

## COMPARATIVE PERFORMANCE OF ONCE- VERSUS TWICE-A-DAY MILK FEEDING IN PREWEANING SAHIWAL CALVES

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**ABSTRACT:** This study aimed to compare the effects of once- versus twice-daily milk feeding in preweaning Sahiwal calves. Sixteen calves from the Livestock Experiment Station Qadirabad, Sahiwal Punjab, Pakistan, received 2 L of colostrum at birth, followed by 3 L of whole milk daily in two equal feedings until day 14. From day 15, calves were randomly assigned to once-daily or twice-daily milk feeding groups, with the twice-daily group serving as the control. To determine Growth parameters (body weight, heart girth, and withers height) each calf was weighed and measured at 2 weeks of age and then at weaning. Daily health scores were recorded for each calf using a standard scoring system. For indirect monitoring of rumen papillae development, blood samples were collected by jugular venipuncture 4h post-feeding every 14 days from Day 28, to assess  $\beta$ -hydroxybutyrate (BHBA) concentrations. Starter intake was also monitored. The results indicated no significant differences between once- and twice-daily fed calves in terms of average daily gain (0.45 vs. 0.44 kg/day for males; 0.40 vs. 0.39 kg/day for females), starter intake (326.2 vs. 301.9 g/day), growth parameters, BHBA levels, or health scores. These findings indicate that once-daily milk feeding is a viable alternative to twice-daily feeding, offering potential labor savings without compromising calf growth, health, or starter intake.

**Key words:** Feeding frequency; Once versus twice; Calves; Labor cost.

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### INTRODUCTION

Traditionally, calves are fed milk twice daily, while only a small percentage of operations use once-milk feeding (NAHMS, 2014). Early studies showed convenience and the labor-saving benefits of once-daily milk feeding (Wilson *et al.* 1968; Galton and Brakel 1976), Labor is the second largest expense in heifer rearing, accounting for 13.8% of the total cost to raise a heifer in a milking operation (Gabler *et al.* 2000).

Multiple studies have reported no significant differences in growth, rumen development, blood parameters, health scores, behavior, or ruminal microbiota between calves fed once or twice daily (Wilson *et al.* 1968; Ackerman *et al.* 1969; Galton and Brakel, 1976; Stanley *et al.* 2002; Kehoe *et al.* 2007; Mushtaq *et al.* 2023; Zened *et al.* 2023). Moreover, once-daily feeding has been associated with increased calf starter intake due to long interval between milk feeding (Saldana *et al.* 2019), potentially enhancing rumen development and weaning transition.

Casein and milk fat curd formation in the abomasum supports slow nutrient absorption over 18–24 hours, making once-daily feeding feasible when using whole milk or milk replacers (Davis and Drackley, 1998). This practice is increasingly promoted in Europe and New Zealand to reduce labor (Ngahiwi, 2017).

However, data on this practice in Sahiwal calves is lacking. Therefore, this study aimed to evaluate the effects of once- versus twice-daily milk feeding on calf starter intake, growth, structural development, blood BHBA levels and health outcomes in preweaning Sahiwal calves.

### MATERIALS AND METHODS

**Experimental station:** This study was conducted at livestock experiment station (LES) Qadirabad district Sahiwal,

**Experimental design:** Sixteen newborn sahiwal calves (10 males and 6 females) with an average birth weight of  $23.10 \pm 3.41$  kg were enrolled in the study at LES Qadirabad. Within 3 hours of birth, all calves were administered 2.0 L of high-quality colostrum to ensure adequate passive immunity transfer. Plasma total protein levels were measured using an optical refractometer (Fisherbrand Analog Refractometer), confirming levels above 5.5 g/dL ( $6.0 \pm 0.29$  g/dL). The calves were housed individually in pens measuring 142 cm  $\times$  112 cm  $\times$  112 cm (length  $\times$  width  $\times$  height), equipped with wheat straw bedding and natural ventilation. From birth until 14 days of age, they were fed 3 L of milk daily in two equal feedings at 04:00 and 15:00 hours. At 15 days of age, calves were randomly assigned to one of two treatment

groups (n = 8 per group): 1) 2× Feeding: 3 L of milk administered twice daily (04:00 and 15:00 hours); 2) 1× Feeding: 3 L of milk administered once daily (15:00 hours). Between days 63 and 70, milk intake was reduced by half for all calves. Weaning occurred at 70 days of age. Fresh water and a mash starter ration (Table.1) were provided ad libitum throughout the study. The Crude protein (CP) of feed was analyzed using the Kjeldahl method (AOAC2003); neutral detergent fibre and acid detergent fibre were analysed using methods of Van Soest *et al.* (1991), metabolisable energy (ME) was calculated from the total digestible nutrient values of the feed ingredients by multiplying first by 0.04409 and then using the equation as described in NRC(2001). Daily feed intake was recorded by offering a weighed quantity of feed once daily and measuring feed refusals the next morning. Average daily gain (ADG) was calculated by weighing each calf after a 4-hour morning liquid feeding at 2 weeks and again at 70 days using an electronic scale (B-TEK Weight Technology, Karachi, Pakistan | Sang D60 Calibration & Interfacing). Structural development was assessed by measuring heart girth and withers height at 2 weeks and at weaning (70 days). Heart girth was measured using a measuring tape (Fiber tape GWF-1506), wither was determined using a long steel ruler. Calf health was monitored daily, recording occurrences of respiratory issues and scours on a scale of 1–4, as described by Kertz and Chester-Jones (2004). If a calf maintained a scour score of 3 or higher for more than 3 days, it was treated with antibiotics as recommended by the veterinarian. To indirectly monitor rumen papillae development, blood samples were collected via jugular venipuncture 4 hours post-feeding every 14 days starting from Day 28. Samples were analyzed for  $\beta$ -hydroxybutyrate (BHBA) concentrations using a portable meter (PrecisionXtra, Abbott Laboratories, Maidenhead, Berkshire, UK) with appropriate test strips for measuring ketone bodies.

**Table1. Composition of calf starter ration.**

<b>Ingredient</b>	<b>Composition%</b>
Corn grain dry, fine grinded	39.5
Wheat bran	7.0
Rice Polish	6.0
Rape seed meal	5.0
Canola meal	15
Corn gluten30%	15
Molasses(cane)	10
Sodium bicarbonate	1.0
VitTM PremixA	1.5
Crude protien(CP%)	19.1
Metaboliseable energy(ME,Mcal/kg)	3.2
Neutral detergent fibre(%)	19.1
Acid detergent Fibre(%)	12
Protein energy ratio(g of CP/Mcal of ME)	59.6

<sup>A</sup>Vitaminpremixcompositionperkg:vitaminA:28000000IU;vitaminD3: 5400000IU;vitaminE9000:9000IU;vitaminB1:1000mg;

calcium pentothenate: 12500mg; nicotinicacid: 17500mg; folicacid: 250mg; vitaminK3:2500mg;vitaminB2:7500mg

**Statistical Analysis:** Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). For a change in body growth measurements, initial and final body weight (BW), ADG, milk frequency (MF), calf sex, and interaction of MF and calf sex were tested as fixed effects, while the week of the study was included as a random effect. Intake and BHBA concentration data were analyzed using repeated measures analysis. The week of the study was included in the model to account for seasonal changes because calves were enrolled in the study on a rolling basis. For starter intake, health scores and BHBA parameters of calves measured over age, the effect of calf sex was not significant and was removed from the model. Milk frequency was included as fixed effects. The goodness of fit criteria based on second-order Akaike information criterion values were used to select covariance structures for repeated measures. Autoregressive (1) was used as covariance structures. Week of the study and calf were used as random factors for all variables repeated over age. The least squares means are presented in tables. Significant differences were declared at  $p \leq 0.05$ . For significant results, multiple comparisons were made using Tukey-adjusted p-values.

## RESULTS AND DISCUSSION

**Average daily gain and structural development:** The effects of milk feeding frequency (MF) and calf sex on body weight (BW), wither height (WH) and heart girth (HG) of preweaning Sahiwal calves are presented in Table 2. There were no significant interactions ( $p \geq 0.20$ ) between MF and sex for any of the growth parameters, indicating that feeding frequency and sex acted independently.

**Body Weight (BW):** Initial and final BW were not significantly influenced by MF ( $p = 0.79$  and  $p = 0.68$ , respectively). Similarly, no MF  $\times$  sex interaction was observed ( $p = 0.80$  and  $p = 0.75$ ), suggesting that once or twice daily milk feeding did not affect calf weight at the beginning or end of the preweaning period.

However, calf sex significantly affected BW change ( $p = 0.02$ ) and ADG ( $p = 0.02$ ). Male calves gained more weight (31.7 and 30.6 kg) than female calves (28.1 and 27.3 kg) in both feeding groups. The corresponding ADG was also higher for males (0.45 and 0.44 kg/day) compared to females (0.40 and 0.39 kg/day), reflecting approximately 27% greater weight gain in males. This is consistent with established knowledge that male calves tend to have higher growth rates due to physiological and hormonal differences. These findings are in agreement with previous studies, including Wood

*et al.* (1971) and Galton and Brakel (1976), who reported no significant differences in calf growth between once and twice-daily feeding regimens, and highlighted that sex plays a more critical role in weight gain.

**Wither Height (WH):** No significant differences were observed in initial or final WH due to MF ( $p = 0.25$  and  $p = 0.45$ , respectively) or sex ( $p = 0.67$  and  $p = 0.62$ , respectively). The total change in WH and corresponding ADG were also statistically similar across groups (MF  $p = 0.89$ ; sex  $p = 0.08$ ), indicating that skeletal growth was not notably affected by feeding frequency or calf sex.

These results corroborate findings from Kehoe *et al.* (2007), who also reported no differences in wither height when calves were fed milk once or twice daily. This suggests that structural skeletal development, as measured by WH, may be more genetically regulated and

less responsive to variations in milk feeding schedules during the preweaning period.

**Heart Girth (HG):** Initial and final HG did not differ significantly between feeding frequencies ( $p = 0.31$  and  $p = 0.37$ , respectively) or calf sex ( $p = 0.93$  and  $p = 0.42$ , respectively). The total change and ADG in HG were also not significantly affected by MF ( $p = 0.69$ ) or sex ( $p = 0.38$ ). This indicates that thoracic growth, which can be associated with internal organ development and general body conformation, remains stable regardless of milk feeding frequency and sex in the early growth phase. The lack of significant differences across treatment groups suggests that once-daily milk feeding is sufficient to support normal heart girth development in Sahiwal calves during the preweaning stage.

**Table.2 Effect of milk feeding frequency on avg daily gain and structural growth of preweaning sahiwal calves.**

Parameter	1X15 <sup>A</sup>		2XC <sup>A</sup>		SEM	p-Value <sup>B</sup>		
	Male	Female	Male	female		MF	S	MF*S
Body weight kg								
Initial	23.5	22.1	22.9	22.4	1.95	0.79	0.76	0.80
Final	55.2	50.2	53.5	49.7	2.01	0.68	0.07	0.75
Change	31.7	28.1	30.6	27.3	2.91	0.87	0.02	0.47
ADG/d	0.45	0.4	0.44	0.39	0.074	0.87	0.02	0.47
Wither height(cm)								
Initial	69.2	68.4	70.2	68.2	1.32	0.25	0.67	0.34
Final	81.6	80.6	81.4	79.2	1.57	0.45	0.62	0.56
Change	12.4	12.2	11.2	11.0	1.01	0.89	0.08	0.23
ADG/d	0.18	0.17	0.16	0.16	0.026	0.89	0.08	0.23
Heart girth (cm)								
Initial	70.2	69.8	72.2	68.9	1.65	0.31	0.93	1
Final	88.2	84.6	85.3	84.4	1.4	0.37	0.42	0.61
Change	16.0	14.8	13.1	15.5	1.13	0.69	0.38	0.52
ADG/d	0.23	0.21	0.19	0.22	0.029	0.69	0.38	0.52

SEM, standard error of the mean; ADG, average daily gain.

<sup>A</sup> 1X15, milk fed once a day initiated on 15<sup>th</sup> d of birth; <sup>A</sup>2XC, milk fed twice a day (Control group).

<sup>B</sup> p-value represents the main effects of dietary treatments: MF, milk frequency (once vs twice a day); S, sex of calves (male vs female); MF\*S, milk frequency by calf sex interaction

**Calf starter intake:** When daily calf starter DMI was analyzed, it was observed that After 4 wk of age, calves fed milk once a day had a numerically higher calf starter intake (326.2g/d) compared with calves fed twice a day (301.9 g/d); however, we found no difference between treatments (Figure 1;  $P = 0.57$ ). Feeding calves once a day may encourage more starter intake due to the long interval between milk feedings.

Several studies have shown that feeding milk either once or twice daily does not affect starter consumption (Willett *et al.* 1969; Stanley *et al.* 2002; Kehoe *et al.* 2007). Kehoe *et al.* (2007) reported higher values for calf starter intake compared with the present study; this difference could be attributed to breed difference in the present experiment. In addition, Willett

*et al.* (1969) reported greater starter consumption in calves fed milk once a day compared with twice a day, but the difference was not significant.

**Health:** The effects of milk feeding frequency on the health of preweaning Sahiwal calves are summarized in Table 2. Health parameters assessed included fecal and respiratory scores, which are commonly used indicators of digestive and respiratory health in young calves. There was no significant effect ( $p = 0.92$ ) of milk feeding frequency on fecal scores. Calves fed once daily (1X15) had a mean fecal score of 1.54, while those fed twice daily (2XC) had a slightly lower average score of 1.44. However, the difference was not statistically significant. These findings indicate that both feeding strategies

supported normal gastrointestinal function and that increasing milk feeding frequency did not impair digestive health in preweaning calves. This is consistent with findings from earlier studies where fecal consistency and diarrhea incidence were not influenced significantly by feeding frequency, provided that milk intake and hygiene were maintained at appropriate levels (Kehoe *et al.* 2007; Khan *et al.* 2007). This finding is also supported by previous studies that reported no differences in fecal scores or days with scouring between calves fed once daily and those fed twice daily (Owens and Stake, 1971; Leaver and Yarrow, 1972; Stiles *et al.* 1974). It also suggests that once-daily feeding does not increase the risk

of digestive upset under proper management conditions. Similarly, there was no significant difference ( $p = 0.96$ ) in respiratory scores between the two groups. The average respiratory score was 1.02 for calves fed once daily and 1.01 for those fed twice daily, indicating negligible respiratory issues in both groups.

These results suggest that milk feeding frequency does not impact the health of preweaning Sahiwal calves. Maintaining environmental hygiene, adequate ventilation, and proper nutrition likely played a more critical role in preventing respiratory and digestive infections than feeding frequency itself.

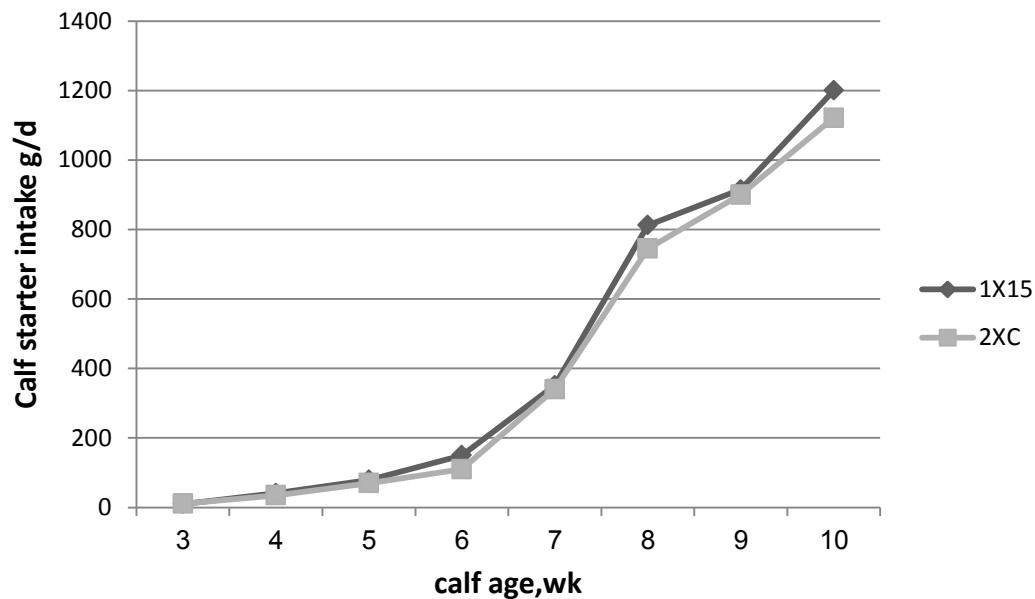


Figure 1. Calf starter intake for calves fed milk once or twice daily (SEM = 49,  $P = 0.57$ ).

Table 3. Effect of milk feeding frequency on health of pre weaning sahiwal calves.

Variables	Milk feeding frequency		SEM	P-Value
	1X15	2XC		
Fecal score	1.54	1.44	0.08	0.92
Respiratory score	1.02	1.01	0.01	0.96

Lower numbers indicate normal calf health.

**Blood  $\beta$ - Hydroxybutyrate (BHBA):** Blood BHBA concentration is used as an indirect indicator of rumen development. The once a fed calves recorded numerically a higher blood ketone concentration than twice fed calves, and this was associated with a higher intake of starter ration, due to long interval of milk feeding in once fed calves but the effect was not significant (Fig. 2). The blood BHBA concentration increased with advancing age; it increased earlier in early weaned calves than in

late-weaned calves. Again, this was most likely due to the acceleration in solid-feed intake at the earlier weaning age. Khan *et al.* (2007) reported an increased plasma BHBA concentration with increasing age in Holstein calves. Quigley *et al.* (1991) reported an increased blood BHBA concentration with an increased grain intake and this was greater in early weaned (Day 28) Holstein calves than in late-weaned (Day 56) calves assessed in a 14-week trial.

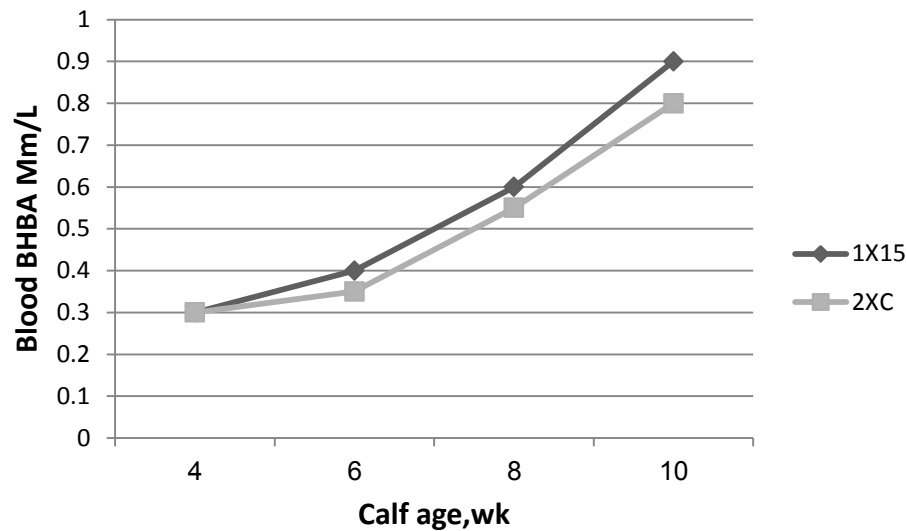


Figure 2. Blood ketones level for calves fed milk once or twice daily (SEM = 0.01, P = 0.68).

**Conclusions:** The results of this study demonstrate that feeding milk once daily to preweaning Sahiwal calves yields comparable outcomes to twice-daily feeding in terms of body weight, average daily gain, calf starter intake, structural growth, health scores and blood BHBA levels. These findings suggest that both feeding strategies can be effectively implemented, either independently or in combination, depending on the specific needs and capabilities of the farm. Therefore, from 15 days of age onward, once-daily milk feeding can be considered an effective and labor-saving option for Sahiwal calves.

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