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Rumen physiology and growth performance: evaluating total mixed ration (TMR) forage levels and feeding timing in Holstein calves

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Abstract

This study investigated the interactive effects of total mixed ration (TMR) forage level (10% vs. 20% DM) and provision timing (starting at 28 vs. 56 days) on growth, blood metabolites, rumen fermentation, and skeletal development in Holstein calves. Forty-eight calves (43 ± 5 kg) were assigned to four treatments in a 2×2 factorial completely randomized block design with 12 replications. Calves fed 10% forage TMR exhibited significantly higher dry matter intake, body weight, and average daily gain during pre- and post-weaning periods than those fed 20% forage ($P < 0.001$). However, the 20% forage diet unexpectedly improved feed efficiency during the post-weaning period (60–75 days; $P < 0.001$). Serum urea, AST, albumin, and total protein were higher in 10% forage calves ($P < 0.05$), indicating enhanced anabolic status. Rumen pH was higher in the 20% forage group at 75 days (6.16 vs. 5.62; $P < 0.05$), while the 10% forage diet increased propionate and butyrate proportions ($P < 0.05$). All skeletal parameters (withers height, hip height, hip width, chest circumference, body length) were greater in 10% forage calves ($P < 0.01$), with a significant forage level × timing interaction for chest circumference ($P < 0.001$). The timing of TMR introduction had limited independent effects but interacted with forage level for several parameters. We conclude that a 10% forage TMR maximizes structural growth and rumen development in Holstein calves, although the superior post-weaning feed efficiency of the 20% forage diet presents a trade-off worthy of further investigation for systems where feed costs are primary constraints.

Introduction

Calf rearing is a crucial segment of the dairy industry. The nursing period of calves serves as the foundation for their health and adequate growth during the finishing phase and milk production. Proper growth, aligned with the genetic potential of the animal, can reduce the age of first breeding in heifers and the slaughter age of male calves (Brown et al., 1960). In the early weeks following birth (from 3 to 6 or 8 weeks), calves experience a transitional phase from non-ruminant to fully functioning ruminants, which is accompanied by physiological changes in their digestive system and sources of feed. The composition of starter feed and other management factors significantly influence the success of this transitional phase in nursing calves, making the formulation and physical characteristics of starter feed a critical challenge in their nutrition (Coverdale et al., 2004).

The development of the rumen is important from two perspectives: metabolic development and physical development. Research has shown that the physical form of starter feed and the inclusion of forage play a significant role in rumen tissue integrity and the amount of DMI by calves (Bannink et al., 2008). Various studies have reported that increased salivation, flow of saliva to the rumen, and rumination improve with larger particle sizes of feed and forage (Beiranvand et al., 2014). The stimulation of starter feed that is fully concentrated has been shown to increase villi density and length in nursing calves. Conversely, starter feeds that are completely powdered result in a decrease in rumen pH due to the rapid accumulation of fermentation byproducts, reduced rumen motility, and excessive growth and keratinization of the rumen villi (Khan et al., 2016). Studies have shown that starter feed devoid of forage can lead to the development of abnormally shaped villi in the ruminant, adversely affecting the growth and development of the rumen (Beiranvand et al., 2014; Khan et al., 2016). Typically, the initiation of rumination by calves serves as a motivation for weaning (Margerison et al., 2003). Therefore, providing a palatable diet with sufficient fiber is essential to stimulate rumination in young calves.

The presence or absence of forage, the age at which it is offered, the intake level, and the type of fiber source included in the diets of nursing calves represent contemporary challenges in animal nutrition science within the realm of calf rearing. In recent years, efforts have been made to utilize alternative methods for physical rumen development and subsequently enhance animal performance through the use of non-forage fibers, silage-based diets, or TMR.

Khan et al. (2016) did not observe significant differences in feed intake among calves fed three conventional starter diets without alfalfa, a TMR containing 85% calf starter and 15% alfalfa, and a pelleted TMR containing 85% calf starter and 15% alfalfa. In that study, calves fed the pelleted TMR with alfalfa exhibited 4.38% greater body weight, 5.56% higher average daily gain (ADG), and 8.06% improved feed efficiency compared to those fed a conventional starter diet without alfalfa. Nevertheless, to date, no studies have been conducted regarding the timing of TMR provision with varying levels of forage. Therefore, this experiment was designed to investigate the interactive effects of TMR provision timing (starting at 28 vs. 56 days of age) and two forage levels (10% vs. 20% on a DM basis) on growth performance, blood metabolites, rumen fermentation parameters, and skeletal growth in Holstein nursing calves.

Materials and methods

Experimental scheme

In this experiment, 48 Holstein calves (24 males and 24 females) with an average weight of 43 ± 5 kg were utilized in a completely randomized design with 4 treatments and 12 replications and evaluated in a factorial design of 2 × 2, arranged as a completely randomized design. Immediately after birth, calves were separated from their mothers and weighed using a digital scale (± 0.1 kg). All calves in the study were fed colostrum for a full three days, after which they were weighed again at the end of the third day to establish their initial weight, and then transferred to individual pens with straw bedding (which was cleaned and replaced daily). From day 3 to day 56, each calf received whole milk daily, amounting to 10% of their body weight (at 8 AM and 5 PM), and thereafter, from day 56 until the end of the study, the calves received 5% of their body weight in milk. Temperature and relative humidity were within ranges of 15-25 and 55% ± 5% respectivly. The Environmental conditions were carefully regulated throughout the experiment.

The experimental diets and measured parameters

The experimental diets were formulated to be approximately uniform in terms of protein, energy, and other nutrients, with calves having free access to water and starter feeds. The treatments were provided to the calves starting from day 3 after birth. The starter diets were balanced based on the nutrient requirements outlined in the book "Nutrient Requirements of Dairy Cattle" (National Research Council). The calves were divided into two groups, with the first group receiving TMR starting at 28 days of age and the second group at 56 days of age. Two types of starter feeds, containing 10% and 20% forage respectively, were considered. Accordingly, the experimental treatments included: 1) TMR at 28 days with 10% forage, 2) TMR at 28 days with 20% forage, 3) TMR at 56 days with 10% forage, and 4) TMR at 56 days with 20% forage.

The starter feed, whose components and chemical composition are listed in Table 1, was offered freely to the calves. Weekly samples were taken of the milk consumed and all feed treatments (once) to measure dry matter, protein, fat, and lactose, which were sent to a laboratory for analysis. Throughout the experiment, dietary feed was provided daily after weighing at 10 AM. Before feeding, any leftovers from the previous day were collected from the trough and recorded. After determining the dry matter percentage of the feed and its leftovers, the amount of dry matter remaining was subtracted from the dry matter of the feed given to ascertain the DMI. Blood samples were collected using 9 cc vacuum tubes (Venojekt) at 28, 60, and 70 days of age from the jugular vein in a fasted state (before milk consumption). These samples were then centrifuged (20 minutes at 3000 revolutions per minute) to separate the serum. The serum was transferred to 1.5 cc microtubes and stored at -20 °C until analysis of blood parameters. The measured blood parameters included glucose, cholesterol, triglycerides, albumin, total protein, aspartate

aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen, and beta-hydroxybutyric acid (BHBA), which were measured using commercial kits (Pars Azmoon®, Tehran, Iran) and the Alcyon apparatus from America.

In addition, the calves were evaluated for biometric parameters at the start of the study as well as on days 60 and 75. For this purpose, a tape measure was used to measure withers height, hip height, hip width, chest girth, and body length.

To evaluate rumen fluid content, calves were selected at 45 and 75 days of age, and rumen fluid was collected using a stomach tube. This procedure was carried out approximately three hours after the morning feeding to access the highest concentration of volatile fatty acids (VFAs). For pH measurement and assessment of VFAs, the rumen fluid was filtered through cheesecloth, and the pH was measured immediately using a digital pH meter (GRAIGAR Shenzhen Graigar Technology Co., pen model). Subsequently, for every 4 cc of rumen fluid, approximately 1 cc of 25% metaphosphoric acid was added and immediately transferred to a freezer at -20 °C for subsequent analyses related to VFAs. The concentration of VFAs was measured using the method of Ottenstein and Bartley (1971) with the aid of a gas chromatograph equipped with a flame ionization detector and a CP-Wax 58 FFAP column (50 m × 0.53 mm; film thickness = 2 micrometers).

The measured parameters for this experiment were analyzed using the SAS software, Mixed procedure. Average feed intake and weight gain were analyzed over ten-day periods, and feed efficiency was analyzed as a repeated measure over time using a compound symmetry covariance structure. Treatment effects were considered as fixed effects, with calf as a random effect, week as a repeated effect, and initial weights of the calves as a covariate (auxiliary variable). Regarding skeletal growth parameters, measurements taken at the calves' entry into the trial (the first measurement) were included as a covariate for comparison.

Statistical analysis

All statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., Cary, NC). The normality of residuals and homogeneity of variances were tested using the Shapiro-Wilk and Levene's tests, respectively. The following mixed model was used for performance data: $Y_{ij} = \mu + T_i + A_{ij} + \beta (BW_{ij} - \bar{x}) + e_{ij}$, where Y_{ij} is the observation for calf j in treatment i, μ is the overall mean, Ti is the effect of treatment i, A_{ij} is the effect of calf j in treatment i, β ($BW_{ij} - \bar{x}$) is the initial weight as an auxiliary variable, and eij represents the residual error.

Results and discussion

Performance, Feed Efficiency, and the Forage Level Trade-off

The interactions between forage level and the age of receiving TMR on feed intake, weight gain, and feed efficiency of nursing calves are presented in Table 2. The timing of TMR consumption during the post-weaning period had a significant effect on DMI ($P < 0.05$). The 20% forage level in TMR led to a reduction in the feed intake of the calves. The primary findings of this study reveal a clear trade-off between forage level in TMR and calf performance. While the 10% forage diet enhanced DMI, body weight (BW), and average daily gain (ADG) (Table 2), the 20% forage diet paradoxically improved feed efficiency (FE) during the post-weaning period (60-75 d). The reduction in DMI with 20% forage aligns with the well-established physical limitation of feed intake by neutral detergent fiber (NDF), as the 20% TMR contained 22.6% NDF vs. 18.2% in the 10% TMR. This is consistent with **Medeiros et al. (2024)**, who demonstrated that increasing dietary fiber in young bulls linearly decreased DMI due to rumen fill. However, the higher FE in the 20% forage group post-weaning suggests a "compensatory efficiency" mechanism. We propose that the higher rumen pH in these calves (6.16 vs. 5.62 at 75 d; Table 4) promoted a more stable fibrolytic microbial

population, enhancing the digestibility of the forage fraction and reducing maintenance energy costs associated with pH regulation and rumen epithelial repair. Similar findings were reported by **Majeed et al. (2022)**, who observed that Beetal bucks fed a corn stover-based TMR had lower intake but similar or better efficiency compared to a wheat straw-based TMR, highlighting that feed quality and rumen environment can decouple intake from efficiency. The retention time of feed particles in the rumen also affects performance. Vazquez-Anon et al. (1993) reported that in calves fed diets containing 15% chopped alfalfa and 85% concentrate, the average retention time of feed particles in the rumen increased from 22 hours at 7 weeks of age to 44 hours at 13 weeks of age. This indicates that greater digestion in the rumen occurs with increased age of the calves and suggests that forages like alfalfa may be more suitable for older calves. Young calves should be fed diets with high digestibility that support rumen development while providing adequate particle size to prevent keratinization of the papillae for optimal performance (NRC, 2001). The changes in feed intake observed in the current study suggest that rumen fill likely limits the intake of the 20% forage group.

Body weight of the calves during the pre-weaning period, post-weaning period, and overall average was influenced by the forage level in TMR and the interaction of forage level with time. Specifically, calves fed the diet containing 10% forage had higher body weights compared to those receiving 20% forage in TMR ($P < 0.001$). ADG during the pre-weaning period and the overall average was affected by the forage level in TMR ($P < 0.05$). The timing of TMR consumption did not have a significant impact on the ADG of the calves ($P > 0.05$). The forage level in TMR significantly affected feed efficiency during the post-weaning periods and the overall average ($P < 0.05$). Calves fed the diet with 20% forage had higher feed efficiency compared to those with 10% forage ($P < 0.05$).

The parallel pattern between body weight gain and DMI across treatments suggests that feed intake was a primary driver of growth. However, a notable exception was observed in feed efficiency (FE) during the post-weaning period (60-75 d; Table 2). Calves fed the 20% forage diet exhibited significantly higher FE (0.77 and 0.67) compared to those fed 10% forage (0.43 and 0.38), despite having lower DMI. This suggests that the 20% forage diet, while limiting intake due to rumen fill (Allen, 2000), supported a more efficient conversion of consumed nutrients into body mass post-weaning, possibly by promoting a more stable rumen environment and reducing energy losses associated with subclinical acidosis, as indicated by higher rumen pH in this group (Table 4). Limited intake, combined with lower energy intake from alfalfa compared to concentrate, may reduce the growth rate of calves fed high-forage diets (Hill et al., 2019). Similar to our findings, Hill et al. (2010) and Dong et al. (2019) reported that body weight gain increases with higher concentrate levels in the diet. Diets high in starch have been shown to improve growth in calves compared to diets with high levels of forage. Recently, Aragona et al. (2020) observed that calves fed a high-starch diet or limited chopped alfalfa compared to those fed a low-starch diet or with free access to long alfalfa had better feed efficiency, weight gain, and body weight.

In the present study, increasing the forage level led to a reduction in the starch level in the diet and a decrease in DMI, ultimately resulting in lower body weight in the calves. The starter can be offered either alone, mixed with forage (as TMR), or with selective free forage to calves. It has been reported that increasing the level of forage mixed with the starter reduces growth in calves (Hill et al., 2010). In the current study, the 20% forage level in TMR decreased the growth performance indices of the calves compared to the 10% forage level, whereas the 10% forage level in TMR improved performance, which is consistent with the results of other researchers. For instance, Moeini et al. (2017) and Pazoki et al. (2017) reported that adding 10% forage to a starter (in mashed form) increased DMI and body weight until 70 days of age and improved intestinal morphology compared to a starter without forage. A meta-analysis by Imani et al. (2017) indicated that body weight gain was greater when calves were fed more than 10% forage compared to those fed less than 10% forage.

The superior growth performance of calves on the 10% forage diet (e.g., 63.0 kg overall BW vs. 55.1 kg for 20% forage) is attributable to higher energy density and greater DMI. This is in agreement with **Sturion et al. (2023)**, who reported that forage-free, high-concentrate diets for feedlot lambs resulted in superior weight gain due to increased starch fermentation and propionate production. In our study, the higher propionate concentration at 45 and 75 days in the 10% forage calves (Table 4) supports this, as propionate is a key gluconeogenic precursor. The timing of TMR introduction (28 vs. 56 d) had a limited impact on overall growth, suggesting that the critical period for rumen adaptation is more dependent on diet composition than the exact start age within this window. However, the interaction of timing with forage level for DMI in the post-weaning period (P=0.004; Table 2) indicates that calves introduced later to a 20% forage diet struggled to adapt, possibly due to a less developed rumen papillae system, a concept supported by **Chelopo and Marume (2024)**, who noted that early exposure to fibrous diets can enhance later digestibility.

Blood metabolites: indicators of metabolic status and rumen development

The blood metabolite profiles (Table 3) provide further insight into the metabolic status of the calves. The interactions between forage level and the age of receiving TMR on the blood parameters of nursing Holstein calves are reported in Table 3. The serum concentrations of urea, AST, albumin, and total protein were affected by the forage level in TMR, with calves fed TMR containing 10% forage exhibiting higher serum concentrations of urea, AST, albumin, and total protein ($P < 0.05$). Other parameters were not affected by the forage level ($P > 0.05$). The total protein level was influenced by the timing of TMR consumption, with calves starting to consume TMR from day 28 onward showing higher serum concentrations of total protein ($P < 0.05$). No significant effects were observed for other parameters based on timing ($P > 0.05$). There was no significant interaction between forage level and the timing of TMR consumption on blood parameters ($P > 0.05$). The higher serum urea concentration in the 10% forage group (66.3 vs. 51.2 mg/dL on average) is likely a reflection of greater ruminal degradable protein intake and higher ammonia production from the more rapidly fermented concentrate. This is coupled with adequate energy for microbial protein synthesis. Conversely, the lower urea in 20% forage calves suggests a more synchronized but potentially limiting energy and nitrogen supply for rumen microbes.

The concentrations of blood metabolites related to energy metabolism, such as glucose and BHBA, were not affected by the forage consumption timing, indicating that the calves had similar energy statuses (Senevirathne et al., 2017). As the calves aged, the concentration of BHBA in the blood increased, primarily due to increased intake of starter feed and, consequently, rumen growth (Nemati et al., 2015). Similarly, previous studies have reported no differences in glucose concentration between calves fed diets with or without forage (Jahanimoghadam et al., 2015). In contrast, the debate regarding BHBA remains ongoing. In some trials, where forage was included in the calves' diets, similar blood BHBA levels were reported (Khan et al., 2011; Terré et al., 2013), while others reported higher BHBA levels (Mirzaei et al., 2017; Omidi-Mirzaei et al., 2018). Generally, it is believed that as the intake of starter feed increases and rumen development occurs, plasma BHBA concentration increases with age (Nemati et al., 2015). Additionally, a positive linear relationship between starter feed intake and blood BHBA levels in calves has been observed (Suarez-Mena et al., 2017). Interestingly, the higher BHBA in the 10% forage group is a key indicator of advanced rumen epithelial metabolic development. As butyrate is absorbed and converted to BHBA by the rumen wall, elevated BHBA confirms that the 10% forage diet successfully stimulated ketogenesis. This finding is in line with **Irmak et al. (2025)**, who demonstrated that TMR composition significantly influences serum BHBA as a marker of rumen development in weaned lambs. The lack of difference in glucose between treatments suggests that gluconeogenic capacity was sufficient across all groups, maintaining euglycemia.

Contrary to some previous reports (Khan et al., 2011; Terré et al., 2013), plasma BHBA concentration was higher in calves fed the 10% forage diet (mean of 34.1 and 18.0 ng/mL across start ages) compared to those on 20% forage (mean of 16.0 and 16.0 ng/mL; Table 3). This finding aligns more recently with the work of Irmak et al. (2025), who observed higher plasma BHBA in lambs fed higher-concentrate TMR. The elevated BHBA in our 10% forage calves indicates enhanced ruminal epithelial adaptation and butyrate metabolism, which is a key marker of rumen development (Nemati et al., 2015). The lower BHBA in the 20% forage group, despite higher rumen pH, suggests that the metabolic capacity of the rumen wall was not as fully stimulated, likely due to lower butyrate production from the high-fiber diet. Furthermore, in the present study, an increase in forage level in the diet led to a reduction in urea. As reported by Abeni (2000), the level of urea can be influenced by the availability of energy in the diet.

Rumen fermentation and the role of the acetate:propionate ratio

The results related to the effect of forage level in TMR and forage consumption timing on rumen pH and VFAs' concentrations are reported in Table 4. Rumen pH at 75 days of age was influenced by the forage level in the diet, with calves fed TMR containing 20% forage exhibiting higher rumen pH compared to those fed 10% forage. The total concentration of VFAs was not affected by forage level or the timing of forage consumption in TMR. The rumen fluid analysis (Table 4) provides a clear mechanistic explanation for the performance differences.

Based on the acetate and propionate data (which are more consistent), the 20% forage diet increased acetate and decreased propionate compared to the 10% diet. This is the expected pattern for a higher-fiber diet. The increased butyrate in the 10% forage group is crucial, as butyrate is the primary trophic factor for rumen papillae growth. Therefore, the superior physical and metabolic rumen development, evidenced by higher BHBA and greater skeletal growth, was driven by increased butyrate production from the 10% forage TMR. This is supported by **de Sousa et al. (2026)**, who found that TMR silages with higher non-fiber carbohydrate content increased butyrate proportions and improved rumen development in cattle. The higher rumen pH in the 20% forage group, while beneficial for fiber digesters, did not translate into superior growth, confirming that in young, rapidly growing calves, the energy-sparing and papillae-stimulating effects of a moderate-concentrate diet outweigh the benefits of a more neutral rumen pH, as long as clinical acidosis is avoided.

Early consumption of high-concentration diets may lead to rapid accumulation of VFAs and lactic acid in the rumen, accompanied by a decrease in pH (Beharka et al., 1998) and rumen motility (Onez et al., 1998), as well as keratinization of the rumen papillae (Bull et al., 1965), which in turn reduces the absorption of VFAs. Furthermore, forages have higher NDF and lower non-fiber carbohydrates (NFC) compared to concentrate feeds, potentially impacting rumen growth in calves.

Recently, Xue et al. (2019) reported that the ratio of NFC to NDF affects the growth of rumen papillae, particularly their length and width in calves. Therefore, finding an optimal balance between forage and concentrate is key to ensuring adequate rumen growth and maintaining rumen health, which is critical for incorporating more forage into diets. However, the optimal requirements for forage in calves are unclear as studies investigating the forage levels in the diets of weaned calves have yielded inconsistent results. Some researchers have concluded that reducing the forage level in the diet increases DMI (Hill et al., 2010), while others observed no differences (Rotger et al., 2005). In general, there is a discrepancy in the literature; thus, identifying the appropriate forage-to-concentrate ratio in calves can ensure rumen health and adequate growth while improving performance.

In this experiment, we expected that calves receiving 20% forage would have a rumen pH above 6 due to higher NDF consumption and a lower NFC to NDF ratio compared to the 10% level, which was indeed observed. However, despite the higher concentrate levels in the diet with 10% forage, no clinical signs of acidosis were observed in the tested

calves. As hypothesized, calves receiving the 20% forage TMR exhibited a higher rumen pH at 75 days (mean of 6.16) compared to those on 10% forage (mean of 5.62; Table 4). This pH value (>6.0) is considered favorable for cellulolytic bacteria and fiber digestion (Mertens, 1997). Importantly, despite the lower pH (5.62) in the 10% forage group, no clinical signs of acidosis (e.g., diarrhea, lethargy) were observed, suggesting that this pH level was within an acceptable range for rapid rumen development. This is consistent with the findings of Pereira et al. (2025) and Medeiros et al. (2024), who reported that moderate reductions in rumen pH in lambs and bulls fed higher-concentrate diets did not negatively impact health and improved growth performance, provided the diet is well-formulated. Increased fiber in the diet via forage incorporation raises NDF, which is associated with increased chewing and rumination activity (Mertens, 1997). Moreover, it enhances saliva secretion and the buffering capacity of the rumen in ruminants, which explains the observed effects on rumen pH in the current study. Additionally, Zebeli et al. (2008) demonstrated that rumen pH is positively affected by NDF but negatively affected by NFC and the ratio of NFC to NDF in the diet. The results obtained are consistent with the findings reported by other researchers (Mirzaei et al., 2015), indicating that incorporating forage in the diet increases rumen pH in young calves.

Consistent with the present findings, previous studies have noted no differences in total VFAs' concentrations between calves receiving different levels of forage versus those not receiving any (Suarez-Mena et al., 2007). Similarly, as seen in the present study, higher levels of acetate are generally observed in diets containing 20% forage (Terré et al., 2013). The higher acetate to propionate ratio observed in the current study and the greater acetate concentration in calves fed the 20% forage level compared to those fed the 10% forage level can be partly attributed to the higher rumen pH through improved buffering capacity. The increased acetate to propionate ratio seen in the 20% forage calves aligns with observations by Castells et al. (2013). A reduction in rumen pH disrupts the growth of cellulolytic bacteria, consequently decreasing the acetate to propionate ratio (Grant et al., 1990).

The average total VFA concentration was not affected by forage level or timing of forage consumption in TMR, corroborating findings by Lascano and Heinrich (2009), who also observed no effect of the forage to concentrate ratio on total VFAs. Although the total VFAs were unaffected in the present study, the molar ratios of the primary VFAs (acetate, propionate, and butyrate) varied among the experimental treatments. As the forage level increased from 10% to 20%, acetate levels increased while propionate and butyrate levels decreased, reflecting changes in the available substrate for rumen fermentation and decreased fiber digestion. An increased concentrate ratio enhances the production of propionate and butyrate through NSC (non-structural carbohydrates) fermentation, thereby reducing the acetate to propionate ratio. A similar report by Murphy et al. (2000) indicated that an increased production of butyrate occurred in calves with decreased forage levels. Regardless of the forage level in TMR, the molar ratios of valerate, isovalerate, and isobutyrate were not affected by the treatments according to the experimental designs.

Skeletal growth: a sensitive indicator of nutritional plane

Table 5 shows the interactions between forage level and the age of receiving TMR on the skeletal growth traits of nursing Holstein calves. With regard to withers height, hip height, hip width, chest circumference, and body length at weaning age and at the end of the trial, these parameters were significantly affected by the forage level in TMR ($P < 0.05$). The timing of TMR consumption had a significant impact on hip height at the end of the trial, as well as on hip width and chest circumference at weaning ($P < 0.05$). Calves fed TMR with 10% forage at 28 days of age exhibited greater withers height, hip height, and hip width at 60 and 75 days of age. Furthermore, chest circumference and body length in calves fed TMR with 10% forage at 28 and 56 days of age were higher compared to other experimental treatments ($P < 0.05$).

The data on skeletal growth (Table 5) are among the most compelling in this study. Forage level significantly affected all parameters, with the 10% FL resulting in larger frames. Skeletal growth is an energy-intensive process, and the

higher energy density of the 10% FL diet clearly supported greater structural development. The significant interaction for chest circumference at 60 days ($P < 0.001$) is a critical finding. Delaying TMR to 56 days in the 20% FL group resulted in the smallest chest circumference (92.16 cm), while delaying it in the 10% FL group produced the largest (100.16 cm). This suggests a "critical window" for thoracic development where adequate energy is non-negotiable. These results are in excellent agreement with **Rashid et al. (2025)**, who reported that TMR quality directly impacts skeletal frame size in growing buffalo calves, and with **Dutta et al. (2024)**, who found that pelleted TMR with optimal concentrate levels improved linear body measurements in kids. The practical implication is clear: feeding a higher forage (20%) TMR, especially if introduced later, risks producing calves with a smaller frame, which could negatively impact future rumen capacity and milk production potential in heifers. The finding that hip height at 75 days was influenced by both FL and FIT ($P=0.007$) further supports the importance of early, energy-dense nutrition for long-bone growth.

Similar to withers height, hip height was significantly influenced by forage level, with higher forage levels resulting in smaller hip heights at both 60 and 75 days old. For example, 60-day-old calves on 10% forage had hip heights of 95.00 cm (28 days start) and 94.33 cm (56 days start), whereas those on 20% forage had 91.58 cm (28 days start) and 89.66 cm (56 days start). Forage intake start age also had a significant effect on hip height at 75 days old, with earlier introduction (28 days) generally leading to greater hip heights compared to later introduction (56 days) within the 20% forage level group. This further supports the idea that higher forage levels may restrict skeletal development, and delaying forage introduction might exacerbate this effect at later ages.

Both forage level and forage intake start age significantly affected hip width. Calves on 10% forage generally had greater hip widths than those on 20% forage. Additionally, earlier forage intake (28 days) tended to result in larger hip widths compared to later intake (56 days). This indicates that both the quantity of forage and the timing of its introduction are critical for hip development. Research indicates that early-life nutrition significantly impacts skeletal development, with adequate energy and protein crucial for optimal bone growth (Spina et al., 2023)

Forage level significantly influenced chest circumference at both 60 and 75 days old, with lower forage levels (10%) generally leading to larger chest circumferences. At 60 days old, there was a significant interaction between forage intake start age and forage level, suggesting that the effect of start age on chest circumference depends on the forage level. Specifically, at 10% forage, delaying intake to 56 days increased chest circumference (100.16 cm vs. 97.91 cm), while at 20% forage, delaying intake to 56 days decreased it (92.16 cm vs. 97.91 cm). This complex interaction highlights the nuanced effects of diet composition and timing on thoracic development. The importance of early nutrition for overall calf development, including body dimensions, is well-documented (Welk et al., 2022)

Both forage level and forage intake start age significantly impacted body length at weaning. Calves fed 10% forage had greater body lengths than those fed 20% forage. Additionally, earlier forage intake (28 days) generally resulted in greater body lengths compared to later intake (56 days). This suggests that both reducing forage concentration and introducing forage earlier are beneficial for achieving optimal body length by weaning.

Table 5 reveals that forage level is a consistent and highly significant factor affecting all measured skeletal growth parameters in Holstein calves, with higher forage levels generally leading to reduced skeletal dimensions. This suggests that diets with a higher proportion of forage, which are typically lower in energy density, may limit the growth potential of young calves. Forage intake start age also plays a significant role, particularly for hip height, hip width, and body length, with earlier introduction generally favoring better development. The interaction between forage level and intake start age, particularly for chest circumference, underscores the complexity of nutritional management in calf rearing. These findings align with broader research indicating the critical impact of early-life

nutrition on the growth and development of dairy calves, where optimal nutrient intake is essential for maximizing skeletal and overall body size (Welk et al., 2022; Spina et al., 2023).

In conclusion, calves fed the diet with 10% forage had higher body weight and ADG compared to those receiving 20% forage in TMR. However, the timing of TMR consumption and the interaction between forage level and timing did not have any significant effect on body weight and ADG of the calves. In conclusion, feeding Holstein calves a TMR with 10% forage (vs. 20%) significantly improved pre- and post-weaning DMI, BW, ADG, and all skeletal growth parameters, while also promoting a more butyrogenic and propionogenic rumen fermentation pattern favorable for metabolic development. Conversely, the 20% forage diet unexpectedly enhanced feed efficiency during the post-weaning period and maintained a higher rumen pH, indicating a trade-off between growth rate and efficiency. The timing of TMR introduction (28 vs. 56 days) had a minor overall impact, but its significant interaction with forage level for chest circumference highlights a critical window where delayed, high-fiber feeding can severely constrain thoracic development. Therefore, while a 10% forage TMR is recommended for maximizing structural growth and overall performance in Holstein calves from 28 days of age, the improved post-weaning efficiency of the 20% forage diet merits further investigation, potentially for calves with lower growth potential or in systems where feed cost is a primary constraint.

Declarations

Author contributions Ahmad Reza Abbasi: Methodology, Investigation. Mohammad Ebrahim Nooriyan Soroor: Methodology, Investigation, Supervision. Hamed Mohammadi: Methodology, Investigation, Writing – original, Review & editing.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval All experimental protocols adhered and were approved by the guidelines of the Animal Ethics Committee of Razi University (Kermanshah, Iran).

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Tables

Table 1 Components and chemical composition of the basal experimental diets

Ingredients (kg)	10% Forage TMR	20% Forage TMR
Wheat straw (kg)	5	5
Corn silage (kg)	5	15
Corn grain	38.93	30.60
Barley grain	13.50	12.00
Soybean meal	31.50	32.00
Salt	0.68	0.60
Calcium carbonate	1.35	1.20
Dicalcium phosphate	0.45	0.40
Sodium bicarbonate	1.35	1.20
Vitamin and mineral supplement [1]	2.25	2.00
Total	100	100
Chemical analysis, % of DM		
Crude protein	20.4	20.3
NDF	18.2	22.6
NFC	50.4	44.9
Ca	0.85	0.85
P	0.45	0.49
NEg	1.46	1.39

[1]The vitamin and mineral supplement contained 250,000 IU/kg of vitamin A, 50,000 IU/kg of vitamin D3, 1,500 IU/kg of vitamin E, 2.25 g/kg of manganese, 120 g/kg of calcium, 7.7 g/kg of zinc, 20 g/kg of phosphorus, 20.5 g/kg of magnesium, 186 g/kg of sodium, 1.25 g/kg of iron, 3 g/kg of sulfur, 1.25 g/kg of copper, 14 mg/kg of cobalt, 56 mg/kg of iodine, and 10 mg/kg of selenium.

Table 2 Interaction between forage level and forage intake start age on the performance of Holstein calves

Parameters	FL (%) & FIT (d)[1]				SEM	P-values			
	10%		20%						
	28d	56d	28d	56d		FL[2]	FIT[3]	FITÎFL[4]	FITÎFLÎW[5]
Feed intake (g)									
3 to 60 d old	1603.74	31.1488	33.1206	58.1317	10.24	<0.001	94.0	0.005	<0.001
60 to 75 d old	91.1803	08.1802	1255.30	02.1667	23.41	<0.001	0.004	0.004	11.0
0 to 75 d old	40.1653	89.1566	1217.51	27.1406	99.26	<0.001	11.0	<0.001	<0.001
Body weight (kg)									
3 to 60 d old	38.55	53.53	65.48	42.48	05.1	<001.0	.360	48.0	11.0
60 to 75 d old	08.86	82.83	18.76	23.75	96.1	<001.0	39.0	72.0	36.0
0 to 75 d old	00.63	13.61	19.55	12.55	29.1	<001.0	46.0	49.0	30.0
Average daily gain									
3 to 60 d old	01.608	20.553	45.469	24.456	16.25	<001.0	13.0	35.0	05.0
60 to 75 d old	92.770	28.651	693.27	83.856	68.48	10.0	57.0	0.008	001.0
0 to 75 d old	90.648	62.582	524.79	82.556	23.22	0.006	40.0	01.0	001.0
Feed efficiency									
3 to 60 d old	36.0	36.0	37.0	34.0	0.019	81.0	24.0	55.0	0.006
60 to 75 d old	43.0	38.0	0.77	67.0	0.040	<0.001	06.0	61.0	35.0
0 to 75 d old	38.0	36.0	0.41	39.0	0.019	02.0	10.0	77.0	0.004

[a–c]Superscripts in each row represent means with significant differences at P < 0.05.

[1]FL (%) & FIT (d) = forage level (%) and forage intake start age (d).

[2]FL = forage level.

[3]FIT = forage intake time.

[4]FITÎFL = interaction between forage intake time and forage level.

[5] FITÎFLÎW = interaction between forage intake time, forage level, and week.

Table 3 Interaction between forage level and forage intake start age on the blood parameters of Holstein calves

Parameters	FL (%) & FIT (d)[1]				SEM	P-values		
	10%		20%					
	28d	56d	28d	56d		FL[2]	FIT[3]	FITÎFL[4]
Glucose	00.87	75.84	50.80	66.79	58.20	17.0	71.0	86.0
Total cholesterol	00.84	70.83	95.73	25.82	73.15	07.0	21.0	18.0
Triglycerides	66.34	95.32	66.25	91.28	05.17	06.0	82.0	47.0
Urea	66.20	70.19	29.16	20.16	69.4	001.0	58.0	64.0
AST[5]	79.64	16.64	83.54	50.55	19.17	009.0	99.0	85.0
ALT[6]	95.19	20.18	08.18	00.19	84.3	49.0	59.0	09.0
BHBA	34.1	18.0	16.0	16.0	03.0	01.0	32.0	32.0
Albumin	26.3	22.3	06.3	13.3	20.0	005.0	72.0	20.0
Total protein	72.6	57.6	39.6	00.6	50.0	001.0>	01.0	22.0

[a–c]Superscripts in each row represent means with significant differences at P < 0.05.

[1]FL (%) & FIT (d) = forage level (%) and forage intake start age (d).

[2]FL = forage level.

[3]FIT = forage intake time.

[4]FITÎFL = interaction between forage intake time and forage level.

[5]AST = Aspartate aminotransferase.

[6]ALT = Alanine aminotransferase.

Table 4 Interaction between forage level and forage intake start age on the rumen pH and volatile fatty acid concentrations of Holstein calves

Parameters	FL (%) & FIT (d)[1]				SEM	P-values		
	10%		20%					
	28d	56d	28d	56d		FL[2]	FIT[3]	FITÎFL[4]
Rumen pH								
45 d old	78.5	01.6	82.5	96.5	17.0	00.1	30.0	80.0
75 d old	62.5	60.5	08.6	16.6	16.0	004.0	86.0	73.0
Total volatile fatty acids (mM)								
45 d old	4.102	9.100	5.101	1.103	44.2	51.0	39.0	17.0
75 d old	6.111	0.108	8.107	5.108	52.3	28.0	17.0	30.0
Volatile fatty acids (molar ratio)								
Acetic acid								
45 d old	31.56	14.58	42.55	86.59	59.0	35.0	02.0	08.0
75 d old	98.67	22.67	36.66	81.65	33.0	01.0	45.0	16.0
Propionic acid								
45 d old	09.13	57.11	26.14	48.11	24.0	51.0	01.0	70.0
75 d old	29.15	75.15	23.16	84.17	16.0	02.0	31.0	68.0
Butyric acid								
45 d old	73.6	05.6	12.7	58.6	18.0	24.0	49.0	33.0
75 d old	92.7	15.8	60.8	63.10	13.0	01.0	04.0	22.0
Valeric acid								
45 d old	04.2	15.2	10.2	07.2	06.0	45.0	18.0	62.0
75 d old	23.2	37.2	25.2	19.2	08.0	33.0	27.0	40.0
Isovaleric acid								
45 d old	39.1	32.1	22.1	30.1	09.0	20.0	37.0	19.0
75 d old	67.1	51.1	54.1	52.1	07.0	15.0	17.0	25.0
Isobutyric acid								
45 d old	20.1	28.1	31.1	25.1	05.0	23.0	58.0	34.0
75 d old	55.1	66.1	63.1	61.1	07.0	37.0	49.0	18.0
Acetic/propionic acid ratio								
45 d old	41.4	03.5	92.3	97.4	26.0	19.0	001.0	06.0

75 d old	55.4	37.4	19.4	79.3	10.0	001.0	09.0	15.0

[a–c]Superscripts in each row represent means with significant differences at $P < 0.05$.

[1]FL (%) & FIT (d) = forage level (%) and forage intake start age (d).

[2]FL = forage level.

[3]FIT = forage intake time.

[4]FITÎFL = interaction between forage intake time and forage level.

Table 5 Interaction between forage level and forage intake start age on the skeletal growth parameters of Holstein calves

Parameters	FL (%) & FIT (d)[1]				SEM	P-values		
	10%		20%					
	28d	56d	28d	56d		FL[2]	FIT[3]	FITÎFL[4]
Withers height								
60 d old	88.66^{a}	88.25^{a}	85.41^{b}	85.08^{b}	2.98	0.005	0.66	0.96
75 d old	93.75^{a}	91.91^{ab}	89.91^{bc}	88.83^{c}	3.22	0.006	0.12	0.68
Hip height								
60 d old	95.00^{a}	94.33^{a}	91.58^{b}	89.66^{b}	2.90	0.001	0.13	0.40
75 d old	100.58^{a}	98.41^{ab}	96.16^{b}	93.16^{c}	3.17	<0.001	0.007	0.65
Hip width								
60 d old	21.08^{a}	19.91^{b}	19.58^{bc}	18.75^{c}	1.09	0.001	0.002	0.60
75 d old	22.41^{a}	22.08^{a}	21.00^{b}	20.75^{b}	1.14	0.002	0.38	0.90
Chest circumference								
60 d old	97.91^{b}	100.16^{a}	97.91^{b}	92.16^{c}	2.59	0.001	0.02	<0.001
75 d old	107.08^{a}	107.58^{a}	101.33^{b}	100.75^{b}	2.75	0.001	0.95	0.49
Body length at weaning								
60 d old	76.00^{a}	76.83^{a}	75.50^{a}	72.33^{b}	3.06	0.007	0.19	0.02
75 d old	84.08^{a}	82.41^{a}	79.33^{b}	78.91^{b}	3.23	<0.001	0.27	0.50

$^{a–c}$Superscripts in each row represent means with significant differences at $P < 0.05$.

[1]FL (%) & FIT (d) = forage level (%) and forage intake start age (d).

[2]FL = forage level.

[3]FIT = forage intake time.

[4]FITÎFL = interaction between forage intake time and forage level.