

Effects of intensified or conventional milk feeding on pre-weaning health and feeding behavior of Holstein female calves around weaning

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Abstract

Health, inflammatory, and stress indices as well as feeding behavior around weaning were measured for Holstein female calves fed intensified milk or conventionally during the pre-weaning period. Calves (n = 48) were randomly assigned to one of two experimental treatments including a conventional (CF) or an intensified feeding (IF) groups. In CF group, calves (n = 24) received 0.52 kg of dry matter (DM) per day from pasteurized whole milk (23.00% crude protein (CP) and 27.00% fat) until day 50 of age. In IF group, calves (n = 24) fed 0.97 kg of DM per day on average for the first three weeks, and then, milk allowance decreased gradually to reach 0.52 kg DM per day and continued until day 50. All calves were gradually weaned from day 51 to 56. Blood samples were taken on days 14, 28, and 57 at 06:30 AM for serum amyloid A (SAA), cortisol, alanine aminotransferase (ALT), and iron analyses. Conventionally fed calves had more days with fever during the pre-weaning period. Blood SAA and cortisol levels were higher in CF calves on day 14. However, SAA levels were higher for IF calves on day 57. Intensified milk-fed calves spent more time for standing than CF calves. A trend to be significant was observed for non-nutritive oral behavior in IF calves. In summary, dairy calf health can be improved by intensified milk feeding during the pre-weaning period; however, this method has the potential to reduce calves welfare around weaning transition.

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Introduction

Dairy calves are susceptible to many infectious diseases during the early weeks of age, especially the first two weeks. According to the National Animal Health Monitoring System, calf mortality in the pre-weaning period is still high in many dairy farms (approximately 7.50 to 10.00%).¹ It is believed that future milk production and herd life may be affected by calf health during the early weeks of age. It has been shown that early life dullness can be a risk factor of calf mortality after 90 days of age and shorter herd life.² Feeding and other management practices in dairy farms are considered as main factors influencing calves health and well-being. It seems that a higher rate of mortality in the critical pre-weaning period is mostly related to calves poor welfare.³ Poor nutrition or under-nutrition accompanied by disease, weaning, and transportation, is one of the most important stress factors for dairy

calves.⁴ Calves respond to inflammation and stressors by an increased level of some acute-phase proteins such as serum amyloid A (SAA). These proteins are also recognized as animal health biomarkers and used for early diagnosis of diseases.⁴ In addition to health status, expressing natural behavior in relatively natural life is defined as an index of animal welfare.³ Rearing dairy calves in intensive farming systems had a significant effect on the expression of calves behavior due to separating from dams and raising according to farm management practices.⁵ Conventionally, calves are fed about 10.00% of body weight which is equal to 4.00 - 5.00 kg of milk or milk replacer per day. This system is based on solid feed intake and early rumen development and has been used for many years by producers.⁶ However, during the last two decades, this feeding method has been challenged by several studies.⁷⁻⁹ Studies on milk feeding programs have shown that conventional feeding methods can reduce calves weight gain¹⁰ and

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welfare as well.³ Other authors have observed abnormal behavior in restricted milk or conventionally fed calves.¹¹ Opponent system called an intensified milk feeding program provides as much as twice more nutrients rather than a conventional feeding method, supporting greater weight gain and well-being of calves.¹² Soberon et al. have reported that there is a positive relationship between pre-weaning average daily gain (ADG) and heifer's subsequent milk production.⁹ They have announced that 25.00% of the variation in the first lactation can be explained by ADG amounts in the pre-weaning period. Besides many advantages, intensified feeding programs reduce calves starter intake, causing low rumen development.¹³ As mentioned above, improved growth performance of calves in different feeding programs has been reported in many studies; however, the effect of early milk feeding methods on calf's health is under debate. On the other hand, a few studies have investigated the feeding behavior of calves around the weaning transition.

The first objective of this study was to evaluate if the early life milk feeding amounts influence health indices of female calves during the pre-weaning period and if the feeding behavior of calves around weaning can be affected by early feeding methods.

Materials and Methods

Calves, housing, and treatments. This study was conducted on a commercial dairy farm (Behroozi Dairy Farm, Tehran, Iran) from May to September 2016. All experimental procedures and guidelines involving animals were approved by the animal experiment committee at University of Tehran, Karaj, Iran. Forty-eight Holstein female calves were randomly assigned to either an intensified (IF, n = 24; 38.20 kg of initial weight) or a conventional feeding (CF, n = 24; 39.50 kg of initial weight) patterns. After birth, calves were separated from their dams within 1 hr and transported to individual pens (1.40 × 2.35 m) which were evenly distributed in a barn. There was an opening in front of pens that calves would be able to access water and starter buckets. All calves fed 3.00 or 3.50 L of high-quality colostrum, measured by a colostrometer (Kruuse, Langeskov, Denmark), according to their appetite at the first meal and received 2.00 L 12 hr later. Pasteurized whole milk [23.00% crude protein (CP) and 27.00% fat] was fed to each group three times a day at 07:00, 15:00, and 23:00 in equal amounts. In CF group, calves fed 0.52 kg of dry matter (DM) per day from pasteurized whole milk (about 10.00% of body weight) until the beginning of the weaning process at day 50 of age. Intensified milk-fed calves received 0.91, 0.98, and 1.02 kg of DM per day for the first, second, and third weeks, respectively. Therefore, they fed 0.97 kg of DM per day on average in the first three weeks (about 20.00% of body weight). Milk was offered according to each calf's

appetite, refusals were recorded daily and milk consumption of calves was calculated. Milk allowance decreased gradually to reach 0.52 kg of DM per day like CF calves and continued until day 50. Allocated milk volume was gradually decreased to wean calves on day 56. All calves had free access to clean water and starter throughout the study. Starter refusals were recorded and renewed every day after milk feeding at 07:30 AM. Daily starter allocation increased (about 50.00%) when the refusals were less than 20.00% of the offered amount. Ingredients and chemical composition of starter are presented in Table 1. Chopped alfalfa hay (10.00%) was included in the starter on day 42 of age and mixed starter and hay were fed to calves for the rest of the study.

Table 1. Ingredients and chemical composition of the starter diet on a dry matter (DM) basis.

<i>Ingredient</i>	<i>Percentage</i>
Corn grain, ground	47.24
Barley grain	10.00
Soybean meal	26.00
Fish meal	2.00
Wheat bran	3.00
Beet pulp	6.00
Dicalcium phosphate (Ca 23.00%+P 18.00%)	0.50
Limestone	1.10
Mineral vitamin mix*	1.10
Sodium bicarbonate	1.10
Salt	0.50
Zeolite	1.10
<i>Chemical composition</i>	
Dry matter	89.09
Crude protein	20.31
Ether extract	3.02
Neutral detergent fiber	14.76
Acid detergent fiber	6.97
Not fibrous carbohydrate	52.55
Calcium	0.95
Phosphorus	0.57
Gross energy, Mcal kg⁻¹ of DM	3.60

* Mineral vitamin mix composition: 10,000,000 IU kg⁻¹ of vitamin A; 2,000,000 IU kg⁻¹ of vitamin D3; 6,000 IU kg⁻¹ of vitamin E; 0.50 g kg⁻¹ of vitamin B1; 0.50 g kg⁻¹ of vitamin B2; 48.00 g kg⁻¹ of Mg; 35.00 g kg⁻¹ of Zn; 30.00 g kg⁻¹ of Mn; 23.00 g kg⁻¹ of Fe; 10.00 g kg⁻¹ of Cu; 0.60 g kg⁻¹ of I; 0.40 g kg⁻¹ of Co; 0.10 g kg⁻¹ of Se.

Measurements and analysis. Milk and starter intakes of each calf were measured daily. Starter samples were taken at different times (at beginning, middle, and end of study), mixed and one sample (0.10 kg) was sent to a laboratory for subsequent analysis. Dry matter, CP, and ether extract were analyzed according to the Association of Official Analytical Chemists¹⁴ method at the Nutritional Laboratory of Urmia University, Urmia, Iran. Neutral detergent fiber and acid detergent fiber were measured by Ankom system based on Van Soest *et al.*¹⁵ Blood samples were taken through the jugular vein and drawn in 6.00 mL evacuated tubes without any additives on day 14, 28, and 56. Tubes were located askew and allowed to clot. Samples

were centrifuged at 3,000 *g* for 15 min and partitioned serum was collected by sterile samplers and stored at -20.00 °C for the next analysis. Serum amyloid A was measured using an enzyme-linked immunosorbent assay method (Bioassay Technology Laboratory, Shanghai, China) at the Nutritional Laboratory of Urmia University, Urmia, Iran. Serum cortisol concentration was measured using Immulite (Siemens Healthcare Diagnostics, Deerfield, USA). Alanine aminotransferase (ALT) and serum iron (Fe) concentrations were measured by an autoanalyzer (model 912; Hitachi, Tokyo, Japan). The fecal score for fluidity was determined by the procedure of Larson *et al.*¹⁶ as follows: 1: Normal, 2: Soft, 3: Runny, and 4: Watery. Scouring was conducted every morning before milk feeding at 06:30 AM. Other health indices such as days with diarrhea, days treated, days with fever, and rectal temperature at weaning were also recorded daily.

Feeding behavior. Feeding behavior of calves was measured using the method described by Castells *et al.*¹⁷ Eight calves in each group were selected randomly to evaluate feeding behavior. Direct observation was used to record the duration of each behavior (lying without chewing, starter eating, ruminating, standing, and non-nutritive oral behavior). Observations were divided into two periods including pre-and post-weaning four times. Times included two weeks before weaning, a week before weaning, a week after weaning, and two weeks after weaning. During the pre-weaning period, behaviors were recorded twice a week immediately after morning milk feeding and starter offering for 1 hr. For the post-weaning period, behaviors were also recorded twice a week immediately after a starter offering for 1 hr. Observed behaviors at the beginning of each min were assumed for a total of one min. Therefore, each calf was evaluated 8 hr for each feeding behavior.

Statistical analysis. All data recorded throughout the time analyzed using a completely randomized design with repeated measurement method of the MIXED procedure of SAS (version 9.20; SAS Institute Inc., Cary, USA). The effects

of treatments (feeding method), time and interaction of treatment with time were included in the model. Treatment and time were used as fixed effects and the individual calf was considered as a random effect. For feeding behavior, the effects of the period (pre-and post-weaning), time (weeks before and after weaning), and individual calf were included in the model. Least square means (\pm SEM) were reported and differences between treatments were considered at $p < 0.05$ and tendency at $p \leq 0.1$ by Tukey test.

Results

Milk DM intake. Data are belonging to early pre-weaning (EPW; day 1 to 21), late pre-weaning (LPW; day 22 to 56), and overall pre-weaning (OPW; day 1 to 56). Milk DM intake increased as calves aged and intensified milk-fed calves consumed more DM from milk than CF ones during the first three weeks and EPW ($p < 0.01$), LPW ($p = 0.02$), and OPW ($p < 0.01$) periods (Table 2).

Health. Fecal fluidity was not affected by the milk feeding method during the OPW period; but IF calves numerically had a higher fecal fluidity score. The milk feeding method also had no significant effect on the incidence of diarrhea. Conventionally fed calves significantly had higher days with fever ($p < 0.05$) than IF calves. A tendency to be significant was observed in the case of days treated for CF calves ($p = 0.06$). The rectal temperature at weaning was numerically higher for CF calves and a tendency was observed ($p = 0.06$; Table 3). Serum amyloid A was measured as one of the most important components of inflammatory reactions. A treatment \times time interaction was detected for SAA. Conventionally fed calves showed higher levels of SAA than IF calves on day 14 ($p < 0.05$), whereas, the concentration of SAA was higher in IF calves on day 57 ($p < 0.01$). Serum cortisol was considered as a marker of stress status. Cortisol level increased with calf age ($p < 0.05$). Conventionally fed calves had higher serum cortisol level on day 14 ($p < 0.05$) as observed previously

Table 2. Average daily milk and starter dry matter (DM) and nutrient intakes in Holstein female calves fed different patterns of nutrition during the pre-weaning period.

Intake ¹	Feeding pattern ²			<i>p</i> -values		
	Intensified	Conventional	SEM	Treatment	Time	Treatment \times Time
<i>Milk dry matter (kg per day)</i>						
Week 1	0.91	0.52	0.01	<0.01	<0.01	<0.01
Week 2	0.98	0.52	0.01	<0.01	<0.01	<0.01
Week 3	1.02	0.52	0.01	<0.01	<0.01	<0.01
Day 1 to 21	0.97	0.52	0.009	<0.01	<0.01	<0.01
Day 22 to 56	0.50	0.48	0.009	<0.02	<0.01	0.01
Pre-weaning	0.68	0.49	0.009	<0.01	<0.01	<0.01

¹ Periods include Week 1: From 1 to 7 days of age; Week 2: From 8 to 14 days of age; Week 3: From 15 to 21 days of age; Pre-weaning: From 1 to 56 days of age.

² Different patterns of nutrition were used in the pre-weaning period. In the intensified feeding, the calves ($n = 24$) were fed whole milk at the rate of 20.00% of body weight (BW) for the first three weeks of age. After that, milk allowance reduced gradually to 10.00% of BW and continued until day 50. Then, calves were weaned gradually between 51 and 56 days of age. In conventional feeding, the calves were fed at 10.00% of BW from the beginning of the experiment until day 50. Calves weaning procedure was similar to the intensified feeding group.

for SAA. A similar serum ALT concentration was observed for both groups ($p > 0.05$) and its level was increased as calves aged ($p < 0.01$). There was a treatment \times time interaction for Fe concentration. Serum Fe level was higher for CF calves on day 14 ($p < 0.05$; Table 4).

Table 3. Health indices of Holstein female calves fed intensified milk or conventionally during the pre-weaning period.

Items	Feeding pattern*		SEM	p-value
	Intensified	Conventional		
Fecal fluidity score	1.20	1.14	0.03	0.29
Days with diarrhea	1.47	1.14	0.35	0.51
Days treated	2.09	3.57	0.55	0.06
Days with fever	0.38	0.71	0.10	0.03
Rectal temperature	39.00	39.30	0.12	0.06

* Different patterns of nutrition were used in the pre-weaning period. In the Intensified feeding, the calves ($n = 24$) were fed whole milk at the rate of 20.00% of body weight (BW) for the first three weeks of age. After that, milk allowance reduced gradually to 10.00% of BW and continued until day 50. Then, calves were weaned gradually between 51 and 56 days of age. In conventional feeding, the calves were fed at 10.00% of BW from the beginning of the experiment until day 50. Calves weaning procedure was similar to intensified feeding group.

Feeding behavior. The duration of each feeding behavior (min) and an average of a total of 8 hr observation are presented in Table 5. The effects of the period (pre-and post-weaning) and time (weeks before and after weaning) were also reported. Duration of lying without chewing decreased from pre-weaning to post-weaning period ($p < 0.01$); however, it was not affected by treatments ($p < 0.05$). Starter eating time increased as calves aged ($p < 0.01$), but there was not a significant difference between treatments on average of 8 hr observation time ($p < 0.05$). Intensified milk-fed calves spent more time for standing than CF calves during 8 hr observation time around weaning ($p < 0.05$). A tendency to be significant was observed for non-nutritive oral behavior ($p < 0.05$). Intensified milk-fed calves numerically spent more time on non-nutritive oral behavior than those fed conventionally during the pre-weaning period.

Discussion

Dairy calves can safely consume a high volume of milk during the early weeks of age. Greater DM intake from milk resulted in a greater weight gain in IF calves during EPW and OPW periods (unpublished data).¹⁸ Providing high amounts of milk during the early weeks of age did not affect the fecal fluidity score of calves in the current study. The effects of feeding methods on the health of calves have been investigated by several studies; however, the results are controversial. In agreement with this study, some studies have reported that increasing milk feeding level has no effect on fecal fluidity score,⁷⁻⁸ but in some cases, the increased fecal score was observed following the use of intensive milk feeding programs.¹⁹ This inconsistency in the results can be related to differences in management practices and environmental conditions. These factors likely play an important role in the fecal score and incidence of diarrhea rather than milk feeding levels.²⁰ On the other hand, increased fecal scores in some studies may be due to a simple response to supply more nutrients. Conventionally fed calves had greater days with fever and days treated than those fed intensified milk (0.71 versus 0.38). It is well known that feeding calves below maintenance can influence the immune system and predispose calves to infectious diseases.¹² In this case, several studies suggested that inadequate metabolizable energy intake can impair immune function. Godden *et al.* have indicated that pasteurized whole milk-fed calves have lower days treated and mortality rather than milk replacer-fed calves.²¹ In this study, loss of body weight was observed in CF calves and further confirmed that feeding limited amounts of milk does not cover even the maintenance requirements, therefore, this situation can impair immune function. Other researchers have reported that lower milk consumption during the early weeks of age can reduce calves growth rate and predispose them to illness.²² Serum amyloid A, an acute-phase protein, as a biomarker of inflammation and stress is used to early

Table 4. Serum amyloid A (SAA), cortisol, alanine aminotransferase (ALT), and iron concentrations of Holstein female calves fed intensified (IF) milk or conventionally (CF) during the pre-weaning period.

Trait*	Treat	Age (day)			SEM	p-values		
		14	28	57		Treatment	Time	Treatment \times Time
SAA (mg L ⁻¹)	IF	6.11 ^b	8.29	9.40 ^a	0.37	0.29	< 0.01	< 0.01
	CF	6.98 ^a	8.47	7.59 ^b	-	-	-	-
Cortisol (nmol L ⁻¹)	IF	41.84 ^b	54.25 ^b	52.41	5.32	0.01	0.01	0.02
	CF	48.28 ^a	66.54 ^a	58.39	-	-	-	-
ALT (Ukat L ⁻¹)	IF	0.1	0.13	0.23	0.01	0.77	< 0.01	0.79
	CF	0.1	0.14	0.23	-	-	-	-
Iron (μ mol L ⁻¹)	IF	7.84 ^b	24.52	22.02	4.48	0.2	0.04	0.02
	CF	22.49 ^a	22.73	19.26	-	-	-	-

* Different patterns of nutrition were used in the pre-weaning period. In the intensified feeding, the calves ($n = 24$) were fed whole milk at the rate of 20.00% of body weight (BW) for the first three weeks of age. After that, milk allowance reduced gradually to 10.00% of BW and continued until day 50. Then, calves were weaned gradually between 51 and 56 days of age. In conventional feeding, the calves were fed at 10.00% of BW from the beginning of the experiment until day 50. Calves weaning procedure was similar to intensified feeding group.

^{ab} Superscript letters in columns indicate a significant difference between treatments.

Table 5. Feeding behavior of Holstein female calves around weaning.

Behavior (min)	Feeding pattern ¹		SEM	p-values		
	Intensified	Conventional		Treatment	Period ²	Time ³
<i>Lying without chewing</i>						
Two weeks before weaning	70.60	59.90	10.50	0.90	< 0.01	0.33
One week before weaning	51.80	69.90	10.50	0.60	< 0.01	0.33
Week after weaning	37.60	50.90	10.50	0.90	< 0.01	0.33
Two weeks after weaning	32.80	44.40	10.50	0.90	< 0.01	0.33
Average of 8 hr	48.20	56.30	5.50	0.10	-	-
<i>Starter eating</i>						
Two weeks before weaning	11.00	17.25	4.50	0.80	< 0.01	< 0.01
One week before weaning	27.00	20.25	4.50	0.80	< 0.01	< 0.01
One week after weaning	42.20	47.75	4.50	0.90	< 0.01	< 0.01
Two weeks after weaning	54.20	49.50	4.50	0.90	< 0.01	< 0.01
Average of 8 hr	33.10	33.60	2.30	0.90	-	-
<i>Ruminating</i>						
Two weeks before weaning	13.20	12.58	7.40	1.00	0.17	0.67
One week before weaning	19.00	19.00	7.40	1.00	0.17	0.67
One week after weaning	12.20	12.30	7.40	1.00	0.17	0.67
Two weeks after weaning	9.20	9.30	7.40	1.00	0.17	0.67
Average of 8 hr	13.40	13.30	3.80	0.90	-	-
<i>Non-nutritive behavior</i>						
Two weeks before weaning	8.40	10.20	3.80	0.90	0.09	0.84
One week before weaning	11.40	4.40	3.80	0.60	0.09	0.84
One week after weaning	7.00	2.90	3.80	0.90	0.09	0.84
Two weeks after weaning	8.00	3.20	3.80	0.90	0.09	0.84
Average of 8 hr	8.70	5.20	2.00	0.09	-	-
<i>Standing without chewing</i>						
Two weeks before weaning	17.20	13.33	4.70	0.90	0.62	0.48
One week before weaning	14.80	13.08	4.70	0.90	0.62	0.48
One week after weaning	19.08	9.08	4.70	0.03	0.62	0.48
Two weeks after weaning	15.80	9.08	4.70	1.10	0.62	0.48
Average of 8 hr	19.90	11.15	2.40	0.02	-	-

¹ Different feeding methods were used in the pre-weaning period. In the intensified feeding method, whole milk was fed to calves (n = 24) at the rate of 20.00% of body weight (BW) for the first three weeks of age. After that, milk allowance was reduced gradually to 10.00% of BW and continued until day 50. Then, calves were weaned gradually between day 51 and 56 of age. In conventional feeding, the calves were fed at 10.00% of BW from the beginning of the experiment until day 50. Calves weaning procedure was similar to intensified feeding group. ²Period: Pre- and post-weaning. ³Time: Weeks related to weaning.

diagnosis of diseases in animals.⁴ For example, SAA is particularly used to early diagnosis of mastitis in cattle.²³ Researchers have found a higher concentration of SAA in calves with diarrhea.²⁴ Increased level of SAA in CF calves on day 14 indicated that they are more susceptible to diseases in the critical first two weeks of life. In another study comparing two limited or IF systems, higher levels of haptoglobin, an acute-phase protein, were observed in limited fed calves.²⁵ On the other hand, the SAA level was higher in IF calves at weaning, indicating more weaning stress. Also, we observed serum cortisol levels to be higher in CF calves than IF ones further indicating more stress experienced by conventionally fed calves on day 14 and 28. Presumably, under-nutrition or nutritional stress is responsible for elevated cortisol levels in CF calves.^{10,25}

Higher blood concentration of some enzymes such as ALT can be a sign of gastrointestinal or liver inflammation being reported in calves with enteritis.²⁶ Similar levels of ATL between treatments in the current study indicate that feeding a high volume of milk to dairy calves does not

affect calves gastrointestinal health²⁷. Serum Fe level was lower in IF calves than CF ones on day 14 when the milk intake was at the highest level. Iron is transported by a protein called transferrin around the body to provide it for growing cells.²⁸ However, to avoid bacterial growth, serum Fe is stored in liver as ferritin during the acute phase of inflammation.²⁸ Reportedly, lipopolysaccharide challenge and following inflammation can alter serum Fe level which can be alleviated by supplementing omega-3 fatty acids.³⁰ In this study, decreased concentration of Fe in serum of IF calves on day 14 may not be related to any inflammatory reaction in these calves because of lower SAA and ALT concentrations and it can be due to lower Fe intake from milk during the time that milk consumption is in the highest level, while starter intake is in the lowest level. Results of our study were in line with others indicating lower blood iron concentration in neonatal calves.³¹ On the other hand, this iron deficiency may be more severe in IF calves than CF ones on day 14 because of consuming more milk which is naturally deficient in iron content.

Investigating the feeding behavior of calves in this study showed that feeding method during early life did not affect lying behavior (resting) of calves around weaning (two weeks before or after weaning). Average lying behavior during 8 hr observation was numerically higher for CF calves than IF ones (56.30 versus 48.20 min, respectively). In consistent with others,³² resting time during the weeks before weaning was longer than that of after weaning (from 122.40 to 70.40 min and 129.80 to 95.30 min for IF and CF calves, respectively). Calf age, bedding quality³³, and health status are the most important factors affecting resting behavior. This behavior is considered an important part of growing animal's welfare.^{34,35} Although a tendency to be significant was observed for lying behavior, it seems that intensified feeding during early life might reduce the welfare of calves around weaning. Starter eating time was not affected by the early feeding method and it was equal for both groups. Eating time increased with calves age and significantly was higher in the weeks after weaning (from 37 to 97 min and from 37.50 to 97.25 for IF and CF calves, respectively). Some studies have reported that providing a high volume of milk in the pre-weaning period can reduce calves solid feed intake.^{13,18} However, it seems that differences in solid feed consumption between high or low milk intake calves are eliminated after weaning.^{7,36} In agreement with this study, Khan *et al.* have shown that the step-down method can be useful to promote solid feed intake in intensified milk-fed calves.⁸ Others have reported larger meal size in restricted milk-fed calves compared to *ad libitum* fed calves during the week after weaning and have suggested that differences in meal characteristics are not persistent.³⁷ Similar eating time between treatments in this study provided further evidence that the early milk feeding method does not affect post-weaning feeding time. Rumination behavior is essential for stabilizing rumen fermentation and emptying.³⁸ No differences were detected between two feeding methods (intensified or conventional feeding) regarding ruminating time. Our results are in line with others found a high correlation between solid feed eating and ruminating time.³⁹ Non-nutritive oral behavior can be a sign of calf's poor welfare.⁴⁰ Tongue rolling, self-licking, or facilities licking were considered as non-nutritive oral behaviors. Studies investigating the welfare of pre-weaned calves have reported that restricted milk-fed calves exhibit more non-nutritive oral behavior⁴¹ and less lying time.⁴² Monitoring the feeding behavior of calves around weaning in this study showed that there was not a significant difference between treatments; however, a tendency was observed. Intensified milk-fed calves numerically spent more time expressing abnormal behaviors than CF group during 8 hr observation (8.70 versus 5.20 min on average for IF and CF calves, respectively). Hulbert and Moisés have reported that calves on a high plan of nutrition increase non-

nutritive behaviors around weaning which is in line with our results.⁴³ It has been shown that limited energy intake can cause abnormal behaviors such as ingesting foreign things in beef calves. Miller-Cuchon *et al.* have indicated that in the week after weaning, calves fed restricted amounts of milk in the pre-weaning period consume solid feed more quickly.³⁷ In the current study, conventionally fed calves consumed more DM from starter feed during pre-weaning period. Increased non-nutritive oral behavior in IF calves in this study may be attributed to lower energy intake during transition from milk to solid feed. Spending time for standing behavior in IF calves was also longer than CF ones. Increased standing behavior around weaning can be related to hunger and reduced welfare in IF calves.⁴⁴ Early solid feed intake is crucial for rapid rumen development and smooth transition from milk to solid feed intake after weaning.¹⁹ In the present study, impaired rumen development in IF calves due to lower starter intake in EPW and OPW periods may be effective in the expression of hunger behaviors such as standing. Hungry and exhibiting subsequent behaviors may be related to delayed rumen development and lower nutrients digestibility in IF calves.^{13,44} With regard to the results of this study and as mentioned by other authors,⁴⁵ it is suggested that the weaning time of intensified milk fed calves should be reconsidered.

Dairy calves not only ingest high amounts of milk safely but also they are less likely to become ill in the first week of age. However, concerning some feeding behaviors of calves and serum SAA levels, it seems that the intensified feeding method has the potential to reduce calves welfare during weaning transition.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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