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Effects of Free-Choice Provision of Different Forage Sources in Preweaning Period on Performance and Some Rumen Parameters of Simmental Calves

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ABSTRACT: The objective was to evaluate the effect of free-choice provision of different forage sources [(alfalfa hay (AH) or grass hay (GH)] in preweaning period on performance and rumen fermentation of Simmental female calves. Twenty-one individually housed Simmental calves (46.69 ± 4.14 birth weight, 3 days old) were randomly allocated into 3 treatments of 7 calves each: Control: pelleted starter without forage, GH: pelleted starter + GH and AH: pelleted starter feed + AH. The study continued from 3 days of age to 56 days of age. All calves were fed 2×2.6 L of whole milk (5.2 L/day) until the end of the experiment. Starter and forage were offered ad libitum in different buckets during the experiment. Feed intake was monitored daily, and body weight was measured at the beginning and at the end of the experiment. Ruminal fluid samples were taken from calves on the last day of the experiment. Inclusion of forage in the diet had no significant effect on starter intake, final body weight, body structure, average daily gain (ADG) and feed conversion ratio. Fecal scores were also not affected by the treatments. At the end of the study, calves fed pelleted starter had lower rumen pH than calves fed forage supplemented diets ($P < 0.001$). On the other hand, calves fed with forage-supplemented diets had higher acetate and acetate to propionate ratios than calves fed starter diets alone ($P < 0.01$). Based on the conditions of our study, we conclude that AH and GH intakes at levels 5.74% and 6.60% of total dry matter intake (DMI) improve rumen health without affecting starter feed consumption and animal performance, which may lead to improved performance in the future life of the animal.

Keywords: Different forage source; Growth performance; Preweaning calves; Rumen parameters

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INTRODUCTION

In recent years, regardless of the milk feeding regime, only concentrate supplementation has attracted much attention for rapid development of suckling calves (Ghaffari and Kertz, 2021). However, it has been observed that such practice especially with finely ground or pelleted starter feed, increases the accumulation of fermentation products (Laarman et al., 2012), lowers the pH in the rumen (Laarman and Oba, 2011) and impairs the development of rumen epithelium of calves (Greenwood et al., 1997). To avoid these negative effects, it is recommended to provide forage to suckling calves or to increase the fiber content of starter feed for calves. In addition, it has been found that providing forage rather than increasing the fiber content in a concentrate feed seems to be a better strategy to improve performance and intake of calf starter concentrates (Terré et al., 2013). Research has shown beneficial effects of forage inclusion on growth performance (Chen et al., 2021), feed conversion rates (Coverdale et al. 2004), feeding behavior (EbnAli et al., 2016), rumen epithelial abnormalities (Beiranvand et al., 2014; Mirzaei et al., 2015) and chewing activity and salivary secretion that increase rumen pH (Van Ackeren et al. 2009).

Jami et al. (2013) suggested that forage addition to the diet of very young calves may be beneficial due to fact that fibrolytic rumen bacteria are present in 3 days of age of calves. Jahani-Moghadam et al. (2015) found improvement in the performance of calves when a source of forage was offered to calves younger than 2 weeks of age. Because of the limited digestibility of fibre and its accumulation in the rumen, provision of forage to young calves may negatively affect starter intake (Drackley, 2008) before weaning. However, Castells et al. (2012) showed that calves consuming a small amount of forage, exception of alfalfa hay, resulted in an increase in starter intake and improved average daily gain (ADG). Subsequent studies reported that forage supplementation in the preweaning period did not alter (Castells et al., 2013; Wu et al., 2017; Hill et al., 2019; Horvath and Miller-Cushon, 2019) starter intake and ADG. Various factors, including the source, amount, timing and type of forage provided led to different results (Xiao et al., 2020).

Most previous studies investigating the effects of grass hay (GH) and alfalfa hay (AH) as the main forage source on calf performance during the milk feeding period found that these forages were given as total-mixed rations (TMR) at different times after

birth (Beiranvand et al., 2014; Nemati et al., 2016; Omidi-Mirzaei et al., 2018). However, when forage at 3 days of age was offered ad libitum and separated from the starter feed, it remains unclear whether feeding of GH and AH would result in a better performance and rumen environment. Therefore, the objective of this study was to investigate the effects on calf performance, rumen fermentation parameters and structural growth of GH and AH offered ad libitum and separately from starter feed from 3 days of age. We hypothesized that GH and AH offering by 3 days of age and under the same management conditions would result in better calf performance compared to a starter feed without forage.

MATERIAL AND METHODS

Ethical Statement

This study was conducted with the approval of the Local Ethics Committee for Experimental Animals of Ondokuz Mayıs University dated 16.07.2020 with the number 2020/43.

Animals, Management and Treatments

The experiment was conducted in a commercial dairy farm (Atakum, Samsun, Turkey). Twenty-one female Simmental calves were used for the current experiment. Calves were separated from their mothers soon after birth, weighed and placed in individual calf pens (1.2 × 2.5 m) with sawdust bedding renewed daily. Calves received 2.5 L of colostrum via nipple bottles within 1.5 h of birth and another 2.5 L colostrum within 6 to 12 h after first feeding. Dairy calves (3 days old and 46.69 ± 4.14 kg body weight) were randomly assigned to one of three treatments based on date of birth and live weight (n=7 calves each). The treatments were as follows: Control: pelleted starter feed (contains 31.5 % corn, 15% wheat bran, 11% barley, 10.5% distillers dried grains with solubles (DDGS), 13.25% soybean meal, 5.65% sunflower meal, 5% canola meal, 5.5% molasses, 1.9% limestone, 0.5% salt, 0.1% vitamin and mineral premix and 0.1% toxin binder) without forage, GH: pelleted starter + chopped grass hay (GH; consisted of 70% Gramineae and 3% Leguminosae and 27% other families) and AH: pelleted starter + chopped alfalfa hay (AH). Animals were fed 5.2 L/day of whole milk in two equal portions by nipple bottle daily from day 3 to day 56 at 07:00 a.m. and 05:00 p.m. Starter feed and forage were offered ad libitum in two separate buckets once daily (07:00 h) throughout the study. The amount of feed offered to each calf was adjusted daily

Table 1. Nutrient composition of starter and different sources of forage used in the experimental

Item	Starter	Grass hay	Alfalfa hay
Dry Matter, g/kg of product	900.00	890.70	910.07
Composition			
Crude protein, g/kg of DM	180.44	80.27	160.07
Crude ash, g/kg of DM	40.85	120.37	110.25
Crude fat, g/kg of DM	30.24	10.47	10.51
Acid detergent fibre, g/kg of DM	90.41	380.22	350.81
Neutral detergent fibre, g/kg of DM	210.77	610.24	500.09
Metabolizable energy ^{1,2} (MJ/kg)	11.72	8.99	9.43

¹: Starter metabolizable energy was calculated based on NRC (2001) values for individual feedstuff.

²: Metabolizable energy contents of forages were estimated by using the following regression equations $ME_{ADF} \text{ MJ/kg DM} = 14.70 - 0.15 \times ADF$ (Kirchgessener and Kellner, 1981).

based on achieving a 10% excess oforts over the previous day to provide for ad libitum consumption. The whole milk was supplied from the main milk cooling tanks on the farm, heated to 40-42 °C and served without pasteurization and analyzed regularly. The whole milk contained 3.55% fat, 3.21% crude protein (CP), 5.55% lactose and 12.60% dry matter (DM). The bottles were cleaned and disinfected after each use. Forages were prepared from the same source and chopped before being offered to calves with a forage machine. All calves had ad libitum access to fresh water from a water bucket throughout the study. The nutritient composition of the starter feed, AH and GH is shown in Table 1.

Measurement and Sampling

Individual intake of starter, GH and AH intakes was recorded daily by measuring offers and refusals and calculated based on DM. To measure DM and chemical analyses, at least 8 representative samples of AF and GH (pooled within the study period) were gathered immediately 3 times before the morning feeding over the study period. Animals were weighed on day 3 after birth and then at the end of the experiment (day 56) before the morning feeding using an electronic scale. Average daily gain (ADG) was determined by dividing the difference between the two weights by the experimental period. Feed conversion ratio was calculated by dividing total DMI (milk DM + starter DM + forage DM) of calves by total body weight gain. On the 3rd and 56th day of the experiment, the rump height and withers height of the calves were measured in according to Larson et al. (1997). A nickel-plated brass measuring stick aligned with spirit level was used to determine the rump and withers height.

Rumen fluid (20 mL) was collected via stomach tube 4 hours after morning feeding on day 56 and then

filtered through a 4-ply cheesecloth. Ruminal pH was immediately measured for all samples using a portable pH meter. Approximately 5 mL of filtrate liquid was placed in a centrifuge tube and then 1 mL of 25% metaphosphoric acid was added. After the samples were mixed, they were kept for 30 minutes and centrifuged at 5000 rpm for 10 minutes. Approximately 1.5 mL of the supernatant was collected, transferred to Eppendorf tubes and stored at -18°C for individual VFA analyses.

Fecal scores of calves were determined based on fecal scoring system by inspection at equal intervals each week from the day of entry into the study (day 0) to the day of exit from the study (day 56); ‘‘1 = watery, diarrhea, 2 = soft, amorphous, 3 = soft, semi-formed, 4 = hard, formed and 5 = hard, pelleted’’ (Turkmen, 2015).

Laboratory Analyses

Representative samples of starter, GH and AH were oven dried at 60°C for 48 hours and ground through a 1 mm sieve using a Wiley mill. The samples were analyzed for dry matter (DM), CP ($N \times 6.25$), crude fat (CF) and ash according to AOAC (1990); and neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991). The DM, CP, fat and lactose content of the milk samples were measured using the FOSS Milko Scan FT1 instrument.

For individual volatile fatty acid (VFA) analyses, samples stored in a freezer (-18 °C) were thawed at room temperature before analysis, centrifuged at 13.000 x g for 5 min, and the upper parts were removed using a micropipette and transferred to the sample tubes (vials) of a gas chromatography instrument. Analyses were performed in an Agilent-6890N gas chromatography instrument in according to Filippek and Dvorak (2009).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences program (SPSS, 2007). Normality of data distribution was verified by using the Shapiro-Wilk and Kolmogorov-Smirnov tests, and homogeneity of variance was assessed using Levene's Test. Means were compared by one way analysis of variance (ANOVA) followed by Tukey's test in cases of normal distribution. Where distribution was not normal, non-parametric Kruskal Wallis test was used for comparing means. Results are shown as means \pm standard error of the mean. Significant differences were declared at $P < 0.05$. Mathematical model for statistical analysis: $Y_{ij} = \mu + a_i + e_{ij}$ (Y_{ij} = j observed value of i treatment; μ : population mean; a_i : effect of i treatment; e_{ij} : random error variable).

RESULTS

The composition of the forages and starter feed is presented in Table 1. GH had lower concentrations of CP and energy, higher NDF, and ADF compared with

AH. The values for DMI, BW, ADG and feed conversion rate are shown in Table 2. Total DMI was not affected when forage sources were offered as separate and free choice compared with control calves. There were no significant differences among treatments in terms of overall starter feed intake (Table 2). In the present study, overall forage DMI of calves was similar for calves fed and AH diets. In addition, animals were offered chopped forage ad libitum, and forage intake was 5.74 and 6.60% of total DMI (data not shown in Table 2) for AH and OH calves, respectively. We observed no difference among the treatments for the mean weekly starter intake of calves during the experiment, despite the numerical differences (Fig 1). AH calves had higher forage intake at weeks 2, and 5 ($P < 0.05$) of the experiment than the GH calves ($p < 0.05$, Fig. 2). Initial and final BW, and ADG were not different among dietary treatments. Depending on the similarity of the experimental treatments in terms of total DMI and ADG, feed conversion ratio also was not different among treatments. At the end of the

Table 2. The effect of alfalfa hay or grass supplementation on growth performance and fecal score in calves

	Treatment ¹			SEM	P-value
	Control	GH	AH		
Initial BW, kg	46.91	46.00	47.16	0.97	0.08
Final BW, kg	85.30	88.50	87.16	1.48	0.76
ADG ² , kg/d	0.64	0.70	0.67	0.02	0.47
Intake, kg DM/d					
Starter	0.31	0.37	0.36	0.02	0.61
Forage	-	0.038	0.044	0.003	0.50
TDMI ³ , kg/d	0.97	1.07	1.07	0.02	0.28
Feed conversion ratio ⁴	1.41	1.42	1.52	0.41	0.53
Fecal score ⁵	2.39	2.53	2.44	0.03	0.31
Withers height, cm	83.66	81.66	80.58	0.82	0.29
Rump height, cm	92.25	90.25	91.41	0.79	0.59

¹: Control = diet without forage, GH = diet containing starter and grass hay, and AH = diet containing starter and alfalfa hay; ²: Average daily gain; ³: Total dry matter intake (including milk, starter and forage); ⁴: kg of TDMI / kg of BW gain; ⁵: Fecal score: 1 = watery, diarrhea 2 = soft, amorphous, 3 = soft, semi-formed, 4 = hard, formed and 5 = hard, pellet.

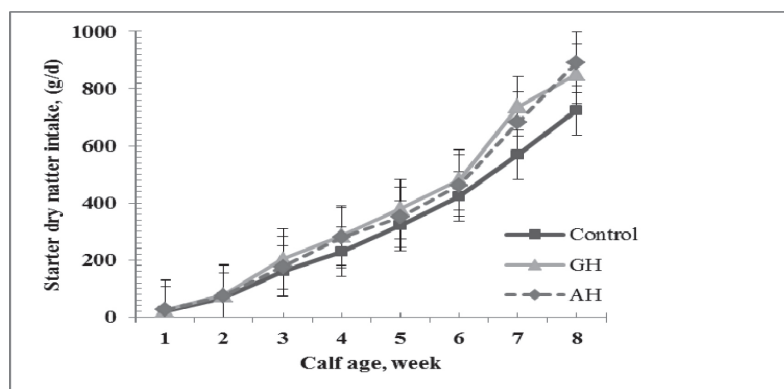


Figure 1. Evolution of starter dry matter intake of calves fed one of the following diets: diet containing starter (Control), diet containing starter and grass hay (GH) and diet containing starter and alfalfa hay (AH) as free during the 8 week of study

study, it was found that the measured rump and withers heights were similar in the control and treatment groups (Table 2). Fecal scores were not affected by the GH and AH (Table 2).

The effects of two forage sources on rumen pH and VFA are shown in Table 3. Calves that had access to chopped GH and AH had higher rumen pH compared to the control group ($P < 0.001$). Compared to the control, calves fed chopped GH and AH had higher acetate ($p < 0.001$) and acetate to propionate ratios ($p < 0.01$). In the current study, the experimental treatments had no effect on propionate and butyrate concentrations.

DISCUSSION

In the feeding of ruminants, the use of forages is obligatory in almost all periods because of their high fiber content for maintaining the health of the digestive system. However, the inclusion of forage in calf diets is controversial because the rumen of calves is not so developed before weaning.

Newborn ruminants are monogastric animals as long as they are fed with milk. The offer of forages has the aim to develop the rumen. This may lead to accumulation of forage in the rumen and subsequently

reduced intake of starter feed (Drackley, 2008). However, in this study, starter feed intake and total DMI were not affected by forage sources in calves fed GH and AH from day 3 to 56 days of age. This may be due to the fact that intake of the studied forages was relatively low (GH and AH at 5.74% and 6.60% of total DMI, respectively) and did not cause any physical restriction in the rumen in regulating starter feed intake. The present results on starter feed intake were in agreement with the findings of Wu et al. (2017) who reported that addition of alfalfa or oat hay to calf diet on 3rd and 15th day of preweaning neither increased nor decreased the starter feed intake. Similarly, (Mirzaei et al. (2015) and Castells et al. (2013) reported that supplementation of forage in preweaning period had no positive effect on starter feed intake and total DMI. However, the present results do not agree with the findings of Gahremani et al. (2021) who indicated that supplementary of wheat hay promotes the consumption of starter feed in pelleted form. Chen et al. (2021) also found that supplementation of forage in preweaning period increased the consumption of starter feed in pellet form. Many other studies (Castells et al., 2015; Nemati et al., 2016; Terré et al., 2015; Lin et al., 2018; Omid-Mirzaei et al., 2018) reported increased intake of starter feed in calves fed forage. The differences between the present results and the previous reports were

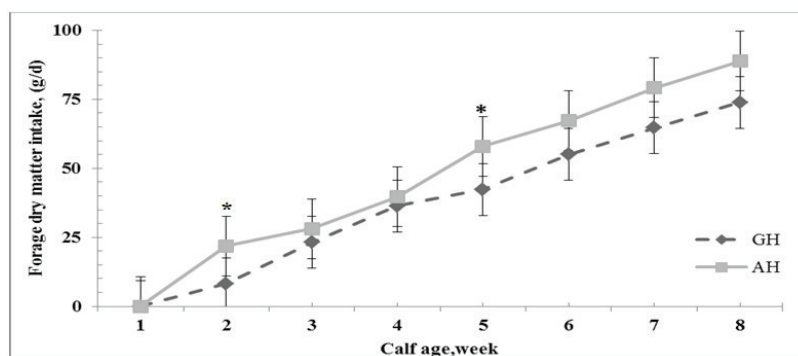


Figure 2. Evolution of forage dry matter intake of calves fed one of the following diets: = diet containing starter and grass hay (GH) and diet containing starter and alfalfa hay (AH) as free during the 8 week of study. For each time point, * denotes significant differences ($P < 0.05$) between treatments

Table 3. The effect of alfalfa hay or grass supplementation on rumen fermentation in calves

	Treatment ¹			SEM	P-value
	Control	GH	AH		
Rumen pH	5.40 ^b	5.96 ^a	6.01 ^a	0.06	<0.001
Individual VFA, mol/100 mol					
Acetate	46.64 ^b	50.79 ^a	52.02 ^a	0.63	<0.001
Propionate	33.87	32.64	32.25	0.50	0.41
Butyrate	14.06	12.74	13.17	0.61	0.68
Acetate:propionate	0.38 ^b	1.56 ^a	1.62 ^a	0.03	0.003

¹: Control = diet without forage, GH = diet containing starter and grass hay, and AH = diet containing starter and alfalfa hay

^{a,b}: Means within a row with different superscripts differ ($P < 0.05$).

mainly attributed to differences in the method and timing of forage supply, chemical properties, variation in forage types and physical form of starter feed (Xiao et al., 2020). For example, in a study comparing the effects of forage offers (fed *ad libitum* and separately) on calf growth performance, no treatment effects were found for starter and total DMI (Gasiorek et al., 2020). In addition, some previous studies reported that AH had a positive effect on starter feed intake when added to finely ground starter feed (Beiranvand et al., 2014; EbnAli et al., 2016). However, a similar effect was not observed with pelleted (Horvath and Miller-Cushon, 2019) or textured (Hill et al., 2019) starter feed. In some studies, AH was recommended as a better forage source for calves due to its high content of CP and palatability during preweaning (Imani et al., 2017). However, in another study, AH was not recommended as a forage source because it was shown to result in lower intake of starter feed (NDF content 17.7%) in calves compared to barley straw, rye-grass hay, oat hay, triticale silage and corn silage (Castells et al., 2012). In addition, it was reported that voluntary consumption of legumes was generally higher than that of wheat (Castells et al., 2012). However, in this study, no significant difference was found between GH and AH intakes (5.74% and 6.60% of total DMI, respectively). This could be due to the similar NDF digestibility of GH and AH. It has been shown in previous studies that increased rumen pH was associated with increased intake of starter feed by calves (Khan et al., 2011; Daneshvar et al., 2015). In present study, although the rumen pH was high in the GH and AH groups, starter feed intake of the calves did not change significantly compared to the control group. This was probably due to the fact that control calves had adapted well to starter feed intake and the risk of acidosis was minimised (Karami et al., 2021).

In this study, forage sources had no significant effect on final BW and ADG, perhaps as a result of similar DMI and milk consumption (Khan et al., 2007). The present results are in line with those of Xiao et al. (2020) and Jahani-Moghadam et al. (2015) who reported that forage supplementation after the third day had no effect on weaning age or final weight. Similarly, Nemati et al. (2016) reported that addition of 0, 12.5 and 25% alfalfa hay to starter feed had no effect on ADG at weaning (51st day). In another study, it was reported that wheat straw supplementation (from day 3) had no significant effect on final BW and ADG of calves (Hosseini et al., 2019). In contrast to present results, significantly higher final BW values were reported when the basal diet was supplemented with wheat straw (Gahremani et al., 2021) and oat hay (Castells et al., 2013; Chen et al., 2021). Moreover,

Castell et al. (2012) reported that calves receiving oat hay at 4% of total DMI consumed more starter feed (1.14 vs. 0.76 kg/d, respectively) and had a greater ADG (0.93 vs. 0.76 kg/d, respectively) compared with AH at 14% of total DMI. On the other hand, there are also studies reporting that ADG decreased due to forage reducing calf starter feed consumption (Drackley, 2008; Hill et al., 2008, 2009; Maktabi et al., 2016). The insignificant results in terms of ADG and final weight were probably due to the insignificant effect of forage sources on starter feed consumption of experimental animals (Omidi-Mirzaei et al., 2018). Moreover, feed conversion ratio, calculated on the basis of consumption and weight gain, were similar among the groups. In addition to similar consumption rates, propionate and butyrate concentrations were also similar in the groups receiving the starter feed with and without forage supplementation. Previous studies have reported propionate and butyrate as the primary source of energy in ruminants (Blottière et al., 2003), so it can be concluded that similar energy was supplied to the animals in the groups.

In this study, rump and withers height were not influenced by forage sources, probably due to similar DMI and milk consumption (Khan et al., 2007). The present results are comply with the results of Beiranvand et al. (2014) and Omidi-Mirzaei et al. (2018) who indicated that the structural growth parameters were not affected by increasing the added forage content in the diet. In contrast to the present findings on body measurements, Hill et al. (2010) reported a linear decrease in body length, hip height or hip width with increasing dietary alfalfa content.

Mean rumen pH in the groups receiving starter feed and GH or AH was within the range recommended by Anderson et al. (1987) during the preweaning period. In the control group, rumen pH decreased in response to the rapidly fast fermentable starch and NFC content of the starter feed. The higher rumen pH observed in calves fed forage were consistent with previous data (Nemati et al., 2016; Pazoki et al., 2017; Lin et al., 2018; Takemura et al., 2019). On the other hand, the present results do not comply with the results of Leão et al. (2020) and Omidi-Mirzaei et al. (2018) who reported similar rumen pH values for calves fed with textured starter supplemented with forage sources of different particle size and with the results of Maktabi et al. (2016) who indicated that different forage sources (AH or beet pulp) had not affect on the mean rumen pH in calves fed ground starter. In the current study, the higher rumen pH in calves fed diets supplemented with GH and AH could be due to greater rumination and eating and/or lower VFA concentration (Kara-

mi et al., 2021). In contrast to Drackley (2008), who claimed that AH tended to alter rumen fluid pH during milk-feeding period compared to the other forage sources, no difference was found between the groups consuming GH and AH in this study. No difference in rumen pH between the AH and GH groups in this study may be due to the similar rates of degradation and passage from the rumen of both forage source.

Feeding diets high in NDF and low in NFC resulted in increase in acetate and decrease in propionate in the rumen (Koch et al., 2017; Soltani et al. 2017). However, the absence of a difference in propionate concentrations between the control and experimental groups at day 56 in this study could be attributed to the similar starter feed intake of calves. On the other hand, a significant increase in acetate concentration was observed at the end of the experiment in the groups with forage intake compared to the control group at, indicating cellulolytic activity in rumen and better fermentation of fibers (Žitnan et al., 1998). This increase in acetate concentration resulted in higher acetate to propionate ratio. In many studies, higher acetate levels were found in the rumen of calves that received calf starter and forage (Castells et al., 2013; Mirzaei et al., 2016; Nemati et al., 2016). However, the effects of forage supplementation on VFA are controversial. Wu et al. (2017) reported that rumen acetate, propionate and, butyrate content and the ratio of acetate to propionate were not affected by the administration of AH at 3 or 15 days of age. Takemura et al. (2019) found that the acetate to propionate ratio in the rumen was higher and the butyrate content was lower in calves consuming forage than in calves consuming starter feed. These differences could be due to the forage source, NDF and OM digestibility, amount, level and particle size of the forage and physicochemical structure of calf starter feed (Khan et al., 2016).

Because rumen fluid and VFA concentrations were only measured in a single time frame (day 56) in this study, the present results do not provide comprehensive information on the effects of GH and AH on rumen pH and rumen fermentation during the suckling period. Therefore, there is a need for studies in which rumen fluid will be collected at different time points to accurately describe the dynamic changes in rumen fermentation during the suckling period.

In the present study, forage supplementations had no significant effect on fecal scores. The present results on fecal scores are in agreement with the findings of Hosseini et al. (2016) and Hosseini et al. (2019) who reported no detectable effect of forage supplementation on fecal scores of calves.

In the current study, the effect on calf growth performance and rumen fermentation of free-choice provision of GH or AH separately from starter feed at 3 days after birth were evaluated. Based on the conditions of our study, we conclude that AH and GH intakes at levels 5.74% and 6.60% of total DMI may improve rumen development without affecting starter feed consumption and animal performance, which may lead to improved performance in the future life of the animal.

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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