Effect of weaning age and milk feeding volume on growth performance of Nili-Ravi buffalo calves

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ABSTRACT

The objective of this study was to evaluate the growth performance of early or late weaned Nili-Ravi buffalo calves offered feeding regimes of low or high milk volumes. For this purpose, 48-day-old buffalo calves were randomly allocated to four treatments of twelve calves each. Calves were weaned either at day 56 (early weaned) or 84 (late weaned). Within each weaning age calves were offered milk at either 10% (low milk) or 15% (high milk) of their body weight. Early weaned calves were offered milk adjusted weekly until day 35; the calculated milk intake at day 35 was reduced by one-third in each of the last two weeks leading to the weaning of the calves at day 56. Late weaned calves were offered milk, adjusted weekly, until day 63 and weaned at day 84 by decreasing milk by one-third each week for the last two weeks. At week sixteen, late weaned calves had higher body weight and average daily gain than early weaned calves (p < .05). These same measurements were also higher in the high milk than the low milk fed calves (p < .05). Highest body weight and average daily gain was recorded in high milk late weaned calves and poorest in low milk early weaned calves. Early weaning or low milk volumes minimised the feeding costs, however, resulted in lower body weights and average daily gains. Thus, choices of pre-weaning feeding regimen should be made with feeding cost and performance of replacement calves kept in mind.

Introduction

Calves are the future of our dairy herds. Males are future breeding bulls and females are used as herd replacements. Underfeeding results in high mortality and increased age at puberty in buffalo heifers. High-mortality rates in young calves and the disposal of male buffalo calves results in a loss of quality germplasm. Balanced feeding, improved management and minimising the incidence of disease can be helpful in reducing the age at first calving (Heinrichs et al. 2005). The pre-weaning feeding cost of buffalo calves under conventional systems in Pakistan most often exceeds the market price available for weaned calves (Bhatti et al. 2009). Therefore, commercial dairy farmers prefer to sell the milk of their freshly calved buffaloes rather than feeding it to newly born calves. New born male buffalo calves are generally retained only for milk letdown from their dams and not as future bulls. Thus, if let-down of dam’s milk is not an issue, disposing of buffalo calves, especially males at an early age, is common practice for farmers in Pakistan. Thus, feeding strategies are required to reduce the cost of rearing buffalo calves to weaning. Early weaning of Sahiwal calves and offering them solid feed or using milk replacer reduced pre-weaning feeding costs, however, this practice resulted in poor weight gains at weaning (Bhatti et al. 2012a). However, Rashid et al. (2013) reported that buffalo calves can be weaned successfully at 8 weeks without compromising their growth performance.

The objective of the study was to identify an economical weaning strategy for buffalo calves by offering them low (10% of body weight) or high milk volumes (15% of body weight) and weaning them early (at 8th week) or late (at 12th week).

Materials and methods

Male and female buffalo calves (n = 48) born at the Livestock Experiment Station, Bhuneki, Pattoki during
the months of September-November 2013, were separated from their mothers and given fresh colostrum at 10% of their body weight within four hours of their birth up to day three. Calves were randomly allocated to four treatments with twelve calves in each. Calves were weaned either at day 56 (early weaned) or 84 (late weaned). Within each weaning age calves were offered milk either at 10% (low milk) or 15% (high milk) of BW. Early weaned calves were offered milk adjusted weekly until day 35; the calculated milk intake at day 35 was then reduced by one-third each week in the last two weeks prior to weaning. Thus, there were four treatments in a 2×2 factorial design; factor I was level of milk feeding (low or high milk) and factor II was weaning age (early or late weaned). The trial terminated when the calves reached the age of sixteen weeks.

Feeding of calves

Calves were fed milk through nipple feeders fitted in buckets. The quantity of daily milk offered to calves was calculated initially on the basis of their birth weights and then on live body weight measured weekly with a digital scale before morning feeding. Total quantity of daily milk offered to calves was divided into two equal volumes and offered twice daily at 0600 and 1800 h. The milk was warmed to a temperature of 41°C so that the final temperature of milk at the time feeding remained ≥40°C. Calves were offered a concentrate ration containing 19.7% CP and 3.1 Mcal/kg of ME (Table 1) from day 14. All the animals were kept in separate pens. They were provided with free access to clean water at all times. Intake of milk, water and starter ration was measured on a daily basis. Calves were weighed weekly on a digital scale before the morning feeding to monitor their growth and for calculation of quantity of milk to be fed during the subsequent week. The calf scour score was measured on a scale of 1-4 as described by Kertz and Chester-Jones (2004). Record of all the other ailments and medication was maintained.

For monitoring rumen development of calves, blood concentration of β-hydroxy butyric acid (BHBA), as an indicator of functional rumen wall and rumen papillae development, was measured fortnightly starting from the beginning of the fifth week. For this purpose, blood samples were taken from the jugular vein 2-hours post-feeding in the morning. The BHBA was determined immediately using a portable metre (Precision Xtra, Abbott Laboratories Limited, Maidenhead, Berkshire, UK) supplied with appropriate strips for measuring ketone bodies. Blood glucose concentration was measured before and after morning feeding fortnightly starting from the fifth week by using the same metre as that for BHBA but with different strips appropriate for measuring glucose.

Chemical analysis of milk was conducted for dry matter, crude protein, butterfat and lactose with an ultrasonic milk analyser, Master LM2, Milko-tester Ltd, Bulgaria. The starter ration was analysed for dry matter, crude protein, ether extract, nitrogen free extracts and crude fibre as described by AOAC (1990). The NDF and ADF were determined using the procedures of Van Soest et al. (1991).

Statistical analysis

The recorded data on milk and starter consumption, weekly weight was analysed using repeated measures analysis with the MIXED Procedures of SAS (SAS Institute Inc. 2011) with an AR (1) covariance structure as described by Littell et al. (1998). The effect of calf

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Table 1. Composition of calf starter ration.

| Ingredients, %, as fed basis |  |
|-----------------------------|--|
| Maize grains | 20 |
| Canola meal | 25 |
| Soybean meal | 11 |
| Maize oil cake | 20 |
| Wheat bran | 10 |
| Molasses | 10 |
| DI calcium phosphate | 1 |
| CaCO₃ | 0.9 |
| Salt | 1 |
| NaHCO₃ | 1 |
| Vitamin premix* | 0.1 |
| Total | 100 |

| Nutrient analysis |  |
|-------------------|--|
| Dry matter, % | 89 |
| Crude protein, %, on dry matter basis | 19.7 |
| Ether extract, %, on dry matter basis | 2.5 |
| Crude fibre, %, on dry matter basis | 7.3 |
| Neutral detergent fibre, %, on dry matter basis | 12.75 |
| Ash, %, on dry matter basis | 8.7 |
| Metabolizable energy, Mcal/kg** | 3.1 |
| Protein energy ratio, g/Mcal | 63.5 |
| Ca:P ratio | 2:1 |

*Composition of vitamin premix/each Kg of vitamin premix

| Vitamin | Quantity |
|---------|----------|
| Vitamin A | 27,000,000 international unit |
| Vitamin D₃ | 5,400,000 international unit |
| Vitamin E | 9000 international unit |
| Vitamin B₂ | 1000 mg |
| Calcium Pentothenate | 12,500 mg |
| Nicotinic acid | 17,500 mg |
| Folic acid | 250 mg |
| Vitamin K₃ | 2500 mg |
| Vitamin B₁₂ | 7500 mg |

**Metabolizable energy was calculated by formulae described by NRC (2001).
was considered as random. The following statistical model was used for analysis

\[ Y_{ijkl mn} = \mu + F_{1i} + F_{2j} + W_k + (F_1xF_2)_{ij} + (F_1xW)_{ik} + (F_2xW)_{jk} + \text{Sex}_i + \text{Calf}_m + e_{ijkl mn} \]

where

- \( Y_{ijkl mn} \) is the dependent variable,
- \( \mu \) is overall mean,
- \( F_{1i} \) is the fixed effect of factor 1 where \( i = \) level of milk feeding either 10 or 15% of the body weight;
- \( F_{2j} \) is the fixed effect of factor 2 where \( j = \) weaning age either day 56 or 84;
- \( W_k \) is the repeated measure of week \( k \);
- \( \text{Sex}_i \) is sex effect of calf
- \( \text{Calf}_m \) is random effect of calf \( m \) and
- \( e_{ijkl mn} \) is residual error.

For the non-repeated data (average growth rate, weaning weight, total weight gain, body measurements, scour score, total milk intake and starter ration, feeding cost), GLM procedures were used.

The statistical model was as follows:

\[ Y_{ijkl} = \mu + F_{1i} + F_{2j} + (F_1xF_2)_{ij} + \text{Sex}_k + e_{ijkl} \]

where

- \( Y_{ijkl} \) is the dependent variable,
- \( \mu \) is the overall mean;
- \( F_{1i} \) is the fixed effect of factor 1 where \( i = \) level of milk feeding either 10 or 15% of the body weight;
- \( F_{2j} \) is the fixed effect of factor 2 where \( j = \) weaning age either day 56 or 84;
- \( \text{Sex}_k \) is sex of the calf;
- \( e_{ijkl} \) is residual error.

The data are presented as least square means. Out of 48 calves, three calves died each from each of the low milk early weaned and high milk early weaned groups, two calves died from the high milk late weaned group, two calves did not adapt to nipple feeding (one each from low milk late weaned and high milk late weaned) and one calf could not complete 16 weeks from the low milk late weaned group, leaving 9 calves in each treatment. Of nine calves, five were male and four were female in each treatment, except in the low milk early weaned treatment which consisted of four males and five females. Thus, the data from 36 calves is presented in the results section of this paper.

**Results**

**Body weight, total weight gain and average daily growth rate**

**0–8 Weeks**

High milk fed calves had a 3.9 kg higher (\( p < .05 \)) body weight and 3.8 kg higher (\( p < .05 \)) weight gain than low milk calves at 8 weeks (Table 2). Late weaned calves had a 4.8 kg higher (\( p < .05 \)) body weight and 4.1 kg higher (\( p < .05 \)) weight gain than early weaned calves at 8 week. High milk late weaned calves had the highest and low milk early weaned calves had the lowest body weight, total weight gain and average daily growth rate (Table 2). High milk early weaned calves and low milk late weaned calves did not differ (\( p > .05 \)) in total weight gain and average daily growth rate at 8 weeks. Males buffalo calves had higher (\( p < .05 \)) body weight at 8 weeks than females

| Table 2. Body weight and daily gain of buffalo calves weaned early or late and offered low or high milk volumes. |
|---------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Parameters                      | Weaning age | Milk volume |          | Weaning age | Late weaning |          |          | F1 | F2 | F1xF2 | Sex |
|---------------------------------|------------|-------------|--------|------------|-------------|--------|--------|----|----|-------|-----|
| Birth weight, kg                | Early      | Late        | Low    | High       | SE          |        |        |    |    |       |     |
| 33.9                            | 34.6       | 34.2        | 34.3   | 1.4        |             |        |        | 2.0 | ns | ns    | ns  |
| Body weight, kg                 |            |             |        |            |             |        |        |     |    |       |     |
| Week 8                          | 57.9       | 62.7        | 58.4   | 62.3       | 1.3         | 57.5   | 58.4   | 59.3 | 66.2 | 1.8   | *   |
| Week 12                         | 64.3       | 83.5        | 70.2   | 77.6       | 1.5         | 62.7   | 65.9   | 77.7 | 89.3 | 2.1   | *** |
| Week 16                         | 72.9       | 92.3        | 79.2   | 86.1       | 2.0         | 71.0   | 74.9   | 87.2 | 97.3 | 2.8   | *** |
| Weight gain, kg                 |            |             |        |            |             |        |        | 2.0 | ns | ns    | ns  |
| 0–8 weeks                       | 24.0       | 28.1        | 24.2   | 28.0       | 1.3         | 22.4   | 25.7   | 26.0 | 30.2 | 1.8   | *   |
| 8–12 weeks                      | 6.4        | 20.8        | 11.8   | 15.3       | 0.7         | 5.2    | 7.5    | 18.4 | 23.1 | 0.9   | *** |
| 12–16 weeks                     | 8.6        | 8.8         | 8.9    | 8.5        | 1.0         | 8.3    | 8.9    | 9.5  | 8.0  | 1.5   | ns   |
| ADG, g/d                       | 39.0       | 57.7        | 45.0   | 51.8       | 2.0         | 35.9   | 42.2   | 53.9 | 61.3 | 2.9   | *** |

\( F1: \) Weaning age; \( F2: \) Level of milk feeding; \( F1 \times F2: \) Interaction of \( F1 \) and \( F2; \) \( * : (p < .001); \) \( ** : (p < .01); \) \( * : (p < .05); \) ns: (\( p > .05 \)).

Early weaned: calves weaned at 8th week; Late weaned: calves weaned at 12th week; Low milk: milk offered at 10% of body weight; High milk: milk offered at 15% of body weight.
(62.9 vs. 57.4 kg), however, the total weight gain and daily growth rate were not influenced \((p > .05)\) by sex of calves.

8–12 Weeks

High milk calves had 7.4 kg higher \((p < .01)\) body weight than low milk fed calves at 12 weeks; their weight gain and average daily growth rate during 8–12 weeks was 3.5 kg and 123 g/day higher than the low milk calves, respectively (Table 2). Late weaned calves had 19.2 kg higher \((p < .001)\) body weight than early weaned calves at the age of 12 weeks; their weight gain and average daily growth rate during the period of 8–12 weeks was 14.4 kg and 514 g/day higher than the early weaned calves, respectively (Table 2). High milk late weaned calves had the highest and low milk early weaned calves had the lowest body weight, total weight gain and average daily growth rate at 12 weeks. Male buffalo calves had higher \((p < .05)\) body weight at 12 weeks than females; however, total weight gain and daily growth rate during the period of 8–12 weeks was not affected \((p > .05)\) by the sex of calves.

12–16 Weeks

At 16 weeks of age, high milk fed calves had 6.9 kg higher \((p < .05)\) body weight than low milk fed calves and late weaned calves had a 19.4 kg higher body weight than early weaned calves; their weight gain and average daily growth rate during weeks 12–16 was, however, not affected \((p > .05)\) by treatments (Table 2). High milk late weaned calves had the highest and low milk early weaned calves had the lowest body weight.

Total weight gain and cumulative daily growth during the whole trial period (0–16 week) was higher \((p < .05)\) in late weaned than in early weaned calves but not affected \((p > .05)\) due to milk volumes. Male calves had higher \((p < .05)\) daily growth rate, total weight gain and final body weights than female calves during the whole trial period.

Growth curves of calves from birth to week 7 did not differ between treatments; however, after week 8, the late weaned calves had higher body weights than early weaned calves (Figure 1). Overall growth performance was better in high milk fed calves than low milk fed calves and was also better in late weaned calves than early weaned calves.

Milk intake

Buffalo milk contained 7.01% fat, 4.15% protein, 5.56% lactose and 16.72% total solids. High milk fed calves consumed 62.81 more \((p > .001)\) milk than low milk fed calves and late weaned calves consumed 180.61 more \((p > .001)\) milk than early weaned calves during the pre-weaning period (Table 3). High milk late weaned calves had the highest and low milk early weaned calves had the lowest total milk intake during the trial period. Daily milk intake (kg/day), and milk dry matter as a percentage of body weight of calves, on different treatments, validated these results (Figures 2 and 3). Maximum intake of milk dry matter as a percentage of body weight was 2% in high milk fed late weaned calves during weeks 7–8 and 1.7% in high milk early weaned calves in the 6th week (Figure 3). Sex of the calves did not affect \((p > .05)\) milk intake of buffalo calves.

Total starter dry matter intake

Calves were offered starter ration in the third week of age but their daily starter intake was negligible (less than 70 g/day) up to 8 weeks (Figure 4). Early weaned calves started eating solid feed earlier than late weaned calves. Total starter DM intake was higher \((p < .05)\) in early weaned than late weaned calves and was also higher in low milk than high milk calves during first 8 weeks (Table 3). During weeks 8–12, early weaned calves had a higher \((p < .001)\) starter intake than late weaned calves, however, this not affected \((p > .05)\) by milk volumes. The starter intake of calves was not affected by any treatment during weeks 12–16. There was no interaction between milk feeding volumes and weaning age for total starter intake from weeks 0–16. Total starter intake was highest in high milk early weaned calves and lowest in high milk late weaned calves. Sex of the calves did not affect \((p > .05)\) starter intake of buffalo calves.
Dry matter intake to weight gain ratio

Dry matter intake to weight gain ratio (DMWR) was not affected \((p > .05)\) by treatments during weeks 0–8 and 12–16, however, it was poorer in early weaned calves than late weaned calves during weeks 8–12. The DMDR in all treatments was better during the pre-weaning period but increased post-weaning (Table 3).

### Table 3. Intake of milk and starter ration and dry matter to weight gain ratio of buffalo calves weaned early or late and offered low or high milk volumes.

| Parameters                  | Main effects          | Simple effects          | P-Values    |
|-----------------------------|-----------------------|-------------------------|-------------|
|                             | Weaning age           | Milk volume             | Early weaning | Late weaning | P-Values | F1 | F2 | F1 × F2 | Sex |
|                             | Early | Late | Low | High | SE | Low | High | SE | F1 | F2 | F1 × F2 | Sex |
| Milk Intake (Fresh), L      |                   |                       |             |           |     |       |      |     |     |      |      |
| 0–8 weeks                   | 187.9 | 230.4 | 190.1 | 228.2 | 5.3 | 175.6 | 200.2 | 204.6 | 256.2 | 7.4 | *** | *** | ns | ns |
| 8–12 weeks                  | 0 | 138.0 | 56.7 | 81.4 | 2.6 | 0 | 0 | 113.4 | 162.8 | 3.7 | *** | *** | *** | ns | ns |
| Total                       | 187.9 | 368.5 | 246.8 | 309.6 | 5.6 | 175.6 | 200.2 | 318.0 | 419.0 | 8.0 | *** | *** | *** | ns | ns |
| Milk intake (DM), kg        |                   |                       |             |           |     |       |      |     |     |      |      |
| 0–8 weeks                   | 31.4 | 38.5 | 31.8 | 38.2 | 0.9 | 29.4 | 33.5 | 34.2 | 42.8 | 1.2 | *** | *** | ns | ns |
| 8–12 weeks                  | 0 | 23.1 | 9.5 | 13.6 | 0.4 | 0 | 0 | 19.0 | 27.2 | 0.6 | *** | *** | *** | ns | ns |
| Total                       | 31.4 | 61.6 | 41.3 | 51.8 | 0.9 | 29.4 | 33.5 | 53.2 | 70.0 | 1.3 | *** | *** | *** | ns | ns |
| Starter intake (DM), kg     |                   |                       |             |           |     |       |      |     |     |      |      |
| 0–8 weeks                   | 1.3 | 0.6 | 1.3 | 0.6 | 0.2 | 1.8 | 0.8 | 0.8 | 0.4 | 0.3 | * | * | ns | ns |
| 8–12 weeks                  | 14.0 | 4.3 | 9.5 | 8.8 | 1.0 | 13.2 | 14.6 | 5.6 | 2.9 | 1.5 | *** | ns | ns | ns |
| 12–16 weeks                 | 32.0 | 29.8 | 30.3 | 31.6 | 2.2 | 30.7 | 33.4 | 29.9 | 29.7 | 3.1 | ns | ns | ns | ns | ns |
| 0–16 weeks                  | 47.3 | 34.7 | 41.1 | 41.0 | 3.1 | 45.7 | 48.8 | 36.3 | 33.0 | 4.4 | *** | ns | ns | ns | ns | ns |
| DM to weight gain ratio     |                   |                       |             |           |     |       |      |     |     |      |      |
| 0–8 weeks                   | 1.4 | 1.4 | 1.4 | 1.4 | 0.0 | 1.4 | 1.3 | 1.3 | 1.4 | 0.0 | ns | ns | ns | ns | ns | ns |
| 8–12 weeks                  | 2.6 | 1.4 | 1.4 | 1.9 | 2.2 | 2.5 | 2.8 | 1.3 | 1.5 | 0.5 | * | ns | ns | * | ns | ns |
| 12–16 weeks                 | 4.6 | 4.5 | 3.8 | 5.4 | 0.8 | 4.3 | 5.2 | 3.3 | 5.6 | 1.1 | 1.1 | ns | ns | ns | ns | ns |
| Total                       | 47.3 | 34.7 | 41.1 | 41.0 | 3.1 | 45.7 | 48.8 | 36.3 | 33.0 | 4.4 | *** | ns | ns | ns | ns | ns |

**Figure 2.** Daily milk intake of buffalo calves weaned early or late and offered low or high milk volumes.

**Figure 3.** Daily milk dry matter intake as percent of body weight of buffalo calves weaned early or late and offered low or high milk volumes.

**Figure 4.** Daily starter dry matter intake as percent of body weight of buffalo calves weaned early or late and offered low or high milk volumes.

Ketone bodies

Blood ketone levels were recorded from the 5th week of age to the 13th week of age fortnightly. Early weaned calves had higher blood BHBA in blood than late weaned calves during the 9–13th weeks. However, blood BHBA was not affected by milk feeding volumes (Figure 5).
Blood glucose level

Blood glucose levels of calves were lower during fasting than post-feeding until 7 weeks of their age. Thereafter, the gap between pre- and post-feeding blood glucose narrowed; more quickly in early than late weaned calves (Figure 6).

Calf scour

Weaning age did not affect (p > 0.05) the number of scour days and average scour score of calves.

Figure 5. Ketone bodies of buffalo calves weaned early or late and offered low or high milk volumes.

Figure 6. Fasting (—–) and Post-feeding (——) blood glucose of Buffalo calves under four different dietary treatment.

Table 4. Number of scour days and average scour score of buffalo calves weaned early or late and offered low or high milk volumes.

| Parameters | Main effects | Simple effects |
|------------|--------------|----------------|
|            | Early age    | Late age       | Milk volume | Early weaned | Late weaned | p-Values |
|            | Low | High | SE | Low milk | High milk | SE | F1 | F2 | F1xF2 |
| Number of scour days | 12 | 10 | 7 | 15 | 1.4 | 8 | 15 | 6 | 14 | 2.1 | ns | ** | ns |
| Average scour score | 1.4 | 1.3 | 1.3 | 1.4 | 0.04 | 1.4 | 1.4 | 1.2 | 1.4 | 0.06 | ns | ns | ns |

Early weaned: calves weaned at 8th week; Late weaned: calves weaned at 12th week; Low milk: milk offered at 10% of body weight; High milk: milk offered at 15% of body weight; F1: Weaning age; F2: Level of milk feeding; F1 x F2: Interaction of F1 and F2.

**p < .01; ns: (p > .05).**

Feeding cost

Early weaned calves cost less for milk consumed, total feeding cost and cost to produce each kg live weight gain but a higher (p < .001) expenditure on starter than late weaned calves (Table 5). On the other hand, high milk fed calves cost more to feed milk; total feeding cost and cost to produce each kg live weight gain than low milk calves. High milk late weaned calves had the highest feeding cost and high milk early weaned calves had the lowest feeding cost for body weight gain.

Discussion

Milk intake

This research aimed to address buffalo calf nutrition. It compared different feeding regimes including weaning stage and amount of milk fed. An initial challenge faced was separating buffalo calves from their dams and weaning them onto artificial feeding through nipples. Feeding buffalo calves through nipples was more difficult than Sahiwal cow calves raised previously (Bhatti et al. 2012a, 2013) in separate experiments. During the current trial, two of the buffalo calves did not accept nipple feeding until the end of the experiment and thus were removed from the study. Because of the reluctance of buffalo calves to drink milk through nipples, their milk intake was generally lower than their actual daily allowance. In the calves offered milk at the rate of 15% of body weight, maximum daily milk intake was never higher than 12% of body weight and in calves offered milk 10% of body weight maximum daily intake during the first two weeks was less than 8%. This low milk intake in buffalo calves resulted in lower growth rate and lower body weights.
than expected on such milk volumes. Mortality was also higher in buffalo calves than in Sahiwal calves raised in our previous studies (Bhatti et al. 2012a; Bhatti et al. 2013). Overall mortality was 20%. Most of the calves died as a result of undiagnosed disease. The symptoms of the disease were red spots on tongue; calves gradually became weak and were unable to stand, their intake reduced and they ultimately died. Three calves died due to hypothermia, dehydration and tympany.

Milk intake was higher in high milk than low milk fed calves and was also higher in late weaned than early weaned calves. Late weaned calves had a longer period of milk feeding than early weaned calves and as a result drank more milk. Rashid et al. (2013) reported that buffalo calves weaned after the 12th week consumed 22.6% more milk than calves weaned in the 8th week. Cheema et al. (2016) reported that Sahiwal calves fed milk at 15% of body weight had higher milk intake than calves fed milk at the rate of 10% of body weight. Bhatti et al. (2013) reported that milk feeding ad libitum in Sahiwal calves resulted in higher intake of milk than low milk volumes.

**Starter intake**

During weeks 0–8 all calves had negligible starter intake with no significant difference among treatments. Some of the calves did not start eating the starter ration until they were completely weaned. The delayed starter intake by calves resulted in their poor growth rate post-weaning. The lower starter intake by buffalo calves could be due to their dislike of feed ingredients used in the starter ration. Rashid et al. (2013) reported a high intake of starter ration (400–1000 g/day) in buffalo calves during the first eight weeks on continuous or limited feeding. In their study, the ingredients used in their starter ration were different than used in the present study and calves were not offered hay or fodder in addition. Studies on rumen development of cow calves indicate that concentrate feeding helps in earlier papillae development in the rumen than seen with hay; however, the physical form and chemical composition of starter diet are also important (Khan et al. 2015). After the 8th week, starter intake of early weaned calves increased abruptly. Rashid et al. (2013) reported that buffalo calves weaned at the 8th week started eating the starter ration more quickly than calves weaned at the 12th week. Khan et al. (2011) reported that starter plus hay intake was rapidly increased in Holstein calves when milk supply was decreased. de Passillé et al. (2011) reported that, due to milk feeding, the starter intake was low in Holstein calves before weaning. The opinion that buffalo calves eat hay before consuming starter ration needs further investigation.

**Body weight, total weight gain and average daily growth rate**

Body weights at 8, 12 or 16 weeks were higher in high milk than low milk fed calves and were also higher in late weaned than early weaned calves. This was due to a greater proportion of nutrient intake coming from milk than the starter ration with calves fed more milk and weaned later and also because of the better DM conversion ratio achieved with milk than starter ration (Table 3). Cutrignelli et al. (2003) reported that buffalo calves weaned at day 50 had higher body weights than those weaned at day 35; however, the difference was statistically non-significant; which they attributed to poor quality hay consumed by the late weaned calves. Rashid et al. (2013) reported no improvement in final body weight of Nili-Ravi male buffalo calves offered 22.6% more milk than calves fed limited milk; the similarity of body weight fed limited milk, in the study, was attributed to their higher starter intake. Bhatti et al. (2009) reported that buffalo calves fed milk at the rate of 10% of body weight through a nipple attained a body weight of 77.2kg by the 12th week. Jogi and Lakhani (1996) reported that Murrah buffalo calves raised by farmers through direct
suckling reached 70 kg body weight at the age of 3 months. However, in the present study late weaned buffalo calves attained a bodyweight of 83.5 by week 12. Jasper and Weary (2002) reported that Holstein calves fed milk ad libitum were 9.4 kg heavier than conventionally fed calves at day 42. Khan et al. (2007) reported that Holstein calves fed more milk using a step-down method were 25.9% heavier than conventionally fed calves. Similarly, de Passillé et al. (2011) reported that low milk (6 L/day) fed calves were lighter than high milk fed (12 L/day) calves.

In the present study, the average daily gain of late weaned calves was higher than in the early weaned calves. However, Rashid et al. (2013) reported that buffalo calves weaned at the 8th, 10th and 12th week displayed similar growth rates (387–401 g/day) up to the age of 12 weeks. Abdullah et al. (2013) reported that the average daily gain of buffalo calves fed whole milk at 10% of body weight up to day 56 and weaned at the age of 12 weeks. It was also reported that the calves were offered milk than when offered solid feed. This indicated a better biological efficiency of nutrient utilisation from milk than from starter ration. Khan et al. (2007) reported better feed conversion efficiency in pre-weaning period than post-weaning in female Holstein calves. Lee et al. (2009) reported that calves fed whole milk were heavier than those offered milk replacer when fed similar amounts of dry matter from milk or milk replacer; better performance of calves on whole milk was attributed to better bioavailability of nutrients and unknown growth factors present in whole milk. Rashid et al. (2013) reported that buffalo calves weaned in the 8th week achieved better feed efficiency than calves weaned in the 12th week of age.

**Blood glucose and β-hydroxy butyric acid (BHBA)**

Young calves are like mono-gastric animals: they use glucose as the primary energy source. With the passage of time due to their transition from mono-gastric to ruminant digestion, short chain fatty acids (VFA’s) become the primary energy source. Thus, before rumen development, fasting glucose is lower than post-fed glucose and after rumen development it is the reverse. Higher fasting glucose, after rumen development, is due to gluconeogenesis and post-fed glucose is lower due to the formation of VFA’s as a result of fermentation. In the present study, early and late weaned calves exhibited higher fasting blood glucose than post-fed values after the 9th and 11th week, respectively. Benschop and Cant (2009) reported that glucose remained the primary energy source in Holstein calves up to the age of week 8. Cheema et al. (2016) reported that in Sahiwal calves fasting blood glucose became higher than post-fed level in the 10th and 11th weeks, while calves were weaned in the 8th and 12th weeks, respectively. With the advancement in age, the blood glucose values decreased from more than 100 mg/dL in the 5th week to less than 70 mg/dL in the 13th week. Rashid et al. (2013) reported decreasing blood glucose levels in buffalo calves from 60–85 mg/dL in the 6th week to less than 45 mg/dL in the 12th week. Cheema et al. (2016) also reported a decreasing blood glucose level in Sahiwal calves with age from more than 100 mg/dL in the 3rd week to less than 100 mg/dL after the 11th week.

Blood concentrations of β-hydroxy butyric acid (BHBA) is an indicator of metabolic function of the rumen wall (Khan et al. 2012). Production of VFA’s or efficient conversion of butyric acid to BHBA, separately or in combination are the main reason for higher concentrations of BHBA in blood (Khan et al. 2012). In the present study, early weaned calves displayed higher
blood BHBA than late weaned calves. This might be due to early rumen development in early weaned calves. Similar results are reported by Cheema et al. (2016) in Sahiwal calves weaned in the 8th or 12th weeks. Anderson et al. (1986) reported that early weaned calves had higher concentrations of VFA’s than late weaned calves. Coverdale et al. (2004) reported that concentrations of VFAs increased in the 1st week post-weaning (8th week of age) due to an increase in solid feed intake. Baldwin et al. (2004) reported that rumen development is associated with solid feed intake and early weaning enhanced the solid feed intake and resulted in early rumen development.

Calf scour
Scouring was more prevalent in high milk fed calves than in low milk fed calves. Jasper and Weary (2002) reported that the level of milk feeding (ad-libitum or 10% of body weight) had no effect on the incidence of calf scour. Constable (2004) reported that there are many reasons for calf diarrhoea, but 30% of diarrhoea is due to bacteraemia, predominantly because of E. coli. Borderas et al. (2009) reported that high or low feeding did not cause any gastrointestinal problems. Reasons for the higher number of scour days in the present study are unclear.

Feeding cost
Early weaned low milk fed calves cost less per kg of live weight gain than late weaned high milk fed calves; this was due to the lower milk and higher starter intakes in the former and starter ration was cheaper than milk. Although costing more to feed per kg of live weight gain, late weaned high milk fed calves had significantly higher weight gain than all the other groups. Lower early growth resulting from lower nutrient intake compromises future performance (Zicarelli 2007). Thus, lower feeding cost should not be the only criteria used in choosing a nutritional regime for raising buffalo calves. The decision to choose a specific feeding package would depend upon available feed resources, anticipated productive performance and net profit from the raised calves.

Conclusions
The highest body weight and average daily gain was recorded in high milk late weaned calves and poorest in low milk early weaned calves. Early weaning or low milk volume consumption minimised the feeding costs, however, resulted in lower body weight and average daily gain. Choices of pre-weaning feeding regimen should be made with the view of the cost of feed and performance of replacement calves. In this study, calves did not consume their daily share of milk due their slow adaptation to t artificial feeding and low consumption of starter ration up until after the eighth week. Had the calves consumed their due share of milk and started eating starter ration earlier, the results of this study may have been different. Therefore, further studies on buffalo calves are suggested before formulating a final recommendation on weaning age and milk feeding regime to achieve peak growth performance.

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Ethical statement
All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors. It is also certified that the manuscript does not contain clinical studies or patient data. The authors declare that they have no conflict of interest.

Disclosure statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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References
Abdullah M, Iqbal ZM, Saadullah M, Haq A, Javed K, Jabbar MA, Tauseef A. 2013. Comparative performance of calves fed milk and/or milk replacer supplemented with calf starter up to weaning age in Nili-Ravi buffaloes. Buffalo Bull. 32:874–877.
Anderson KL, Nagaraja TG, Morrill JL, Avery TB, Galitzer SJ, Boyer JE. 1986. Ruminal microbial development in conventionally or early-weaned calves. J Anim Sci. 64:1215–1226.
AOAC. 1990. Official methods of analysis. Association of Official Analytical Chemists Publ., 15th ed. Gaithersburg (MD), USA.
Azim A, Khan AG, Anjum MI, Nadeem MA. 2011. Effect of milk replacer and early weaning diets on growth
performance of buffalo calves during weaning period. Pak Vet J. 31:23–26.
Baldwin RL, VI, McLoed KR, Klotz JL, Heitmann RN. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J Dairy Sci. 87(E. Suppl):E55–E65.
Benschop DL, Cant JP. 2009. Developmental changes in clearance of intravenous doses of glucose, acetate and β-hydroxy butyrate from plasma of calves. Livest Sci. 122:177–185.
Bhatti SA, Khan MS, Sarwar M. Ehsanullah 2009. Performance of buffalo and cow calves during pre-weaning period under same managemental conditions at the University of Agriculture, Faisalabad. Pak J Zool. (Suppl. 9):623–628.
Bhatti SA, Ahmad MF, Wynn PC, McGill D, Sarwar M, Afzal M, Ullah E, Khan MA, Khan MS, Bush R, et al. 2012a. Effect of diet on preweaning performance of Sahiwal calves. Trop Anim Health Prod. 44:819–826.
Bhatti SA, Ali A, Nawaz H, McGill D, Sarwar M, Afzal M, Khan MS, Ullah E, Amer MA, Bush R, et al. 2012b. Effect of preweaning feeding regimens on post-weaning growth performance of Sahiwal calves. Animal. 6:1231–1236.
Bhatti SA, Nazir K, Basra MJ, Khan MS, Sarwar M, Mughal MAI. 2013. Prospects of raising Sahiwal cow calves for veal production under tropical environment. Trop Anim Health Prod. 45:923–930.
Borderas TF, de Passille AMB, Rushen J. 2009. Feeding behavior of calves fed small or large amounts of milk. J Dairy Sci. 92:2843–2852.
Cheema AT, Bhatti SA, Akbar G, Wynn PC, Muhammad G, Warriach HM, McGill D. Forthcoming 2016. Effect of weaning age and milk feeding level on pre- and post-weaning growth performance of Sahiwal calves. Anim Prod Sci. doi:10.1071/AN15719
Constable PD. 2004. Antimicrobial use in the treatment of calf diarrhea. J Vet Intern Med. 18:8–17.
Coverdale JA, Tyler HD, Quigley JD, Brumm JA. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. J Dairy Sci. 87:2554–2562.
Cutrignelli MI, Bovera F, Marchiello M, Di Lella T, Pacelli C. 2003. Influence of weaning age on growth dynamics of young buffalo bulls until 90 days of age. Ital J Anim Sci. 2(Suppl. 1):334–336.
de Passillé AM, Borderas TF, Rushen J. 2011. Weaning age of calves fed a high milk allowance by automated feeders, effects on feed, water and energy intake, behavioral signs of hunger, and weight gains. J Dairy Sci. 94:1401–1408.
Heinrichs AJ, Heinrichs BS, Harel O, Rogers GW, Place NT. 2005. A prospective study of calf factors affecting age, body size and body condition score at first calving of Holstein dairy heifers. J Dairy Sci. 88:2828–2835.
Jasper J, Weary DM. 2002. Effects of ad libitum milk intake on dairy calves. J Dairy Sci. 85:3054–3058.
Jogi S, Lakhani GP. 1996. Study of body weights, rate of gain and mortality percentage in Murrah buffalo calves. Buffalo Bull. 15:51–54.
Kertz AF, Chester-Jones H. 2004. Invited review: guidelines for measuring and reporting calf and heifer experimental data. J Dairy Sci. 87:3577–3580.
Khan MA, Lee HJ, Lee WS, Kim HS, Kim SB, Ki KS, Ha JK, Lee HG, Choi YJ. 2007. Pre- and post-weaning performance of Holstein female calves fed milk through step-down and conventional methods. J Dairy Sci. 90:876–885.
Khan MA, Weary DM, von Keyserlingk MAG. 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. J Dairy Sci. 94:3547–3553.
Khan MA, Weary DM, Veira DM, von Keyserlingk MAG. 2012. Postweaning performance of heifers fed starter with and without hay during the milk-feeding period. J Dairy Sci. 95:3970–3976.
Khan MA, Bach A, Weary DM, von Keyserlingk MAG. 2015. Invited review: transitioning from milk to solid feed in dairy heifers. J Dairy Sci. 99:885–902.
Lee HJ, Khan MA, Lee WS, Yang SH, Kim SB, Ki KS, Kim HS, Ha JK, Choi YJ. 2009. Influence of equalizing the gross composition of milk replacer to that of whole milk on the performance of Holstein calves. J Anim Sci. 87:1129–1137.
Littell RC, Henry PR, Ammerman CB. 1998. Statistical analysis of repeated measures data using SAS procedures. J Anim Sci. 76:1231–1236.
NRC. 2001. Nutrient requirements of dairy cattle. 7th ed. Washington (DC): National Academy Press.
Rashid MA, Pasha TN, Jabbar MA, Ijaz A, Rehman H, Yousaf MS. 2013. Influence of weaning regimen on intake, growth characteristics and plasma blood metabolites in male buffalo calves. Animal. 7:1472–1478.
SAS Institute Inc. 2011. SAS/STAT User’s Guide, Version 6. Cary (NC): SAS Institute, Inc.
Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 7:3583–3597.
Zicarelli L. 2007. Can we consider buffalo a non-precocious and hypofertile species? Ital J Anim Sci. 6(suppl 2): 143–154.