Effect of the Initial Time of Providing Oat Hay on Performance, Health, Behavior and Rumen Fermentation in Holstein Female Calves

Tianyu Chen †, Jianxin Xiao †, Tingting Li, Jing Ma, Gibson Maswayi Alugongo ‡, Muhammad Zahoor Khan ‡, Shuai Liu §, Wei Wang, Yajing Wang, Shengli Li and Zhijun Cao ★

State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China; chentianyu@cau.edu.cn (T.C.); xiaojinxian-dairy@cau.edu.cn (J.X.); litingting@newhope.cn (T.L.); x20193040572@cau.edu.cn (J.M.); lb20163040001@cau.edu.cn (G.M.A.); zahooorcau@cau.edu.cn (M.Z.K.); liushuaicau@cau.edu.cn (S.L.); wei.wang@cau.edu.cn (W.W.); yajingwang@cau.edu.cn (Y.W.); lishengli@cau.edu.cn (S.L.)

* Correspondence: caozhijun@cau.edu.cn; Tel.: +86-010-6273-3746
† These authors contributed to the work equally.

Abstract: For determining the appropriate time of feeding hay, 210 healthy Holstein calves at day two were randomly divided into three groups: basic diet (calf starter) without hay (CON), and the inclusion of oat hay from the second week (H2) or fourth week (H4) with basic diet. Calves were weaned on day 56 and raised until day 70. Calf starter intake and fecal scores were recorded daily. The body weight, body size, and rumen fluid samples were collected every two weeks before and once a week after weaning. Compared to the basic diet group, the calves that were receiving oat hay from the second week had the highest starter intake (1086.1 g vs. 925.6 g; p < 0.05), body weight (68.4 kg vs. 63.0 kg; p < 0.01) and average daily gain (0.84 kg/d vs. 0.73 kg/d; p < 0.01) throughout the trial period. Compared to H4, calves received oat hay from the second week reduced the frequency (1.48% vs. 3.57%; p < 0.05) and duration of diarrhea (0.21 days vs. 0.50 days; p < 0.05) during post-weaning. Compared to the CON calves, the inclusion of oat hay from the second week increased the ruminal pH (6.38 vs. 6.19; p < 0.01) during the entire trial and increased the acetate (49.07% vs. 44.44%; p < 0.05) during post-weaning. Compared to the basic diet group, calves in H2 treatment spent more time in rumination (275.2 min/day vs. 133.3 min/day; p < 0.01) but less time in abnormal behaviors (80.5 min/day vs. 207.0 min/day; p < 0.01). In conclusion, calves supplemented with oat hay had an improved growth rate and rumen environment compared to calves fed calf starter only.

Keywords: calf; oat hay; time; growth performance; rumen fermentation; calf diarrhea; calf behavior

1. Introduction

The digestive system of newborn calves differs from that of adult cows in anatomical structure and physiological functions. During the first few weeks of life, differences in feed digestion and metabolism between calves and monogastric animals are minimal. Hence, calves are mainly fed whole milk or milk replacer, most likely increasing the feeding costs during this period [1]. An early transition from milk to solid feeds is more desirable to reduce dependence on milk as the primary source of nutrients. However, this can only be achieved with a well-developed rumen. Both calf starter and hay have been widely investigated to determine their role in rumen development [2,3]. While some studies contend that calves should be fed calf starter only in the pre-weaning period [4,5], other studies have averred the importance of feeding hay [6,7]. Proponents of feeding calf starter only argue that the dietary concentrates produce higher levels of VFA, specifically butyric and propionic acid, which promote rumen epithelium development [8]. Moreover,
feeding hay reduces concentrate intake, which disrupts the development of the rumen epithelium [4]. Nonetheless, several recent studies have shown that feeding forage could positively influence daily calves’ growth and rumen development. Coverdale et al. [6] found that calves fed hay were heavier, with greater body weight gain and improved feed efficiency. Such observations can be linked to a better rumen environment, such as increased ruminal pH [9] rather than greater starter intake. Concentrates have been associated with hyperkeratosis of the ruminal papillae [10] and rapid accumulation of fermentation products in the rumen with a decrease in pH [11]. It is believed that feeding forage to pre-weaning calves encourages rumination while increasing the amount of saliva flow into the rumen [12], resulting in a greater ruminal pH.

The anatomical development of rumen depends on the physical form of diet [11]. Forage inclusion in the diet promotes an increase in rumen volume [13,14] and weight [15], which may further improve the growth and performance of calves. Although it is commonly believed that the introduction of hay can stimulate rumen development, the most appropriate age at which hay should be fed to the pre-weaning calves remains irresolute. In a previous study, calves provided alfalfa hay in starter feed at two weeks of age had improved feed intake, ADG and rumination compared to those fed starting at the fourth or sixth week of life [16]. Supplementation of oat hay from two weeks improved ruminal pH, overall rumen development, and pre-weaning performance compared to those supplemented from week six, which were less responsive to the dietary changes [17]. On the other hand, no differences in ADG, DMI and rumen development were observed in calves fed alfalfa hay or oat hay from either day three or 15 of age [18].

By studying the behavior of animals, farmers can have a clearer grasp of the behavior rules of animals, and it is convenient for a farmer to raise and manage animals [19]. Phillips [20] found that providing hay to calves could reduce the behavior of bedding intake, as well as the licking of the bucket and pen. Feeding hay to calves during lactation can increase rumination time and reduce non-nutritional oral behavior [21,22]. Although many studies have reported that feeding hay can improve the calves’ behavior [17,23], when to feed is still not clear, however. Hosseini et al. [16] added 15% alfalfa hay to calves at two, four, and six weeks of age; however, no differences were found in behavior between different hay introduction times.

To provide an approximate time that calves can begin feeding on hay on dairy farms, we used a large sample size (210 Holstein calves) in this trial. We investigated the effects of supplementing hay at two different ages on dairy calves’ growth performance and health indicators. This study hypothesized that feeding hay to pre-weaning dairy calves does not have a negative effect on their performance and is not dependent on the time hay is introduced during the pre-weaning period.

2. Materials and Methods
2.1. Experimental Design

The study was conducted at Zhong yuan Animal Husbandry Co. Ltd. (Shijiazhuang, Hebei, China) from October 2018 to January 2019. Animal care and use were approved by Ethical Committee of China Agricultural University (Yuanmingyuan Road, Haidian District, Beijing; Case number: Aw10601202-1-2). During the whole trial period, the average temperature in October, November, December, and January was 14.5 °C, 7 °C, 0 °C and −1 °C, respectively. Two hundred and ten healthy Holstein female calves were randomly divided into three groups in a randomized complete block design of 70 calves per group. The selected calves (initial BW = 35.8 ± 2.6 kg; serum total protein ≥ 5.5 g/dL) were assigned to the respective treatments, which included calves fed milk and calf starter only (the control group, CON) and calves fed milk, calf starter and oat hay from either week two (H2) or week four (H4).
2.2. Feeding and Housing

The trial period in the current study lasted from birth to the end of week 10. Day one to day 70 was the entire trial; day one to day 56 was pre-weaning; and day 57 to day 70 was post-weaning. Newborn calves were fed 4 L of colostrum within the 1 h after birth and 2 L 8 h later. All calves were injected with 2 mL of vitamin D/day/calf to promote calcium absorption from day one to three. Pasteurized milk (60 °C, 30 min) was provided to calves twice/day at 7:00 and 14:00 h. Calves were fed 6 L/day/calf from day two to day seven, 8 L/day/calf from day eight to 42, 6 L/day/calf from day 43 to 49, and 4 L/day/calf from day 50 to 56, in equal amounts. The calves were weaned completely off milk on day 57 and remained in their individual hutches (Calves were kept in individual hutches with an extended fenced playing area. The inside dimensions of the hutch were approximately 215 cm long by 220 cm wide by 136 cm high. The fenced area was approximately 160 cm long, 110 cm wide and 120 cm high. The distance between the two hutches was 80 cm) in the open area until the end of the experiment on day 70. Calf starter and water were available ad libitum from day three. Oat hay was provided based on the experimental design from either day 15 (H2) or 29 (H4); due to the spilling of the hay on the ground by most of the calves, we could not accurately determine the daily hay intake; hence this data was not included in the final analysis. Calf starter orts were measured and recorded daily before morning feeding. The same batches of oat hay and calf starter were offered daily throughout the experimental period at 8.00 am. The nutrient composition of the calf starter and oat hay is shown in Table 1.

Table 1. Chemical composition (as % of DM) of starter feed and oat hay.

| Component | Starter (%) | Oat Hay (%) |
|-----------|-------------|-------------|
| DM        | 89.48       | 93.20       |
| CP        | 29.86       | 6.18        |
| EE        | 2.27        | 2.75        |
| Ash       | 7.49        | 4.48        |
| NDF       | 9.50        | 44.14       |
| ADF       | 8.47        | 33.70       |
| Energy, MJ/kg | 3.23 | 3.00 |

1 Nutrients analyzed included dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) as a % of DM. The energy was measured with an Oxygen bomb calorimeter.
2 The Calf starter was provided by Yuanxing Co. Ltd. (Inner Mongolia Autonomous Region, China), and contained corn, soybean meal, cotton meal, barley, stone powder, sodium chloride, vitamins and retinoids. The oat hay was cut at a length of less than 2.5 cm using a stationary mixer (20 m³, Trioliet Co. Ltd., Oldenzaal, The Netherlands).
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2.3. Sample Collection and Analysis

2.3.1. Feed Analysis and Body Measurements

Throughout the study, calf starter intake was calculated daily based on the amount offered and refused by each calf from days three to 70. Representative feed samples of calf starter and oat hay were collected every week for further analysis. The samples were used for the determination of dry matter (DM), crude protein (CP), ether extract (EE), crude Ash (Ash), neutral detergent fiber (NDF) and acid detergent fiber (ADF), following the methods of AOAC [24]. The individual body weight, height (vertical distance from the highest point of calf withers to the ground), length (distance from the anterior end of the scapula to the ischium at same side), heart girth (the horizontal circumference of the calf body at the posterior edge of the scapula) and circumference of the cannon bone of the leg (circumference of the upper 1/3 of the metacarpal of the forelimb) were measured before morning feeding and abdominal girth (the vertical circumference of the thickest part of the abdomen) was measured after morning feeding on day one (week one), 14 (week two), 28 (week four), 42 (week six), 56 (week eight), 63 (week 9) and 70 (week 10).
2.3.2. Collection and Analysis of Rumen Fluid Samples

Fourteen (14) healthy calves were randomly selected from each group for rumen fluid sampling on days 14, 28, 56, 63 and 70. Rumen fluid was collected 3 h after morning feeding using an esophageal tube (2 mm wall thickness, 6 mm internal diameter; Anscitech Co. Ltd., Wuhan, Hubei, China). The first 20 mL of rumen fluid was discarded to avoid saliva contamination. The rumen fluid was filtered through four layers of gauze and then divided into two 15 mL centrifuge tubes. The pH value of the rumen fluid was measured immediately with a pH meter (HORIBA Advanced Techno Co. Ltd., Osaka, Japan). The tubes were then stored at \(-20^\circ\text{C}\) for further determination of VFA and NH\(_3\)-N concentration using gas chromatography [25] and the phenol-sodium hypochlorite colorimetric method [26], respectively.

2.3.3. Evaluation of Calf Health

Fecal scores were evaluated and recorded daily based on a standard scoring system [27]. Fecal consistency scoring was done using a scale of 1 to 4. When the calf fecal score was >2, calves were considered to be diarrheic. Specifically, feces was scored as 1 when calves had firm but not hard feces; 2 when feces did not hold form and spread slightly; 3 when feces spread readily to about 6 mm depth; and 4 when calves had feces that had liquid consistency and splattered. Diarrhea rate and frequency were calculated to reflect the diarrheic status of the calves. Pneumonia was scored based on the scoring system designed by the University of Wisconsin at Madison [28]. Coughing (induced or spontaneous, 2 points), nasal discharge (any discharge, 3 points), ocular discharge (any discharge, 2 points), ear and head carriage (ear droop or head tilt, 5 points), and respiratory quality (abnormal respiration, 2 points). Pneumonia was diagnosed if the calves’ total score was \(\geq 4\).

2.3.4. Calf Behavior

The definition of the calf behaviors has been summarized in Table 2. Fourteen calves were selected from each group to observe their behavioral activity on days 57, 63 and 70 of the experiment. The calf behavior was recorded using a video camera (Hikvision Digital Technology Co., Ltd., Hangzhou, China). The time sampling method reported by previous studies [29,30] was used to record the duration of different calf behaviors including standing, lying, eating starter, eating hay, drinking, walking, rumination, abnormal behavior, self-grooming and head out of the pen. For the time sampling, only a portion of the total behavioral observation time was recorded. The duration of behaviors within the first 20 min of each hour was recorded, and the daily average was then multiplied by three. These data were then correlated to the averages of the continuous 1-h sampling. A total of 24 1-h samples were added up to represent the duration of each behavior throughout the day.

| Behavior 1 | Definition of the Behavior |
|------------|-----------------------------|
| Standing   | Four hooves on the ground, whether moving or not |
| Lying      | Lying on the sternum with head held in a raised position or down |
| Eating starter | Head in starter feed bucket accompanied by chewing movements |
| Eating Hay | Head in hay feed bucket accompanied by chewing movements |
| Drinking  | Mouth around drinker |
| Walking    | Stepping and moving |
| Chewing and Ruminating | Chewing irregularly and repeatedly without food in the mouth |
| Abnormal Behavior 2 | Calf licked any surface like fences, floors, windshields |
| Self-Grooming | Calf licked itself with its tongue |
| Head out of Pen 3 | Calf head out of the pen to look around and does not engage in any feeding activities |

1 Adapted from [25] unless otherwise stated. 2 Adapted from [26]. 3 Adapted from [27].
2.3.5. Statistical Analysis

All raw data was entered into an EXCEL sheet. The calf was the experimental unit. Data for BW, structural measurements, ruminal pH, NH₃-N, and VFA concentration at day 1, 14, 28, 42, 56, 63, 70 were analyzed separately by periods (pre-weaning, day 1–56; post-weaning, day 57–70). Data for ADG and starter intake at week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were analyzed separately by periods (pre-weaning, week 1–8; post-weaning, week 9–10). Data for calf behavior were recorded for each calf by day (day 57, day 63, day 70) before analysis. All the data were analyzed by a mixed model (PROC MIXED, version 9.2; SAS Institute, Inc., Cary, NC, USA) with time as a repeated measure. The model included the fixed effects of treatment, time (day or week) and their interactions (treatment × time), and calf as random effect. The data of BW, structural measurements, ruminal pH, NH₃-N, VFA concentration, ADG and starter intake for the entire trial (day 1–70 or week 1–10) used the mixed model with fixed effect of treatment, period (pre-weaning, post-weaning), and their interactions (treatment × period) and calf as random effect. Fecal scores for each calf were recorded daily and used to calculate diarrhea frequency and diarrhea days. Data on diarrhea frequency and diarrhea days were analyzed using a GLIMMIX procedure in SAS (version 9.2, SAS Institute Inc., Cary, NC, USA) with fixed effect of treatment, time (day or week), their interactions (treatment × time), and the random effect of calf [31]. Data on pneumonia occurrence were analyzed using a chi-square test model (PROC FREQ, version 9.2; SAS Institute, Inc., Cary, NC, USA). p < 0.05 showed significant differences, p < 0.01 showed highly significant differences, while trends were indicated as 0.05 < p ≤ 0.10.

3. Results
3.1. BW, ADG and Starter Intake

Table 3 and Figures 1–3 show the effect of oat hay supplementation on BW, ADG and starter intake. Hay supplementation on week two and week four resulted in heavier calves during pre-weaning (p < 0.01) and the entire trial period (p < 0.01) compared with CON. The treatment groups (H2 vs. H4) did not differ in the BW either during the pre-weaning or the entire trial period. However, calves introduced to hay at two weeks tended to be heavier during the post-weaning period (p < 0.01) than CON and H4, probably as a result of greater BW beginning on day 42, up until the end of the experiment (p < 0.05, Figure 1).

Figure 1. Mean BW for Holstein female calves fed a basic diet without (CON: solid circles; n = 70) or with hay inclusion from the second (H2; Solid squares; n = 70) or fourth (H4; Solid triangles; n = 70) week of age. Differences between CON and hay groups are represented by an asterisk (p < 0.05, denoted by **). Differences between H2 and H4 are represented by a cross (p < 0.05, denoted by †).
Table 3. Effects of hay supplementation from either week two or four on BW (body weight), ADG (average daily gain) and starter intake in dairy calves during different periods \(^1\) (CON: n = 70; H2: n = 70; H4: n = 70).

| Items                  | Treat | Time 4 | T * t | Period 5 | T * p |
|------------------------|-------|--------|-------|----------|-------|
| Initial BW (kg)        | CON   | H2     | H4    | SEM      |       |
| BW (kg)                |       |        |       | 0.33     |       |
| Pre-weaning ADG (kg/d) |       |        |       |          |       |
| Post-weaning ADG (kg/d)|       |        |       |          |       |
| Entire trial ADG (kg/d)|       |        |       |          |       |
| Starter intake (g)     |       |        |       |          |       |

\(^{abc}\) Means within a row with different superscripts differ. \(^1\) Pre-weaning: from calf birth to week eight; Post-weaning: from week nine to week 10. Entire trial: from calf birth to week 10. \(^2\) CON = control (basis diet without hay); H2 = inclusion of oat hay from the second week; H4 = inclusion of oat hay from the fourth week. \(^3\) Calf starter was provided by Yuanxing Co. Ltd. (Inner Mongolia Autonomous Region, China), which contained corn, soybean meal, cotton meal, barley, stone powder, sodium chloride, vitamins and retinoids. \(^4\) For all, data were summarized by week. \(^5\) Data were analyzed for the entire trial (pre-weaning, post-weaning) period. * The interaction between treat and time (T * t) or treat and period (T * p).

![Figure 2](image_url)  
**Figure 2.** ADG for Holstein female calves fed a basic diet without (CON: solid circles; n = 70) or with hay inclusion from the second (H2: Solid squares; n = 70) or fourth (H4: Solid triangles; n = 70) week of age. Differences between CON and hay groups are represented by an asterisk (p < 0.05, denoted by **). Differences between H2 and H4 are represented by a cross (p < 0.05, denoted by †).

Similarly, ADG was significantly affected by treatment during the pre-weaning, post-weaning and the entire experimental period (p < 0.01; Table 3). The H2 calves had the greatest ADG, while CON calves had the least ADG from week four to nine (Figure 2). Calves fed hay from week two (H2) had greater ADG during pre-weaning and the entire trial period (p < 0.05) and tended to be greater during post-weaning (p = 0.06) compared to H4 calves. Providing hay at two weeks had an effect on starter intake during the pre-weaning, post-weaning and entire trial periods (p < 0.01). Calves on the H4 and CON diet did not differ in starter intake. However, calves fed H2 had the greatest starter intake compared to H4 and CON calves during the different periods (p < 0.05).
The body structural measurements reported in the current study are summarized in Table 4. Our findings showed that hay supplementation did not affect body height at different periods. Consistently, the body length, heart girth, and circumference of cannon bone were similar among different treatments during the pre-weaning period. However, calves on H2 and H4 groups had greater body length (p < 0.01) during the post-weaning period and the entire trial period. Heart girth had no differences between H2 and H4, but both of them were higher than CON during post-weaning (p < 0.05) and the entire trial period (p < 0.05). Abdominal girth showed greater size (p < 0.01) during the post-weaning period on H2 than CON and H4. Compare to CON and H4, H2 calves had higher (p < 0.01) circumference of cannon bone during post-weaning and the entire trial period.

Table 4. Effects of hay supplementation at different ages on body structural growth in dairy calves during different periods.

| Items                  | Treatment | SEM         | p-Value |
|------------------------|-----------|-------------|---------|
|                        | CON       | H2          | H4      |         |
|                        | Treat     | Time 4      | T * t   | Period 5| T * p   |
| Body height (cm)       | 80.4 b    | 80.2 a      | 80.5 a  | 0.29    | 0.61    | <0.01  | 0.11 <0.01 | - - |
| Pre-weaning            | 88.1 a    | 88.5 a      | 88.4 a  | 0.31    | 0.66    | <0.01  | 0.57 - -   | - - |
| Post-weaning           | 84.4 a    | 84.4 a      | 84.5 a  | 0.29    | 0.87    | - -    | - -       | <0.01 0.68 |
| Entire trial           |           |             |         |         |         |        |           |        |
| Body length (cm)       | 75.0 a    | 75.3 a      | 75.4 a  | 0.30    | 0.57    | <0.01  | <0.05 - - | - - |
| Pre-weaning            | 84.2 b    | 86.1 a      | 85.9 a  | 0.29    | <0.01   | <0.01  | 0.88 - -  | - - |
| Post-weaning           | 79.3 b    | 80.5 a      | 80.6 a  | 0.30    | <0.01   | - -    | <0.01 0.07 | - - |
| Entire trial           |           |             |         |         |         |        |           |        |
| Heart girth (cm)       | 86.5 a    | 86.9 a      | 87.1 a  | 0.32    | 0.32    | <0.01  | 0.06 - -  | - - |
| Pre-weaning            | 100.4 b   | 102.0 a     | 101.3 a | 0.38    | <0.05   | <0.01  | 0.99 - -  | - - |
| Post-weaning           | 92.7 b    | 94.1 a      | 94.2 a  | 0.37    | <0.05   | - -    | <0.01 0.22 | - - |
| Entire trial           |           |             |         |         |         |        |           |        |
| Abdominal girth (cm)   | 92.5 b    | 94.2 a      | 94.1 a  | 0.69    | <0.05   | <0.01  | 0.06 - -  | - - |
| Pre-weaning            | 114.4 b   | 119.0 a     | 117.5 b | 0.64    | <0.01   | <0.01  | 0.60 - -  | - - |
| Post-weaning           | 102.9 b   | 106.3 a     | 105.8 a | 0.54    | <0.01   | - -    | <0.01 0.13 | - - |
| Entire trial           |           |             |         |         |         |        |           |        |
| Circumference of cannon bone (cm) | 10.7 a | 10.8 a | 10.7 a | 0.04 | 0.12 | <0.01 | <0.01 <0.01 | - - |
| Pre-weaning            | 11.5 b    | 11.7 a      | 11.5 b  | 0.05    | <0.01   | <0.01  | 0.72 - -  | - - |
| Post-weaning           | 11.1 b    | 11.3 a      | 11.1 b  | 0.02    | <0.01   | - -    | <0.01 0.27 | - - |

a,b,c Means within a row with different superscripts differ (p < 0.05). 1 Pre-weaning: from calf birth to week eight; Post-weaning: from week nine to week 10. Entire trial: from birth to week 10. 2 CON = control (basis diet without hay); H2 = inclusion of oat hay from the second week; H4 = inclusion of oat hay from the fourth week. 3 For all, data were summarized by day. 4 Data were analyzed for the entire trial (pre-weaning, post-weaning) period. * The interaction between treat and time (T * t) or treat and period (T * p).
3.3. Rumen pH and NH$_3$-N

The effect of hay feeding on rumen pH and NH$_3$-N has been demonstrated in Table 5 and Figures 4 and 5. Both groups of calves fed hay had greater pH during the pre-weaning, post-weaning and entire trial periods compared to CON ($p < 0.05$). However, the time hay was introduced to calves did not result in differences in rumen pH. Calves on CON had a higher NH$_3$-N concentration compared to calves provided hay on week two and week four during the pre-weaning ($p < 0.01$), post-weaning ($p < 0.05$), and entire trial periods ($p < 0.01$). The H2 calves had the lowest NH$_3$-N concentration during the pre-weaning ($p < 0.01$) and entire trial periods ($p < 0.01$).

Table 5. Effects of hay supplementation at different ages on rumen pH and NH$_3$-N in dairy calves during different periods \(^1\) (CON: $n = 14$; H2: $n = 14$; H4: $n = 14$).

| Treatment $^2$ | SEM | Treat | Time $^3$ | T * t | Period $^4$ | T * p |
|---------------|-----|-------|-----------|-------|-------------|-------|
| **pH**        |     |       |           |       |             |       |
| Pre-weaning   | 6.26$^b$ | 6.39$^a$ | 6.49$^a$ | 0.07  | $<0.01$     | $<0.01$ | $<0.01$ | -     | -    |
| Post-weaning  | 6.13$^b$ | 6.37$^a$ | 6.33$^a$ | 0.07  | $<0.01$     | $<0.01$ | 0.57    | 0.98  | -    |
| Entire trial  | 6.19$^b$ | 6.38$^a$ | 6.41$^a$ | 0.04  | $<0.01$     | –       | –       | $<0.01$ | 0.25 |
| **NH$_3$-N (mmol/L)** | | | | | | |
| Pre-weaning   | 17.54$^a$ | 12.23$^c$ | 14.43$^b$ | 0.91  | $<0.01$     | $<0.01$ | $<0.01$ | -     | -    |
| Post-weaning  | 9.02$^a$ | 5.85$^b$ | 6.02$^b$ | 0.52  | $<0.01$     | $<0.01$ | 0.41    | -     | -    |
| Entire trial  | 13.10$^a$ | 8.42$^c$ | 10.07$^b$ | 0.55  | $<0.01$     | –       | –       | $<0.01$ | 0.49 |

\(^{abc}\) Means within a row with different superscripts differ. \(^1\) Pre-weaning: from calf birth to eighth week; Post-weaning: from week nine to week 10. Entire trial: from birth to week 10. \(^2\) CON = control (basis diet without hay); H2 = inclusion of oat hay from the week two; H4 = inclusion of oat hay from week four. \(^3\) For all, data were summarized by day. \(^4\) Data were analyzed for the entire trial (pre-weaning, post-weaning) period. \(*\) The interaction between treat and time (T * t) or treat and period (T * p).

Figure 4. Ruminal pH for Holstein female calves fed a basis diet without (CON: checkered bar; $n = 14$) or with hay inclusion from the second (H2: stripe bar; $n = 14$) or fourth (H4: gray bar; $n = 14$) week of age. Different letters within a time point indicate significant differences between treatments ($p < 0.05$).
Figure 5. Ruminal NH$_3$-N for Holstein female calves fed a basis diet without (CON: checkered bar; n = 14) or with hay inclusion from the second (H2: stripe bar; n = 14) or fourth (H4: gray bar; n = 14) week of age. Different letters within a time point indicate significant differences between treatments (p < 0.05).

3.4. Rumen Volatile Fatty Acids

The effect of hay supplementation on rumen volatile fatty acid at different ages has been summarized in Table 6. Our data reported that acetate was lower (p < 0.05) and propionate (p < 0.01) was higher in the CON group compared to the H2 and H4 groups during post-weaning. Moreover, butyrate tended to be higher in the CON group compared to the H2 and H4 groups during pre-weaning (p = 0.06). No difference was found in valerate between different treatment groups. The total volatile fatty acids (VFA) were higher in the CON (p < 0.05) and the H2 (p < 0.05) group than H4 during pre-weaning. The ratio of acetic acid concentration to propionic acid concentration (C2/C3) was not different throughout the pre-weaning and entire trial periods, but the H2 and H4 groups were significantly (p < 0.05) higher than the CON group during post-weaning.

Table 6. Effects of hay supplementation at different ages on rumen volatile fatty acids in dairy calves during different periods ¹ (CON: n = 14; H2: n = 14; H4: n = 14).

| Items       | Treatment ² | SEM ³ | p-Value ⁴ |
|-------------|-------------|-------|-----------|
|             | CON H2 H4   |       | Treat Time ⁴ T * t Period ⁵ T * p |
| Acetate (%) |             |       |           |
| Pre-weaning | 50.62 51.3 51.94 | 0.012 | 0.77 <0.01 0.13 - - |
| Post-weaning| 44.44 ⁵ b 49.07 a ⁵ 49.1 a ⁵ | 0.014 | <0.05 <0.05 0.57 - - |
| Entire trial| 47.72 50.51 50.6 | 0.009 | 0.26 - - - 0.05 0.70 |
| Propionate (%) |         |       |           |
| Pre-weaning | 26.39 27.19 28.04 | 0.013 | 0.69 <0.01 0.09 - - |
| Post-weaning| 41.29 a ³ 36.48 b ³ 35.84 b ³ | 0.013 | <0.05 <0.65 0.06 - - |
| Entire trial| 34.00 31.88 31.97 | 0.009 | 0.23 - - - <0.01 <0.05 |
| Butyrate (%) |           |       |           |
| Pre-weaning | 13.6 12.69 11.55 | 0.006 | 0.06 <0.01 0.54 - - |
| Post-weaning| 10.42 11.74 12.13 | 0.008 | 0.32 <0.05 0.27 - - |
| Entire trial| 12.38 12.54 11.94 | 0.007 | 0.81 - - - 0.29 0.27 |
| Valerate (%) |           |       |           |
| Pre-weaning | 9.38 8.79 8.38 | 0.007 | 0.61 <0.01 0.26 - - |
| Post-weaning| 3.85 2.7 2.92 | 0.004 | 0.15 <0.01 0.9 - - |
Table 6. Cont.

| Items                  | Treatment | SEM | p-Value   |
|------------------------|-----------|-----|-----------|
|                        | CON       | H2  | H4        |
|                        |            |     |           |
| VFA (mmol/L)           |            |     |           |
| Pre-weaning            | 87.56 a    | 81.81 a | 71.76 b  |
|                        | 3.72      | <0.05 | <0.01 | 0.64 |
| Post-weaning           | 189.03    | 174.62 | 167.22  |
|                        | 17.54     | <0.01 | <0.05 | -   |
| Entire trial           | 137.22    | 126.98 | 120.51  |
|                        | 6.07      | -     | <0.01 | 0.89 |
| C2/C3                  | 2.55      | 2.31 | 2.25     |
|                        | 0.21      | 0.52 | <0.01 | 0.14 |
| Post-weaning           | 1.12 b    | 1.43 a | 1.47 a  |
|                        | 0.15      | <0.05 | 0.29 | 0.2   |
| Entire trial           | 1.82      | 1.87 | 1.86     |
|                        | 0.17      | 0.96 | <0.01 | 0.26 |

a,b Means within a row with different superscripts differ. 1 Pre-weaning: from calf birth to week eight; Post-weaning: from week nine to week 10. Entire trial: from calf birth to week 10. 2 CON = control (basis diet without hay); H2 = inclusion of oat hay from week two. H4 = inclusion of oat hay from week four. 3 C2/C3: the ratio of acetate/propionate. 4 For all, data were summarized by day. 5 Data were analyzed for the entire trial (pre-weaning, post-weaning) period. * The interaction between treat and time (T * t) or treat and period (T * p).

3.5. Calf Health

The effect of hay supplementation on diarrheal frequency and rate in dairy calves during different periods has been summarized in Table 7. Our data revealed that there were no differences for frequency of diarrhea among different treatment groups in the pre-weaning and entire trial periods. However, frequency of diarrhea in the post-weaning period was numerically higher than that in the pre-weaning. Frequency of diarrhea in H2 was lower than H4 in post-weaning (p < 0.05). Likewise, calves provided hay from week two had fewer days with diarrhea than the H4 calves during the post-weaning period (p < 0.05), but no differences from the CON group. No difference in pneumonia occurrence among different treatment groups was found.

Table 7. Effects of hay supplementation at different ages on diarrheal frequency and rate in dairy calves during different periods 1 (CON: n = 70; H2: n = 70; H4: n = 70).

| Items                  | Treatment | SEM | p-Value   |
|------------------------|-----------|-----|-----------|
|                        | CON       | H2  | H4        |
|                        |            |     |           |
| Diarrhea Frequency (%) |            |     |           |
| Pre-weaning            | 7.04      | 7.08 | 7.03     |
|                        | 2.86 a,b   | 1.48 b | 3.57 a  |
| Post-weaning           | 6.20      | 5.96 | 4.64     |
|                        | 3.94      | 3.97 | 3.93     |
| Entire trial           | 4.34      | 4.17 | 4.47     |
|                        | 47.92     | 41.38 | 46.05  |

a,b Means within a row with different superscripts differ. 1 Pre-weaning: from calf birth to week eight; Post-weaning: from week nine to week 10. Entire trial: from calf birth to week 10. 2 CON = control (basis diet without hay); H2 = inclusion of oat hay from week two. H4 = inclusion of oat hay from week four. 3 Diarrhea frequency = ΣIncidence of diarrhea in experimental calves/Calf numbers × days of trial × 100%. 4 Diarrhea days = ΣNumber of days with diarrhea in each calf/Calf numbers × 100%. 5 Pneumonia Occurrence = Number of pneumonia calves/Calf numbers × 100%; since no calves has pneumonia during post-weaning, there is only one period for this indicator.

3.6. Calf Behavior

The effect of hay supplementation at different ages on calves’ behavior during different periods has been summarized in Table 8. Our results demonstrated that calves fed H2 spent less time of standing (p = 0.05) and eating calf starter (p < 0.01) compared to the CON and H4 groups. Moreover, H2 calves devoted less time than H4 calves (p < 0.05) when eating hay. Lying, drinking and walking times were similar between treated groups. More time was spent on rumination by hay groups when compared with the CON group.
Hay-fed calves spent less time ($p < 0.01$) on abnormal behavior, tended to spend more time on self-grooming ($p = 0.07$) and less time with their heads outside the pen ($p < 0.01$) compared to CON group.

### Table 8. Effects of hay supplementation at different ages on behavior in dairy calves (CON: $n = 14$; H2: $n = 14$; H4: $n = 14$).

| Items                        | Treatment | SEM | $p$-Value   | Treat | Time | T * t |
|------------------------------|-----------|-----|-------------|-------|------|-------|
| Standing (min/d)             | CON       | 461.0 a | 0.05        |       |      |       |
|                             | H2        | 407.6 b |            | <0.05 |      |       |
|                             | H4        | 452.3 a | <0.01       |       |      |       |
| Lying (min/d)                | CON       | 877.1  |             | 0.59  |      | <0.05 |
|                             | H2        | 929.3  | <0.01       |       |      |       |
|                             | H4        | 910.2  |             |       |      | <0.05 |
| Eating starter (min/d)       | CON       | 123.4 a | <0.01       |       |      |       |
|                             | H2        | 91.6 b  |             | 0.1   |      |       |
|                             | H4        | 110.1 a |             |       |      | <0.01 |
| Eating Hay (min/d)           | CON       | -      |             | <0.05 |      |       |
|                             | H2        | 94.8 b  | <0.01       |       |      |       |
|                             | H4        | 100.4 a |             |       |      | <0.01 |
| Drinking (min/d)             | CON       | 16.8   |             | 0.86  |      | <0.01 |
|                             | H2        | 15.8   |             |       |      |       |
|                             | H4        | 17.3   | <0.01       |       |      |       |
| Walking (min/d)              | CON       | 20.7   |             | 0.15  |      | <0.01 |
|                             | H2        | 15.0   |             |       |      |       |
|                             | H4        | 15.9   |             |       |      | <0.01 |
| Chewing and Rumination (min/d)| CON    | 133.3 b | <0.01       |       |      | <0.01 |
|                             | H2        | 275.2 a |             |       |      |       |
|                             | H4        | 279.1 a | <0.01       |       |      |       |
| Abnormal Behavior 2 (min/d)  | CON       | 207.0 a | <0.01       |       |      | <0.01 |
|                             | H2        | 80.5 b  |             | 0.37  |      | <0.01 |
|                             | H4        | 69.6 b  |             |       |      |       |
| Self-Grooming 3 (min/d)      | CON       | 13.8   | <0.05       |       |      | <0.05 |
|                             | H2        | 14.8   |             | 0.2   |      |       |
|                             | H4        | 20.5   |             |       |      | 0.12  |
| Head out of Pen (min/d)      | CON       | 180.7 a | <0.01       |       |      | <0.05 |
|                             | H2        | 121.4 b |             |       |      |       |
|                             | H4        | 138.5 b |             |       |      | <0.01 |

*a,b* Means within a row with different superscripts differ. 1 CON = control (basis diet without hay); H2 = inclusion of oat hay from the week two; H4 = inclusion of oat hay from the week four. 2 Abnormal behaviors mainly for non-nutritive oral behavior. 3 Self-Grooming: calf licked itself with its tongue. * The interaction between treat and time (T * t) or treat and period (T * p).

### 4. Discussion

#### 4.1. BW, ADG and Starter Intake

Feed digestion and absorption in pre-weaning dairy calves are not very different from monogastric animals. Calves are mainly dependent on milk or milk replacer during this period. The undeveloped rumen might limit calf starter consumption [4,32], whereas roughages can improve starter intake [33]. Similar to previous studies [16,34], we observed that feeding hay increased calf starter intake. We argue that this increase might be attributed to the higher ruminal pH observed in calves fed hay. Negative effects of low rumen pH on feed intake have been demonstrated in both mature cattle [35,36] and young calves [15,37]. Concomitant with higher starter intake, forage provision improved BW and ADG, especially when introduced at two weeks of age. Compared with CON calves, greater BW and ADG during week six to week nine in H2 and H4 calves may be attributed to improved starter intake, which is in line with findings reported by previous studies [6,15,17].

Feeding forage could improve rumen development, by increasing the muscularis mucosa [13], weight [15] and volume [9,38] of the rumen, which may have subsequently enhanced the feed intake and BW. In the current study, calves with hay inclusion initiated at two weeks of age showed the greatest performance, most likely because of earlier and greater rumen development compared to those not fed or fed forage from four weeks of age. Furthermore, forage inclusion at two weeks of age could improve the rumen development compared to not fed or fed forages from four weeks of age. In contrast to the findings of Lin et al. [17] and Hosseini et al. [16], who likewise investigated the optimal time (0 vs. 2 vs. 4 weeks of age; 0 vs. 2 vs. 4 vs. 6 weeks of age) of dietary forage inclusion and found differences during the pre-weaning period only; we found a greater feed consumption and growth rate in H2 calves not only during the pre-weaning but post-weaning period as well. The low number of calves per treatment in studies by Lin et al. [17] ($n = 6$/treatment) and Hosseini et al. [16] ($n = 10$/treatment) might have contributed to the lack of statistical differences during the post-weaning period. A priori statistical power analysis was performed before the study for starter intake and ADG to ensure the number of animals per treatment was adequate. Based on previously reported values [16] and using $\alpha = 0.05$ and power = 0.80, the projected sample size per group was approximately 47 and 55 experimental units for starter intake and ADG, respectively. Thus, 210 calves ($n = 70$/treatment) were recruited for the current study, which were more
than sufficient to investigate the main objective of current research by increasing statistical power, reducing individual variations and avoiding statistical errors.

4.2. Body Structural Measurements

Body structure can be used to determine the level of growth, development, feeding, and management of dairy calves. Similar to the findings of Hosseini et al. [16], our calves were consistently similar in body structural measurements during the pre-weaning period. However, contrary to their findings, some of the parameters (body length, heart girth, abdominal girth and cannon bone circumference) differed during the post-weaning period. Likewise, Nemati et al. [39] reported that calves fed alfalfa hay had greater heart girth and abdominal girth compared to those not fed. Like the other parameters previously discussed, the H2 treatment had the best growth and body development responses, implying that the time hay is introduced in calves can determine the extent to which calves benefit from feeding on forage. These results need to be interpreted with care because high fiber content and underdeveloped rumen might confound the digestion of hay. Moreover, its bulky characteristics can result in rumen fill and, consequently, a bigger abdominal girth, as observed in our study.

4.3. Rumen Fermentation

The calf starter provides carbohydrates that are fermented by rumen microorganisms to produce VFA. These VFAs, especially propionic and butyric acids, are the primary sources of energy [8]. Upon absorption, a small part of propionic acid is converted into lactic acid and pyruvate in the rumen epithelial cells, while most of it is converted to glucose in the liver, which is the main source of blood glucose in mature ruminants [40]. On the other hand, butyric acid promotes the development of rumen epithelium [41] and increases the surface area of the rumen epithelia in contact with the gut contents [42]. In the present study, calves in the CON group had a greater concentration of VFA compared to the H4 during pre-weaning. These results are in line with findings of Castells et al. [9] who reported higher VFA concentration from a week before weaning (week seven to week eight) in calves fed starter only compared to those fed calf starters and hay. Lin et al. [17] have reported that rumen fermentation capacity gradually increases with age, leading to a concomitant increase in rumen VFA. In agreement with Nemati et al. [39], no significant differences were found in the concentration of VFA during post-weaning in the current study, indicating that early hay inclusion time had no effect on rumen fermentation on post-weaning. However, the effect of forage inclusion on VFA remains controversial. While some researchers have reported a positive effect [9], others showed a negative effect [43]. These divergent observations could be attributed to various factors such as the level of forage supplementation or the forage source and physical form. Different forages have different nutrient profiles. For example, different forages might differ in NDF and indigestible neutral detergent fiber (iNDF) content [44] and their degree of degradation in the rumen [45]. On the other hand, the diet mostly affects rumen microbial composition and population [46], changing the rumen fermentation type. For instance, the CP (15.5% vs. 8.2%) and NDF (43.1% vs. 60.9%) content of alfalfa and grass hay differ, with the former having a higher CP and a lower NDF [45,47], which when fermented result in discrete VFA concentrations. Long oat hay cut is more conducive to a stable rumen environment [12,48]. However, we did not explore the effects of the length of oat hay cut on calves in this study. Further research is needed to investigate the effects of different sources, forms and levels of forage supply on rumen fermentation.

The lower propionate and butyrate concentration observed in H2 and H4 compared to the CON calves was most likely due to the proportion of calf starter in the diet. The greater C2/C3 ratio in hay than CON calves could be ascribed to a lower propionate concentration. Lin et al. [46] found that dietary forage inclusion increased acetate production, possibly because of a greater abundance of cellulolytic bacteria. On the contrary, we observed a similar concentration of acetate between the three groups. Suárez et al. [49] inferred that
acetate concentration might be associated with the roughage to concentrate ratio. However, given the forage spillage and few experimental personnel, we did not record the forage intake nor calculate the forage to concentrate ratio, limiting further interpretation of the results. We encourage future work to assess how the ratio of forage consumption affects rumen fermentation.

Ruminal pH was higher in calves fed hay in addition to the calf starter, consistent with the study of Thomas et al. [50], which is possible because the provision of forage could improve rumination and increase the flow of saliva into the rumen [12]. Those differences persisted throughout the experimental period except for day 14. It is easy to explain the lack of pH differences at day 14 because it was the first time forage was fed to the H2 calves, offered in the morning feeding, while rumen fluid was collected 3 h after morning feeding. Hence calves could not have consumed and digested the forage to evoke changes in the rumen fermentation process. Generally, in ruminants with a developed rumen, it takes 0.5 to 1 h after feed intake to start ruminating [51], resulting in a higher rumen pH. Further works are encouraged to study how long it takes for forage feeding to promote rumen-related movements. A decrease in the rumen pH in calves fed CON compared to those fed hay could be attributed to the increase in lactic acid [8]. The addition of hay can also promote the absorption of VFA in the rumen, as was shown with an increase in the mRNA expression of the VFA transporter gene (MCT 1) [9] in calves fed hay.

The nitrogen cycle occurs in the body, with rumen at the center of the whole process. First, crude protein from the feed is microbially decomposed into ammonia in the rumen, absorbed by the rumen wall and then transported to the liver through the bloodstream to synthesize urea. Most of this urea can then be excreted through the kidneys, while a small amount is returned to the rumen through saliva and blood for re-utilization. However, ammonia accumulates rapidly in the rumen when crude protein degradation exceeds the ability of the microorganisms to utilize the ammonia maximally. Therefore, NH$_3$-N concentration reflects the efficiency of MCP synthesis in the rumen [52]. In our study, calves fed hay might have improved the growth of the important bacteria that utilize ammonia nitrogen [53], leading to decreased rumen ammonia. Moreover, decreased ammonia nitrogen concentration as the calf grows could imply that their rumen microbiota develops and tends to mature rapidly. Our study showed that H2 calves had a higher NH$_3$-N utilization rate than H4 and CON calves, indicating that early roughage feeding promoted the proliferation of nitrogen utilizing microbiota.

4.4. Calf Health

Calf diarrhea is a common gastrointestinal disease on most dairy farms throughout the year. It causes mortality and morbidity that is manifested in compromised growth, development, and survival of calves. In the present study, calves receiving hay at two weeks had lower diarrhea frequency and diarrhea days than the other treatments post-weaning. A previous study found that the relative abundance of Clostridium spp. was higher in calves not fed versus those fed forage [18]. Calves in the H4 group showed higher diarrhea days compared with those in H2 after weaning. The increased self-grooming time in the H4 group might have increased the risk of environmental pathogenic bacteria entering the gut. If the hutch are crowded and have poor ventilation, this will increase the risk of pneumonia [54]. Our hutch were placed in an open area, as it is very effective in the prevention of pneumonia. The reason for the higher occurrence of calf pneumonia (CON: 47.92%; H2: 41.38%; H4: 46.05%) may be the lower temperature during trial (The average temperature in October, November, and December 2018 and January 2019 was 14.5 °C, 7 °C, 0 °C and −1 °C, respectively). Maier et al. [55] also documented higher prevalence of pneumonia in the fall (October–December) compared with spring (April–June). A rapid change in environmental temperature has been found to be an important factor leading to the occurrence of calf pneumonia [56].
4.5. Calf Behavior

Studies on calf behavior can provide a better understanding of their responses to the changes or stimulation from the internal and external environment [57]. Compared with the CON and H4 groups, calves in the H2 group spent less time standing and more time lying down, which might have improved their welfare due to the increased rest time. Although calves in the H2 group spent less time eating starter, they had higher starter intake, implying that their rumen had greater capacity [16]. Hence, these calves might have required more time to digest the large amounts of calf starter consumed in fewer minutes than the other groups. Similar results were reported by Castells et al. [23], who found an increase in lying time was concomitant with increased starter intake. Increase starter intake may enhance the maintenance energy expenditure and heat increment because of increased size of the digestive organs and energy expended within the tissues themselves [58]. Therefore, calves tend to lie down rather than stand up in order to reduce energy consumption. On the other hand, rumination is also an energy-consuming process [59]. Consistently, it has been reported that calves fed hay take more time for rumination [16,17,60], mainly when they are lying. A significant reduction in abnormal behaviors was observed in the present study in the calves fed hay, which are in line with findings documented by Castells et al. [23]. Self-grooming primarily indicates a negative mood and is regarded as one of the most frustrating activities [61], while placing the head outside the pen reflects curiosity and distress in calves [62]. These behaviors indirectly reflect the degree of weaning stress of the calf. Feeding hay can thus reduce the negative effects associated with weaning stress. However, various factors influence weaning stress and the mechanisms that link it to feeding hay and the observed behaviors still need further investigation. Feeding calves hay from two weeks of life can increase rumination time, reduce abnormal behaviors, and improve calf welfare around the weaning period.

5. Conclusions

We concluded that the provision of oat hay to calves improved growth performance and rumen fermentation by increasing body weight, ADG, starter intake, rumen pH value, acetate/propionate ratio and decreased rumen NH₃-N concentration. Calves fed oat hay had greater body length and heart girth compared to the CON group. The abdominal girth and circumference of cannon bone during post-weaning and over the entire trial were found highest in the H2 group. Consistently providing oat hay to calves improved calf welfare as the rumination time increased and the time for abnormal behavior decreased in this experiment.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Ethical Committee of China Agricultural University (protocol code: Aw10601202-1-2; Date of approval: 1 June 2021). Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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