Effects of Forage Level in Total Mixed Ration on Sorting Behavior, Rumen Contents, and Serum Indices in Dairy Cows: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary roughage level in total mixed ration on sorting behavior, rumen internal environment, and serum indices of dairy cows. Twelve mid-lactation [days in milk (119±26) d] multiparous Holstein dairy cows [body weight (629±46) kg] were selected and randomly divided into 4 groups, with 1 cow in each group fitted with a rumen cannula. According to a 4×4 Latin square design, they were fed 4 total mixed rations with roughage levels of 40%, 50%, 60%, and 70% (dry matter basis). A total of 4 periods of animal experiments were conducted, with each period lasting 21 d. The results showed that dietary roughage level had significant (P<0.05) or highly significant (P<0.01) effects on the sorting behavior of dairy cows. When dietary roughage level was 40%, cows preferred feed particles 4 mm and avoided particles <4 mm; however, when dietary roughage level >40%, cows preferred feed particles <4 mm and avoided particles 4 mm. Dietary roughage level had no significant effect on particle size distribution in each layer of rumen contents (P>0.05). With increasing dietary roughage level, rumen liquid outflow rate and rumen solid outflow rate increased significantly in a linear manner (P<0.05), while rumen contents weight and rumen liquid volume showed no significant changes (P>0.05); serum insulin-like growth factor-1 (IGF-1) content increased significantly in a linear manner (P<0.01), leptin (LEP) and non-esterified fatty acid (NEFA) contents showed a linear increasing trend (0.05 P<0.10), and serum growth hormone (GH) and insulin (INS) contents showed a linear decreasing trend (0.05 P<0.10). In conclusion, dietary roughage level is an important factor affecting sorting behavior, rumen contents, and serum indices of dairy cows.

Full Text

Effects of Forage Level in Total Mixed Ration on Sorting Behavior, Rumen Content and Serum Indicators of Dairy Cows

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Abstract

This experiment was conducted to investigate the effects of dietary forage level in total mixed ration (TMR) on sorting behavior, rumen environment, and serum indicators of dairy cows. Twelve multiparous Holstein cows in mid-lactation [days in milk (119±26) d] with body weight (629±46) kg were randomly assigned to 4 groups, with one cow in each group fitted with a rumen cannula. Following a 4×4 Latin square design, the cows were fed four TMR diets with forage levels of 40%, 50%, 60%, and 70% (dry matter basis). The experiment consisted of 4 periods, each lasting 21 days. The results showed that dietary forage level had significant ($P<0.05$) or highly significant ($P<0.01$) effects on sorting behavior. When dietary forage level was 40%, cows preferred particles 4 mm and discriminated against particles <4 mm. However, when dietary forage level exceeded 40%, cows preferred particles <4 mm and discriminated against particles 4 mm. Dietary forage level had no significant effect on particle size distribution in different layers of rumen content ($P>0.05$). With increasing dietary forage level, rumen liquid outflow velocity and rumen solid outflow velocity increased linearly ($P<0.05$), while rumen content weight and rumen liquid volume remained unchanged ($P>0.05$). Serum insulin-like growth factor-1 (IGF-1) content increased linearly ($P<0.01$), serum leptin (LEP) and non-esterified fatty acid (NEFA) contents tended to increase (0.05 $P<0.10$), and serum growth hormone (GH) and insulin (INS) contents tended to decrease (0.05 $P<0.10$). In conclusion, dietary forage level is an important factor affecting sorting behavior, rumen content, and serum indicators in dairy cows.

Keywords: dairy cow; dietary forage level; sorting behavior; rumen content; serum indicators

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Total mixed ration (TMR) feeding technology has been widely applied in large and medium-sized dairy farms in China. One of its significant advantages is effectively reducing sorting behavior inherent in traditional feeding methods, thereby providing stable and balanced nutrients for rumen microorganisms, optimizing rumen function, and improving nutrient utilization efficiency [1]. However, some studies have found that dairy cows still exhibit sorting behavior for short-particle diets during TMR feeding [2-4]. Li et al. [1] analyzed the causes of nutrient intake deviation in 30 lactating herds and found that sorting behavior was one of the important factors causing nutrient intake deviation. Sorting behavior can easily lead to disorderly rumen fermentation patterns, reduce production performance [5], cause cows to consume excessive concentrate and insufficient effective fiber, and increase the risk of subacute ruminal acidosis (SARA) [6-7].

Currently, most studies have investigated the effects of TMR on sorting behavior by altering forage sources and particle size. Leonardi et al. [2] found that long-particle TMR increased sorting behavior, while forage source had no significant effect. Other related studies have reported similar results [2,4,8-11], but Bhandari et al. [12] suggested that TMR particle size had no significant effect on sorting behavior. In contrast, research on the effects of forage level in TMR on sorting behavior is limited. Devries et al. [13] found that cows sorted for short particles when fed both low (50.7%) and high (62.3%) forage level diets, with more severe sorting behavior observed on the low-forage diet. Since dietary concentrate-to-forage ratios vary depending on milk yield or lactation stage, it is necessary to explore the influence of forage level on sorting behavior. This study investigated the effects of feeding dairy cows TMR with different forage levels on sorting behavior, rumen content, and serum indicators to provide references for reducing sorting behavior in production practice.

1.1 Experimental Animals

Twelve multiparous Holstein cows (four fitted with rumen cannulas) with average body weight (629±46) kg and lactation days (119±26) d were used. Cows were housed individually and fed at 05:30 and 17:30 daily, with residual feed controlled within 10% (fresh weight basis). Cows were milked at 05:00 and 17:00 daily and had free access to water.

1.2 Experimental Diets

The experimental diets were TMR formulated according to the Chinese Feeding Standard of Dairy Cattle [14]. The four dietary groups had forage levels of 40%, 50%, 60%, and 70%. Salt licks were placed in each stall to ensure mineral requirements were met. Diet composition and nutrient levels are shown in Table 1 .

1.3 Experimental Design

The 12 cows were randomly divided into 4 groups with 3 cows per group, ensuring each group contained one cannulated cow. A 4×4 replicated Latin square design was employed with 4 experimental periods, each lasting 21 days (13-day adaptation period and 8-day sampling period).

1.4 Measurements and Methods

1.4.1 Routine Nutrient Analysis Crude protein (CP) content was determined by the Kjeldahl method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analyzed according to Van Soest et al. [15]. Dry matter (DM) and ash contents were determined following methods in "Feed Analysis and Feed Quality Detection Technology" edited by Zhang Liying [16]. Gross energy (GE) was measured using an automatic oxygen bomb calorimeter (Parr 6200, USA). Starch content was determined according to GB/T 20194-2006.

1.4.2 Sorting Behavior On day 14 of each period, diet and residual feed samples were collected at 0, 4, 8, and 12 h after morning feeding. Particle size distribution was determined using a 4-layer (19, 8, 4, and <4 mm) Penn State Particle Separator (PSPS, Pennsylvania State University). Dry matter was determined at 105°C to calculate sorting behavior.

Sorting behavior for each layer = actual intake (kg/d) / predicted intake (kg/d) [17];
Predicted intake (kg/d) = [feed offered (kg/d) × diet proportion (%)] / [residual feed (kg/d) × residual proportion (%)] [2].

1.4.3 Rumen Liquid and Solid Passage Rates On day 14 of each period, chromium-labeled Chinese wild-rye hay was prepared using sodium dichromate and ascorbic acid according to Feng Yanglian [18]. Before morning feeding, 300 g of labeled hay was placed into each cannulated cow. Fecal samples were collected rectally at 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 60, 72, 84, 96, 108, and 120 h after feeding, dried at 65°C, and ground through a 1.0 mm sieve. Chromium trioxide (Cr O) content was determined spectrophotometrically according to GB/T 13088-2006. The fecal sample with maximum Cr O content was set as the t=0 sample with content C . Subsequent time points (t) and

Cr O contents were fitted using the equation (Ct = C e^(-kt)) by least squares to determine the solid passage rate constant k.

On day 15 of each period, cobalt-ethylenediaminetetraacetic acid (Co-EDTA) was used as a marker. Before morning feeding, 150 mL of solution containing 18 g Co-EDTA was infused into the rumen of each cannulated cow at multiple sites. Rumen fluid samples (50 mL) were collected at 0, 2, 4, 6, 8, 10, 12, 14, 16, 24, and 30 h after infusion and stored at -20°C. After thawing at 4°C, samples were centrifuged at 10,000 × g for 15 min, and 10 mL of supernatant was collected. Cobalt (Co) content was determined by flame atomic absorption spectrometry according to GB/T 13884-2003. The 0 h rumen fluid Co content served as blank control. Subsequent time points (t) and natural logarithms of Co content [ln(Ct)] were fitted using the equation [ln(Ct) = kt + a] by linear regression. The slope k represented rumen liquid dilution rate (%/h). Rumen liquid outflow velocity (L/h) = rumen liquid dilution rate (%/h) × rumen liquid volume (L).

1.4.4 Fecal Particle Size On days 16-17 of each period, fresh fecal samples were collected and analyzed for particle size using a fecal analysis sieve (Nasco, USA).

1.4.5 Serum Indicators On day 20 of each period, blood samples were collected from the jugular vein before morning feeding, water-bathed at 37°C for 60 min, and centrifuged at 2,720 × g for 15 min to separate serum. -hydroxybutyrate (-OHB) was measured using a kit from Nanjing Jiancheng Bioengineering Institute. Insulin (INS), growth hormone (GH), glucagon (GCG), and insulin-like growth factor-1 (IGF-1) were measured using kits from Tianjin Jiuding Biological Products Co., Ltd. Leptin (LEP), glucagon-like peptide-1 (GLP-1), and 5-hydroxytryptamine (5-HT) were measured using kits from Shanghai Xinran Reagent Company. All indicators were determined by enzyme-linked immunosorbent assay (ELISA) using a microplate reader (ELx800, BioTek, USA). Non-esterified fatty acid (NEFA) content was measured using a kit from Nanjing Jiancheng Bioengineering Institute by colorimetry with a visible spectrophotometer (UV757CRT/PC, Shanghai Precision Scientific Instrument Co., Ltd.).

1.4.6 Rumen Content Composition and Particle Size On day 21 of each period, all rumen contents were removed, placed in insulated square plastic buckets, mixed thoroughly, weighed, and sampled. One portion was used for particle size determination by PSPS, and another was dried at 65°C for nutrient analysis.

1.5 Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.1 software. All data are presented as least squares means. Linear, quadratic, and cubic responses to forage level were tested using CONTRAST statements. $P<0.05$ was considered significant, and 0.05 $P<0.10$ was considered a tendency.

2.1 Effects of Dietary Forage Level on Sorting Behavior

As shown in Table 2 , when dietary forage level was 40%, cows preferred particles 4 mm and discriminated against particles <4 mm. When dietary forage level was 50%, 60%, and 70%, cows discriminated against particles 4 mm and preferred particles <4 mm. Furthermore, with increasing dietary forage level, the degree of discrimination against particles 4 mm and preference for particles <4 mm increased linearly ($P<0.05$). As time after feeding increased, sorting degree for each particle size gradually decreased.

2.2 Residual Feed Particle Size Distribution

As shown in Figure 1 [Figure 1: see original paper], the effects of dietary forage level on residual feed particle size distribution at each time point mainly occurred in the <4 mm and 19 mm layers. As time after feeding increased, the proportion in the 19 mm layer gradually increased, while the proportion in the <4 mm layer gradually decreased.

2.3 Effects of Dietary Forage Level on Rumen Content Nutrient Content and Particle Size Distribution

As shown in Table 3 , dietary forage level had no significant effect on particle size distribution in different layers of rumen content ($P>0.05$). With increasing dietary forage level, ADF content in rumen content increased linearly ($P=0.0163$), CP content decreased linearly ($P=0.0036$), while NDF and OM contents showed no significant changes ($P>0.05$).

2.4 Effects of Dietary Forage Level on Rumen Solid and Liquid Passage Rates

As shown in Table 4 , with increasing dietary forage level, rumen solid outflow velocity, rumen liquid outflow velocity, and rumen liquid dilution rate increased linearly ($P<0.05$), while rumen content weight and rumen liquid volume showed no significant changes ($P>0.05$).

2.5 Effects of Dietary Forage Level on Fecal Particle Size Distribution

As shown in Table 5 , dietary forage level had no significant effect on particle size distribution in different layers of feces ($P>0.05$).

2.6 Effects of Dietary Forage Level on Serum Indicators

As shown in Table 6 , with increasing dietary forage level, serum IGF-1 content increased linearly ($P=0.0019$), while serum 5-HT, -OHB, GLP-1, and GCG contents showed no significant changes ($P>0.05$). Serum LEP and NEFA contents tended to increase (P=0.0604 and P=0.0800, respectively), while serum GH and INS contents tended to decrease (P=0.0743 and P=0.0964, respectively).

3.1 Effects of Dietary Forage Level on Sorting Behavior

Sorting behavior of dairy cows fed TMR is a common concern in production practice. Since dietary concentrate-to-forage ratios vary during the lactation cycle, this study investigated the effects of dietary forage level on sorting behavior. We found that cows exhibited varying degrees of sorting behavior across different forage levels. When dietary forage level was 50%-70%, cows preferred particles <4 mm and discriminated against particles 4 mm, consistent with results from Maulfair et al. [17], Devries et al. [3], Leonardi et al. [2], and Leonardi et al. [10]. Since particles 4 mm were primarily forage and particles <4 mm were primarily concentrate, these results indicate that cows have a natural preference for concentrate and aversion to forage. Furthermore, when dietary forage level was 50%-70%, the degree of preference for concentrate increased linearly with forage level, while Devries et al. [3] found greater concentrate sorting at lower forage levels. This discrepancy may be related to different forage sources used between studies. Our experiment used whole-plant corn silage, alfalfa, oat hay, and Chinese wild-rye hay, whereas Devries et al. [3] used corn silage and grass silage. Additionally, Leonardi et al. [19] found that feed delivery amount was an important factor affecting sorting degree, while our study strictly controlled residual feed within 10% of fresh weight. Leonardi et al. [20] found that adding water to diets with high DM content (80%) to increase moisture significantly reduced sorting behavior. Notably, after adding water, the diet DM content remained as high as 64%, significantly higher than the average DM content in our study (42.8%). Moreover, DM contents were relatively uniform across diets in our experiment, suggesting that diet DM may not be the main factor affecting sorting behavior in this study. Therefore, future research should investigate how dietary DM level influences sorting behavior. In contrast to results at 50%-70% forage level, cows preferred particles 4 mm and discriminated against particles <4 mm when dietary forage level was 40%. This may be related to SARA occurrence when cows were fed the 40% forage diet. To alleviate low rumen pH and maintain rumen environment stability, cows may have selectively consumed long-particle diets rich in forage. Although the critical rumen pH threshold for SARA remains controversial, rumen pH below 5.60-5.80 is commonly used as an indicator of SARA [21]. In our experiment, when dietary forage level was 40%, rumen pH was below 5.80 for 7.9 h/d, significantly higher than other groups; detailed data have been published [24].

3.2 Effects of Dietary Forage Level on Residual Feed Particle Size Distribution

The effects of dietary forage level on residual feed particle size distribution at different time points can directly reflect sorting behavior [17]. After feeding, particle size changes in residual feed mainly occurred in the 19 mm and <4 mm layers. As time after feeding increased, the proportion of particles 19 mm gradually increased, while the proportion of particles <4 mm significantly decreased. This result visually demonstrates that cows preferred particles <4 mm and discriminated against particles 19 mm, leading to increased residual proportion at 19 mm and decreased residual proportion at <4 mm. However, using residual feed particle size distribution as a direct indicator of sorting behavior has limitations. For example, at 40% forage level, as time after feeding increased, the proportion of residual particles 19 mm gradually increased, while the proportion of particles <4 mm significantly decreased. This contradicts the result that cows preferred particles 4 mm and discriminated against particles <4 mm. This contradiction may be related to the influence of residual feed amount on sorting behavior calculations. When residual feed amount is very small, even large changes in particle size distribution have minimal impact on sorting behavior values. Therefore, using the ratio of actual intake to predicted intake as an indicator of sorting behavior is more accurate.

3.3 Effects of Dietary Forage Level on Rumen Content Nutrient Content, Particle Size, and Fecal Particle Size

Research on the effects of forage level on rumen content nutrient content, particle size, and fecal particle size is limited. In this experiment, the lack of significant differences in rumen content particle size across the four forage levels may be related to sampling time. Rumen content samples were collected before morning feeding on day 21 of each period, and rumen contents may have been sufficiently reduced in size by rumination activity. Regarding rumen content nutrient content, ADF content increased linearly while CP content decreased linearly with increasing forage level, whereas NDF content showed no significant differences. These results may be related to the linear increase in ADF intake, linear decrease in CP intake, and lack of significant difference in NDF intake [22]. Furthermore, in this experiment, fecal particle size distribution in different layers was not affected by dietary forage level, a result that requires further verification in future studies.

3.4 Effects of Dietary Forage Level on Rumen Solid and Liquid Passage Rates

Cassida et al. [23] found that saliva secretion rate was the decisive factor affecting rumen liquid outflow velocity. In our experiment, rumen liquid outflow velocity increased linearly with dietary forage level, although daily saliva secretion only increased numerically [22], possibly due to different measurement methods for saliva secretion between studies. In our experiment, rumen content weight and rumen liquid volume showed quadratic trends with increasing dietary forage level, whereas Maekawa et al. [24] found linear increasing trends. Additionally, rumen solid outflow velocity increased linearly with dietary forage level, which is inconsistent with Zebeli et al. [25] who suggested that forage helps increase rumen mat thickness and consistency, thereby increasing solid retention time. Therefore, the effects of dietary forage level on rumen solid and liquid passage rates may be influenced by measurement methods and animal factors, requiring further clarification in future research.

3.5 Effects of Dietary Forage Level on Serum Indicators

GH can enhance animal appetite and regulate energy balance [26]. In our experiment, the linear decreasing trend in GH content with increasing dietary forage level was consistent with the significant linear decrease in feed intake [22]. Leptin, as a product of the LEP gene, plays an important regulatory role in food intake and energy balance [27]. Morton et al. [28] used mice to study the effects of leptin on short-term feed intake and satiety signal sensing, confirming that leptin is an important regulator of feed intake that can inhibit feeding by suppressing hypothalamic neuropeptide Y (NPY) expression. In our experiment, the linear increasing trend in serum leptin content accompanied by a significant linear decrease in feed intake further confirms that leptin is a factor that inhibits feed intake. Additionally, in our experiment, serum IGF-1 content increased linearly while serum INS content tended to decrease with increasing dietary forage level. Relling et al. [29] also observed increased serum IGF-1 content and decreased INS content accompanied by decreased feed intake. These results indicate that IGF-1 and INS are also important regulators of feed intake. Correspondingly, changes in feed intake also affect serum indicators. With increasing dietary forage level, feed intake decreased linearly, reducing fermentable organic matter in the rumen and decreasing ruminal propionate concentration [22], which in turn reduced hepatic gluconeogenesis and serum INS content [30].

4 Conclusions

1. When dietary forage level was 40%, cows preferred particles 4 mm and discriminated against particles <4 mm. However, when dietary forage level was 50%-70%, cows discriminated against particles 4 mm and preferred particles <4 mm.
2. Dietary forage level did not affect rumen content particle size, weight, or volume, but significantly affected rumen liquid and solid outflow velocities.

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Note: Figure translations are in progress. See original paper for figures.