM (**Table 3**). No effect of feeding program was observed on the evolution of total VFA, acetate and propionate contents with the age of calves during the preweaning period (**Figures 3(a)-3(c)**). However, from d 21 to d 63, butyrate ruminal concentration

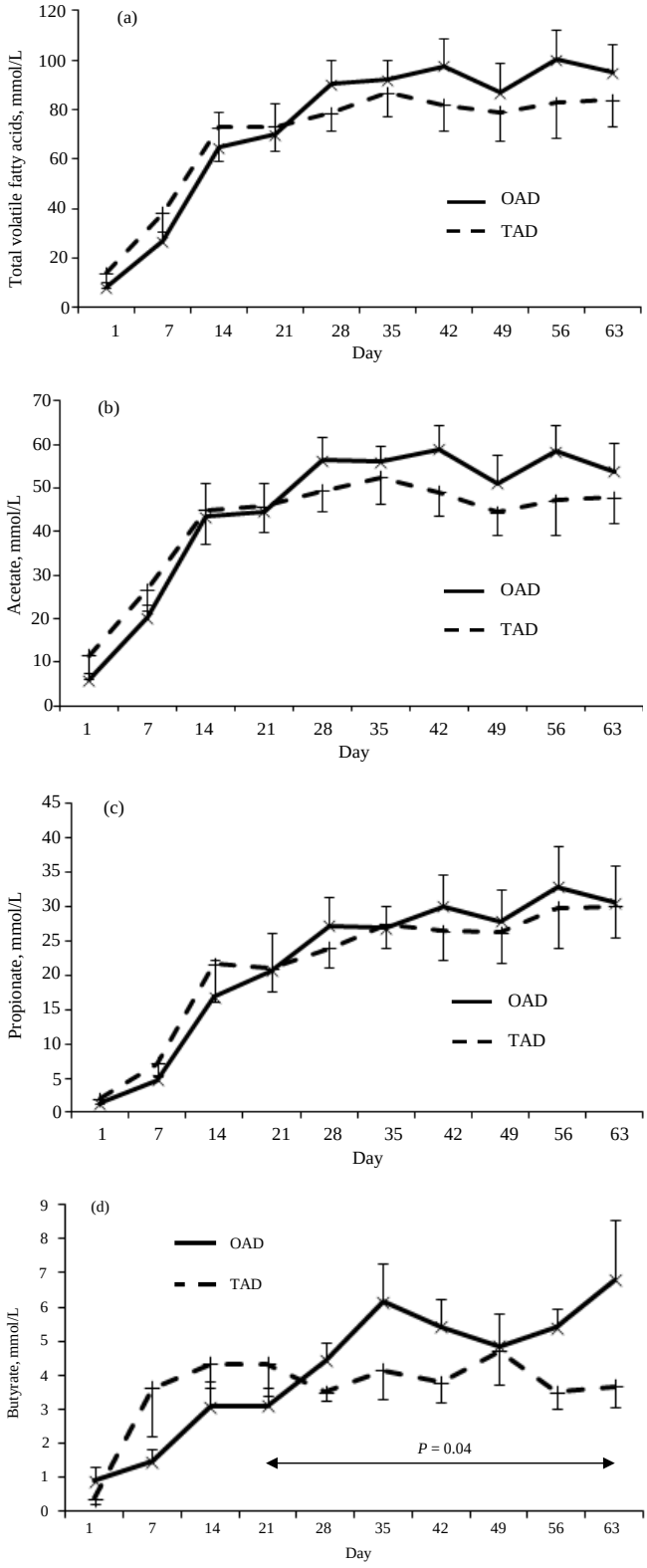

Figure 3. Effect of feeding program on evolution of ruminal total (a) volatile fatty acids (VFA), acetate (b), propionate (c) and butyrate (d) content with the age of calves during the pre-weaning period. Vertical bars show standard errors. OAD = calves received MR once a day (200 g/L of MR); TAD = calves received MR (125 g/L of MR) twice a day; MR = milk replacer.

was significantly higher for OAD than TAD calves: 5.17 and 3.95 mM, respectively (**Figure 3(d)**). Ruminal NH_3-N content did not vary with age (P = 0.364) whereas it tended (P = 0.072) to differ between feeding programs: 71.5 and 48.6 mg/L for OAD and TAD calves from day 1 to day 63, respectively (**Table 3**).

At slaughter, CW tended to differ (P = 0.092) between OAD and TAD calves and was 45.9 and 51.3 kg, respectively (**Table 4**). Total stomachs weight (5.2% of CW on average) and relative weights of reticulo-rumen (62.9%), omasum (16.8%) and abomasum (20.3%) did not differ between feeding programs. The RP density was higher (P = 0.006) for OAD (84.8 RP/cm^2) than TAD calves (64.7 RP/cm^2) and no pathologic abnormality was detected. The absorption surface of ruminal epithelium was 57% higher (P = 0.002) for OAD than TAD calves.

4. Discussion

It should be emphasized that in the present experiment, we studied the effect of two calves-feeding programs differing mainly on MR allowance and distribution frequency. Feeding dairy calves once a day first aimed at saving time for dairy farmer. However, growth performances must be maintained without jeopardizing future replacing heifers. This study showed that both dietary programs reached the growth objective of doubling birth weight at weaning: 93.4 and 99.1 kg for OAD and TAD calves, respectively. Dairy calves had an average ADG of 799 g/d from birth to weaning at 63 days of age, which met the objective of approximately 750 g/d. Similar values were reported by Terré *et al*. [4] who recorded a 790 g/d ADG for calves receiving a conventional feeding program and weaned at 47 days of age, and Jasper and Weary [3] who observed a 780 g/d ADG for calves receiving whole milk *ad libitum* and weaned at 42 days of age. In our experiment, growth pattern of calves differed among diets: over the three first weeks of life, ADG was significantly lower for OAD (313 g/d) than for TAD calves (471 g/d) but did not differ thereafter, averaging 1017 g/d. This suggested that an early switch to a once a day distribution of MR 1) was nutritionally stressful for calves and 2) temporarily did not allow to completely meet the calves requirements since dry feed consumption was numerically lower for OAD

Table 4. Effect of feeding program on rumen development in calves at weaning.

	OAD[1]	TAD	SEM[2]	*P*-value
Carcass weight, kg	45.9	51.3	1.8	0.092
Weight, kg				
Reticulorumen	1.44	1.76	0.11	0.13
Omasum	0.43	0.41	0.04	0.72
Abomasum	0.47	0.56	0.02	0.002
Whole stomach	2.34	2.72	0.13	0.11
% of whole stomach weight				
Reticulorumen	61.3	64.6	1.9	0.38
Omasum	18.6	14.9	1.6	0.22
Abomasum	20.1	20.5	0.6	0.73
Ruminal papillae (ventral sac)				
Length, mm	2.18	1.93	0.18	0.50
Width at basis, mm	0.34	0.30	0.01	0.020
Width at summit, mm	0.21	0.20	0.01	0.78
Density, papillae/cm^2 of mucosa	84.8	64.7	5.0	0.006
Surface, mm^2/cm^2 of mucosa	98.1	62.4	8.6	0.002

[1]OAD = calves received MR once a day (200 g/L of MR); TAD = calves received MR (125 g/L of MR) twice a day; MR = milk replacer. [2]SEM = standard error of the mean.

(2293 g) than TAD (3102 g) calves before day 21. So, in our study dry feed consumption did not begin earlier with OAD program. This result contradicts those of Jasper and Weary [3], Terré *et al*. [17] and Morrison *et al*. [18] who showed that increasing the intake of nutrients from MR delayed or decreased starter intake. However, our results corroborated those of Kmicikewycz *et al*. [6] who showed that increasing feeding frequency from two to four meals per day decreased starter intake before week 3 but increased it thereafter. In our experiment, OAD calves had numerically higher starter concentrate intake than TAD calves from week 6. The significant interaction observed between age and feeding program from d 42 to d 56 underlined this rapid increase of starter intake, suggesting an ability of calves to compensate the low initial dry feed intake. Finally, the total starter DM intake over the period did not differ between feeding programs: on average calves ate 44.9 kg DM of starter from birth to weaning. Stanley *et al*. [19] and Kehoe *et al.* [5] also did not find any change in starter consumption between calves receiving the same daily amount of MR whether once or twice a day. Regarding forage intake, calves ate in average 2.9 kg of wheat straw DM over 9 weeks. On the one hand it represented a negligible energy and protein intake but on the other hand it provided physical fiber which is essential for anatomical development of the rumen of calves [20].

Kosiorowska *et al.* [21] observed a heavier reticulo-rumen and omasum in calves fed twice a day a low volume of whole milk (1.6 kg/d) compared to calves receiving a high volume (3.2 kg/d). In the present study, calves were voluntary fed the same MR volume per meal. It resulted in no alteration of reticulo-rumen relative weight whereas abomasum of OAD calves was heavier than that of TAD calves (P = 0.002): this could be explained by a shorter time for emptying abomasum in TAD than OAD calves. Warner and Flatt [22] showed that the rumen increased from 30% to 70% of total tissue weight of pre-ruminant stomach of cattle during the pre-weaning period. In accordance with these observations, we showed that the reticulo-rumen represented an average of 62.3% of whole emptied stomach weight at weaning.

Ruminal pH pattern from birth to weaning greatly differed between OAD and TAD calves. At birth, rumen is a neutral milieu (7.06 pH units on average) as already observed by Rey *et al.* [12]. In the present experiment, there was a tendency (P = 0.10) for a feeding program × age interaction: pH values drastically decreased since birth to reach a nadir value at d 14 for TAD (pH = 5.73) calves and at d 42 for OAD calves (pH = 5.78). This delay could relate to the numerically lower starter intake before 3 weeks of age in OAD than TAD calves. Moreover, pH was significantly lower for OAD calves from d 42 to d 56, which could also be due to their strongly increasing starter intake. In parallel, total VFA ruminal content dramatically increased from birth to d 14 and tended to stabilize afterwards. Butyrate ruminal content was significantly higher (P = 0.04) for OAD than TAD calves from d 21 to d 63.

Before being a ruminant, growth and development of the ruminal absorptive surface area (papillae) is required to enable absorption and utilization of microbial digestion end products, in particular rumen VFA [23]. Regarding RP morphology, there was no difference between feeding programs: length of papillae in the ventral ruminal sac averaged 2.1 mm which was in accordance with measurements made by Kristensen *et al*. [24]: from 1.7 to 3.5 mm at d 35. Regarding ruminal papillae density, results showed that, the number of papillae per square centimeter was on average higher in the rumen ventral sac for OAD calves than TAD calves (P = 0.006): 84.8 and 64.7 RP/cm^2 respectively, resulting in a larger absorptive surface area at weaning. From a histological point of view, this is particularly advantageous in young pre-ruminant. It is well known that ruminal papillae development is a consequence of fermentative activity and specifically VFA production. Numerous researchers have pointed out that dry feed intake and the resultant microbial end products stimulated rumen epithelial development [25] [26]. A constant presence of VFA maintained rumen papillae growth, size and function [23] and increasing amounts of concentrate in the diet resulted in increased papillae density and height in calves [27]. In the present study, calves ate the same quantity of starter concentrate and wheat straw from birth to weaning and had similar total VFA concentrations, so that concentrate intake and VFA concentration could not explain the higher papillae density observed in OAD calves. But, it was stated that, among VFA, butyrate is most stimulatory [28] [29], followed by propionate. In fact, butyrate metabolism by the epithelium appeared to increase with decreasing rumen pH and increasing butyrate concentrations [30]. In our study, rumen pH was lower from d 42 and butyrate concentration was higher from d 21 in OAD than TAD calves, which could explain the higher RP density in the ventral sac of OAD (84.8/cm^2) than TAD calves (64.7/cm^2), and as a consequence the higher absorption surface. The lower ruminal pH and higher butyrate rumen concentration in OAD compared to TAD calves could be due to changes of solid feed intake pattern, modulating rumen metabolism *i.e.* fermentative end-products production or/and absorption.

To go a step further, redox ruminal status was also measured. This is an original parameter which has already been measured *in vitro* in ruminal fluid [31] or *in vivo* on dry dairy cows [32], lactating dairy cows [14] [33], heifers [34] and *ex vivo* in calves [12]. Indeed, ruminal E_h was positive at birth (+253 mV on average whatever was the diet) and then drastically decreased to reach negative values (−149 and −168 mV at d 63 for OAD and TAD calves, respectively) previously observed in rumen of adult cows. Those results are in total accordance with the observations made by Rey *et al*. [12]. Moreover, ruminal E_h tended to differ between feeding programs from birth to weaning ($P = 0.06$): E_h was significantly higher in OAD than TAD calves from d 42 to d 56. During this period, starter intake was higher ($P = 0.047$), ruminal pH was lower and butyrate ruminal content was higher in OAD calves than TAD calves. As a consequence, the clear-cut relationship observed by Baldwin and Emery [35] between the metabolic rate of rumen microorganisms and E_h was obvious, especially just before weaning when rumen can be considered as physiologically mature.

Total tract digestibility of DM was evaluated on N fecal content as already made by Lohakare *et al.* [11] for dairy calves fed MR twice a day and weaned at 70 days. It was not influenced by feeding program: OM digestibility averaged 78.9% on week 4 and 77.1% on week 9 which is in accordance with results obtained by Lohakare *et al.* [11]. However, our sampling periods for digestibility measurements did not correspond with periods where rumen pH and E_h differed between OAD and TAD calves, which could have masked a link between changes of rumen milieu and total tract digestibility.

5. Conclusion

Feeding dairy calves once daily from the fourth day of age to weaning at d 63 permitted to fulfill the objective of growth (body weight higher than 90 kg at weaning) and starter concentrate intake (an average daily starter intake higher than 2 kg DM/d at weaning). However, a growth depression was clearly observed from d 1 to d 21 for calves fed once a day: during this period, a less MR consumption was not balancing by higher starter concentrate intake. The feeding frequency (one or twice a day) modifies ruminal metabolism, especially a couple of weeks before weaning when physicochemical parameters (pH and E_h) and butyrate concentration differed. This change probably explained the higher development of ruminal papillae in calves fed once daily. Finally, the tested feeding system based on a once daily milk replacer distribution certainly affects the concentrate feeding pattern of calves: it needs to be studied more in details in the future.

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