Soybean oil supplementation and alfalfa hay inclusion in starter feed of Holstein dairy calves: growth performance, digestibility, ruminal fermentation and urinary purine derivatives

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\textbf{ABSTRACT}

Forty newborn Holstein female calves (BW = 39.9 ± 2.1 kg) were assigned to 1 of 4 treatment groups (each consisting of 10 animals) in a 2 × 2 factorial arrangement of supplemental soybean oil [0 vs. 3% soybean oil (SBO) on dry matter basis] and forage level [0 vs. 15% alfalfa hay (AH) on dry matter basis] to evaluate the interaction effect of supplemental fat and forage feeding level in starter feed of dairy calves. Treatments were: (1) neither SBO supplementation nor AH inclusion (NSBO-NAH); (2) SBO supplementation but no AH included (SBO-NAH); (3) no SBO supplementation but AH included (NSBO-AH); and (4) SBO supplementation with AH (SBO-AH). Calves had \textit{ad-libitum} access to water and starters throughout the study and weaned on day 63 of age but remained in the study until day 73 of age. The results showed that SBO supplementation reduced starter intake, average daily gain (tendency) and faecal consistency compared to un-supplemented diets. The lowest digestibility for neutral detergent fibre and crude protein, as well as the lowest wither height and volatile fatty acid production, were found for SBO-AH diet among experimental treatments. Moreover, the lowest urinary purine derivatives excretion but the highest urinary nitrogen excretion found in SBO-AH diet indicated the lowest nitrogen utilisation efficiency among experimental treatments. In summary, based on the current study condition, because the negative effects of SBO supplementation were exacerbated when AH was incorporated in the starter feed, concurrent feeding of SBO and AH is not recommendable in young calves.

\textbf{HIGHLIGHTS}

- Supplemental soybean oil and alfalfa hay inclusion in starter feed of dairy calves was evaluated.
- Supplementation of SBO reduced crude protein digestibility and ruminal propionate concentration and caused looser faecal consistency.
- The inclusion of AH exacerbated the negative effects of SBO supplementation on ruminal fermentation and microbial activity.
- Concurrent supplementation of SBO and AH inclusion is not recommendable in dairy calves.

\textbf{Introduction}

Grains and fats are the main sources of energy in ruminants’ diets. There are some limitations for using high-grain diets because of acidosis incidence and dairy calves had lower ruminal pH compared to mature ruminates (Laarman and Oba 2011) and fat supplementation can be a strategy to reduce ruminal acidosis risk due to reduced grain feeding (Stewart and Schingoethe 1984). Fat supplementation has been shown to be favourable for dairy calves in some studies (Ballou and DePeters 2008; Hill et al. 2011; Ghasemi et al. 2017); however, some others reported lower performance with fat supplementation (Kuehn et al. 1994; Hill et al. 2015; Ghorbani et al. 2020; Yousefinejad et al. 2021). Fat source and level (Miller et al. 1959; Ghorbani et al. 2020), environmental temperature (Ghasemi et al. 2017), the delivery method in starter feeds (Berends et al. 2018) and fatty acid (FA) profile (Quigley et al. 2019) are main factors that have an impact on responses. Fat supplementation may...
have an interactive effect with other nutrients in the diet. For instance, fat supplementation reduced fibre digestion (Fallon et al. 1986) or protein digestibility (Hill et al. 2015). Although it has been stated that supplemental fat had a detrimental effect on fibre digestibility in mature ruminants which was mostly due to the coating effect of fat on fibre digestion (Soliva et al. 2004; Maia et al. 2010); however, the interaction of supplemental fat with dietary fibre content in dairy calves that have less ability to fibre digestion is still uncertain.

Fibre level in the starter is an important factor influencing dairy calves’ well-being. Increased rumen motility, promote rumination, improve the integrity and healthiness of the rumen wall and reduce behavioural problems are the beneficial aspects of allocating the forage in starter feed in the pre-weaning period (Suarez et al. 2007; Beiranvand et al. 2014; Mirzaei et al. 2016). On the other side, displace concentrate intake and shift rumen fermentation in favour of acetate rather than butyrate, delay rumen papillae development, reduce starter feed intake and decrease body weight and dry matter digestibility are the unfavourable outcomes in forage-included starter feeds (Nocek and Kesler 1980; Phillips 2004). Moreover, forage inclusion reduces energy density per unit of starter feed due to lower energy content of forage than concentrate fraction (Beiranvand et al. 2014). Therefore, some negative effects observed in forage included starter feeds may arise in the shadow of the low energy content of starters. It could be postulated that supplemental fat, with a high energy level supplied in the diet, may compensate for the lower energy content in forage-included starter diets. However, supplemental fat, on the other side, may have some toxic effects on rumen microorganisms, adhere to the feed particles and create a physical barrier that makes an obstacle for microbial activity and fibre digestibility (Palmquist and Jenkins 1980; Maia et al. 2010). However, this hypothesis needs to be more evaluated in pre-ruminant animals. More research is warranted to be conducted regarding the concurrent feeding of fat along with forage in dairy calves.

We hypothesised that supplementing soybean oil as a fat source may compensate for the lesser energy content supplied through forage incorporation in the starter feeds. The objective of the current study was to evaluate the effects of different levels of SBO (0 vs. 3%, DM basis) and AH inclusion level (0 vs. 15%, DM basis) on the performance, structural growth, ruminal fermentation characteristics, nutrient apparent digestibility and urinary purine derivatives in young calves.

Materials and methods

Calves and management

The present study was conducted on a commercial dairy farm (Avin-Dasht Dairy Industry Co, Qazvin, Iran). A total of forty 3-day-old Holstein female dairy calves with 39.9 (± 2.1) kg of initial BW were randomly assigned to a completely randomised design with a 2 × 2 factorial arrangement of treatments (10 calves/treatment) with the factors of soybean oil supplementation (0 vs. 3%, dry matter basis) and alfalfa hay inclusion levels (0 vs. 15%, dry matter basis). Calves were separated from their dams shortly after birth, housed in individual pens (1.3 × 2.5 m) which were similar in design and bedded with sand that renewed every 24 h. The vaccination schedule and rearing system protocols were as conventional farm protocol. The calves fed 5 L of colostrum within the first 12 h of life (2.5 L of colostrum within 2 h of life and 2.5 L in a second feeding). The calves received 4 L of whole milk/day from day 3 to 10, 7 L/day from day 11 to 53 and 3 L/day from day 54 to 63 in galvanised tin buckets (twice daily at 09:00 and 18:00 h). The average composition of offered milk was 3.16 ± 0.09% fat, 3.05 ± 0.06% crude protein (CP), 4.84 ± 0.06% lactose, and 11.9% total solids. Calves in all experimental treatments were weaned on day 63 of the study, but the experimental diets were continued 10 days later until day 73 of the study. Calves had free access to water during the whole experimental period.

Experimental treatments

Starter feeds were formulated to meet the National Research Council (2001) recommendations and were different in fat and fibre level. The experimental treatments were: (1) neither soybean oil supplementation nor alfalfa hay inclusion (NSBO-NAH), (2) soybean oil supplemented with no alfalfa hay inclusion (SBO-NAH), (3) no soybean oil supplementation but alfalfa hay included (NSBO-AH) and (4) soybean oil supplemented with alfalfa hay included (SBO-AH). The SBO source (Naz Industrial Vegetable Oil Co., Isfahan, Iran) was with the following fatty acids compositions: C16:0 = 12.1%, C18:0 = 5.2%, C18:1 = 21.8%, C18:2 = 51.2%, C18:3 = 8.1% and other fatty acids = 1.6%. Calves were fed basal starters in meal-form with an average geometric mean particle size of 0.74 ± 0.1 mm (American Society of Agricultural Engineers 1995). Alfalfa hay was chopped to obtain the geometric mean particle size equal to 2.8 ± 0.12 mm. After chopping, it was well-mixed with...
starter feed in forage-supplemented groups before feeding to dairy calves, and then the particle size distribution of starter feed was re-measured which was 0.89 ± 0.2 mm. The composition of the starter concentrates was kept constant within treatment, before and after weaning. The ingredients and chemical composition of experimental diets are presented in Table 1.

**Starter intake, performance, faecal score and digestibility**

The amounts of starter diets offered (at 08:00 h) and refused were recorded (at 07:30 h) daily throughout the experiment. Measurement of BW was taken at 10-day intervals during the experimental period using an electronic balance. Average daily gain (ADG) was calculated as the difference between BW taken every 10 days apart divided by 10. Feed efficiency (FE: kg of BW gain/kg of total dry matter intake) was also calculated in a 10-day interval. Total DMI was considered as liquid feed DMI + starter feed DMI. Samples collected from feeds and orts were dried in a convection oven (60 °C for 48 h). Subsamples of dried feeds and orts were well mixed and ground in a mill (Ogaw Seiki CO., Ltd., Tokyo, Japan) to pass a 1-mm screen and were analysed for CP (method 988.05; Association of Official Analytical Chemists 2002) ether extract (method 920.39; Association of Official Analytical Chemists 2002), and NDF without sodium sulphite, but with the inclusion of α-amylase (Van Soest et al. 1991). The non-fibrous carbohydrate (NFC) component was calculated based on National Research Council (2001) equation as follow; 100 – (CP + NFC + EE + ash).

Faeces were scored daily before the morning milk feeding as follows: 1 = normal, 2 = soft to loose, 3 = loose to watery, 4 = watery, mucous, slightly bloody and 5 = watery, mucous and bloody.

Faecal samples were collected via rectal palpation at 6 and 18 h after the morning meal (10 samples obtained for each animal) (Rastgoo et al. 2020). Faecal samples were dried in a forced dried oven (60 °C; 48 h), and then ground in a Wiley mill through a 1-mm screen. Aliquots of all faecal samples collected for each calf were mixed to obtain one composite sample for each animal. The composite faecal samples then were analysed to determine total nitrogen, EE, ash and NDF. Apparent total tract digestibility of nutrients (OM, NDF, CP and EE) was measured by using acid insoluble ash (AIA) as an internal marker. Apparent nutrients digestibility was calculated based on the concentrations of these nutrients and AIA in the feed (corrected for refusals) and faecal samples using the following formula: AD (%) = 100 – (MD/MF) × (NF/ND), where AD is the apparent digestibility (%), MD is the marker in the diet (%), MF is the marker in the faeces (%), NF is the nutrient in the faeces (%) and ND is the nutrient in the diet (%) (Van Keulen and Young 1977).

**Growth indices recording**

The growth indices including heart girth, body length, body girth, withers height, hip height and hip-width were taken at day 3 (Initial), day 63 (weaning), day 73 (the final day of measurements) of age in the morning and before feeding based on the method described by Khan et al. (2007) for dairy calves.
Rumen fluid (30mL) was collected on day 35 (pre-weaning) and day 70 (post-weaning) of the experiment using a stomach tube fitted to a vacuum pump 3–4 h after morning feeding; the first 10 mL was discarded because of possible saliva contamination and rumen pH was measured immediately (HI 8314 membrane pH metre; Hanna Instruments, Vllafranca, Italy). The rumen samples were squeezed through 4 layers of cheesecloth. A 10-mL aliquot was preserved with 2 mL of 25% metaphosphoric acid and frozen at −20 °C until analysis for volatile fatty acids (VFA). After thawing at room temperature, the rumen samples were analysed for VFA using gas chromatography (model CP-9002; Chrompack, Delft, the Netherlands) with a 50 m (0.32 mm ID) silica-fused column (CP-Wax Chrompack Capillary Column; Varian, Palo Alto, CA) as previously described (Kazemi-Bonchenari et al. 2016).

Urine sampling and microbial protein synthesis measurements

The microbial protein synthesized in the rumen was estimated through purine derivatives (PD) obtained via spot sampling technique in post-weaning calves. As milk contains PD (Gonzalez-Ronquillo et al. 2003) and could cause an error in estimating PD excretion results obtained in pre-weaning calves, the spot urine sampling technique was used for MPS estimation only in the post-weaning period when calves received no milk as described by Makizadeh et al. (2020). Urine volumes were estimated as BW × 26.8/creatinine concentration (mg/L) in post-weaned dairy calves as reported by Dennis et al. (2018). Spot urine samples were collected on four consecutive sampling days (from 69 to 72) during the post-weaning period from each animal. Samples were collected when calves urinated spontaneously (~10mL). An aliquot of 5 mL of each sample was diluted immediately with 45 mL of 0.036N sulphuric acid and stored at −20 °C for analysis.

Later, urine samples were thawed at room temperature and analysed to determine the creatinine (Kit No. 555-A; Sigma Chemical Co.), and uric acid (Kit No. 685-50; Sigma Chemical Co) using spectrophotometer (UV-2600i, Shimadzu, Japan) and UN (using the assay described by Broderick and Kang 1980). Allantoin was measured using high-performance liquid chromatography by the method described by Chen and Gomes (1992). Total excretion of allantoin and uric acid was calculated from estimated daily urine output and determined metabolite concentrations.

The ruminal microbial N synthesis was calculated from daily urinary PD output using the following equation described by Chen and Gomes (1992):

\[
\text{Microbial nitrogen (g N/day)} = X \times (\text{mM/day}) \times 70/0.116 \times 0.83 \times 1000;
\]

where \(X\) is microbial purine absorbed (mM/day), 70 is the N content of purines (mg N/mM), 0.116 is the ratio of purine-N to total N in mixed ruminal microbes which is 11.6:100, and 0.83 is average digestibility of microbial purines (Chen and Gomes 1992).

Statistical analysis

Statistical analyses were conducted for 3 periods: pre-weaning (days 3–63), post-weaning (days 64–73) and the entire period (days 3–73) using PROC MIXED of SAS (version 9.1; SAS Inst. Inc., Cary, NC). The following model was adopted:

\[
Y_{ijkl} = \mu + \text{SBO}_i + \text{AH}_j + P_k + (\text{SBO} \times P)_ik + (\text{AH} \times P)_jk + (\text{SBO} \times \text{AH})_{ijk} + \beta(X_i - \bar{X}) + e_{ijklm};
\]

where \(Y_{ijkl}\) is the dependent variable; \(\mu\) is the overall mean; \(\text{SBO}_i\) is the effect of soybean oil supplementation; \(\text{AH}_j\) is the effect of alfalfa hay inclusion (\(j = 0\) vs. 3%, DM basis); \(P_k\) is the effect of period; \((\text{SBO} \times P)_ik\) is the interaction between soybean oil supplementation and period; \((\text{AH} \times P)_jk\) is the interaction between alfalfa hay inclusion and period; \((\text{SBO} \times \text{AH})_{ijk}\) is the interaction between soybean oil supplementation and AH inclusion; \((\text{SBO} \times \text{AH} \times P)_{ijk}\) is the tripartite effect of soybean oil supplementation, \(\text{AH}\) inclusion and period; \(\beta(X_i - \bar{X})\) is the covariate variable and \(e_{ijklm}\) is the over-all error term. The model contained calf within treatment as a random effect and the first-order autoregressive covariance structure (AR1) was determined as the most appropriate covariance structure for all repeated statements according to Akaike’s information criterion and Bayesian information criterion. The BW and growth indices on the initial day of the experiment (day 3) were used as a covariate for weaning and final measurements of related items. Effects were considered to be significant when \(p \leq .05\) and it has been considered to have tendency was considered when \(.05 < p \leq .10\). All reported values through the tables are least-squares means.

Results

Starter intake and performance

The results showed that the highest and the lowest starter intake during the pre-weaning period were observed in NSBO-AH and SBO-AH treatments,
Table 2. Least square means for starter intake, average daily gain and feed efficiency in dairy calves supplemented with soybean oil (0 vs. 3%, DM basis) with different alfalfa hay levels (0 vs. 15%, DM basis) in starter feed (n = 10 calves per treatment).

| Item                      | NSBO       | SBO        | p-Value² |
|---------------------------|------------|------------|----------|
| Treatments¹               | NAH        | AH         | NAH      | AH      | SEM      |
| Starter feed intake, g/day|            |            |          |         |          |
| Pre-weaning               | 532ab      | 636a       | 612c     | 494d    | 56.96    |
| Post-weaning              | 2028       | 2019       | 1931     | 1841    | 155.44   |
| Entire period             | 727        | 835        | 800      | 686     | 78.61    |
| Milk intake, g DM/day     | 651        | 649        | 650      | 651     | 22.42    |
| Total DMI, (milk + starter), g/day | 1183 | 1287 | 1263 | 1146 | 60.27 |
| Average daily gain, g/day |            |            |          |         |          |
| Pre-weaning               | 640        | 607        | 675      | 554     | 34.19    |
| Post-weaning              | 872        | 715        | 745      | 695     | 74.36    |
| Entire period             | 670        | 622        | 685      | 575     | 31.47    |
| Body weight, kg           |            |            |          |         |          |
| Initial                   | 40.0       | 40.5       | 39.8     | 39.2    | 0.80     |
| Weaning                   | 78.4a      | 76.9ab     | 80.4a    | 72.7b   | 1.88     |
| Final                     | 88.1       | 84.2       | 87.8     | 81.6    | 2.10     |
| Feed efficiency³          |            |            |          |         |          |
| Pre-weaning               | 0.57       | 0.51       | 0.58     | 0.50    | 0.03     |
| Post-weaning              | 0.43       | 0.36       | 0.39     | 0.39    | 0.06     |
| Entire period             | 0.55       | 0.49       | 0.54     | 0.48    | 0.02     |

¹Treatments were; (1) no soybean oil supplementation with no forage included in the starter (NSBO-NAH); (2) no soybean oil supplementation with 15% alfalfa hay included in the starter (NSBO-AH); (3) 3% soybean oil supplemented with no forage included in the starter (SBO-NAH); (4) 3% soybean oil supplemented with 15% alfalfa hay included in the starter (SBO-AH).

²Statistical comparisons – SBO: soybean oil supplementation level in the starter (0 vs. 3%); AH: alfalfa hay inclusion level in the starter (0 vs. 15%); SBO × AH: interaction between soybean oil supplementation and alfalfa hay inclusion levels in the starter.

³Kilogram of body weight gain/kg of total dry matter intake.

Results showed that the calves fed SBO-AH diet had the lowest wither height, both in weaning time and final measurement (p < .05; Table 4). Body length, heart girth, body barrel and hip-width were not influenced by SBO supplementation, forage feeding level, or their interaction (p > .05). Hip height is reduced (p = .05) when calves are supplemented with SBO or fed with AH (p < .01).

Ruminal fermentation profile

The lowest ruminal concentration of VFA (p < .05) during the pre-weaning period was found in calves that received SBO-AH treatment (Table 5). The lowest acetate concentration during the post-weaning period was found for NSBO-NAH treatment (p < .05). The supplemental SBO reduced ruminal concentrations of propionate in pre-weaning (p = .01) and valerate in post-weaning (p = .05) periods. Inclusion of AH in starters increased ruminal acetate but reduced ruminal propionate and butyrate concentrations (p < .05).

Urinary purine derivatives and urinary nitrogen excretion

Results show that TPD, MPS and UN were influenced by the interaction of SBO supplementation and AH inclusion in starter with the SBO-AH treatment showed to have the lowest TPD and MPS (Table 6). However, the greatest UN excretion among experimental treatments was found for SBO-AH treatment (p < .05).
Urinary allantoin concentration was also tended to be reduced in SBO-AH treatment ($p = .08$).

**Discussion**

**Interaction of SBO supplementation and AH inclusion**

The lowest starter intake during pre-weaning period was observed in SBO-AH treatment (494 g/day) compared to other treatments. It has been suggested that the lower feed intake in fat supplemented diets may be related to the decreased palatability and reduced nutrient digestibility (Ghorbani et al. 2020). Furthermore, in dairy cows, a negative effect of fat called ‘greasiness’ on intake has been proposed by Drackley et al. (1994). In addition, fatty acid oxidation in the liver is indicated to have the potential to control the appetite in mature ruminants (Harvatine and Allen 2005). Some studies indicated the inflammatory effect of some individual fatty acids in dairy calves that can be related to reduced starter intake (Tsai et al. 2017). In addition to the unfavourable effect of SBO on starter intake, intake was more exacerbated when AH was included in diet accompanied with SBO supplementation. This is probably related to under-developing ruminal conditions during the pre-weaning period (Cersosimo et al. 2019; Yousefinejad et al. 2021). Pre-weaning dairy calves having a limited cellulolytic activity that could further negatively influence ruminal microbes’ activity and consequently fibre digestibility when an unsaturated fatty acid source such as linseed oil (Ikwuegbu and Sutton 1982) or soybean oil (Ghorbani et al. 2020) was supplemented in the diet. Physical coating of the fibre by fat or toxic effects of fat on ruminal microbes may be responsible in part for the lower digestibilities of fibre and protein found in the SBO-AH group (Soliva et al. 2004; Maia et al. 2010). In agreement with previous works (Hill et al. 2015; Ghorbani et al. 2020; Yousefinejad et al. 2021) our results suggest that SBO supplementation was associated with reduced digestibility of OM and NDF in dairy calves, which was more negatively influenced when forage was included in the starter. Regarding the interaction between SBO supplementation and AH inclusion in the starter feed in SBO-AH treatment, it can be postulated that incorporating the AH increased NDF content and SBO supplementation reduced NDF digestibility, from the other side indicating that supplemental SBO can be more detrimental for NDF digestibility when forage is included in starter feed and the least NDF digestibility is found to be SBO-AH treatment. Reduced digestibility of nutrient can be a factor to reduce starter intake in dairy calves (Ghorbani et al. 2020).

In the current study, the lowest ADG (554 g/day) during pre-weaning period was found in SBO-AH treatment. The lowest ADG (554 g/day) during pre-weaning period was found in SBO-AH treatment. The lowest ADG (554 g/day) during pre-weaning period was found in SBO-AH treatment. The lowest ADG (554 g/day) during pre-weaning period was found in SBO-AH treatment. The lowest ADG (554 g/day) during pre-weaning period was found in SBO-AH treatment. The lowest ADG (554 g/day) during pre-weaning period was found in SBO-AH treatment. The lowest ADG (554 g/day) during pre-weaning period was found in SBO-AH treatment.

### Table 3. Least square means for faecal score, rectal temperature and nutrients digestibility in dairy calves supplemented with soybean oil (0 vs. 3%, DM basis) with different alfalfa hay levels (0 vs. 15%, DM basis) in starter feed ($n = 10$ calves per treatment).

| Item                          | Treatments | SEM | $p$-Value |
|-------------------------------|------------|-----|-----------|
| Faecal score                  |            |     |           |
| days 3–13                     | 2.30       | 2.03|           |
| days 13–23                    | 1.73       | 1.93|           |
| days 23–33                    | 2.06       | 1.90|           |
| days 33–43                    | 1.40       | 1.26|           |
| days 43–53                    | 1.26       | 1.46|           |
| days 53–63                    | 1.36       | 1.50|           |
| days 63–73                    | 1.33       | 1.26|           |
| Entire period (days 3–73)     | 1.63       | 1.62|           |
| Body temperature, °C          |            |     |           |
| Pre-weaning (days 3–63)       | 38.61      | 38.72|          |
| Post-weaning (days 63–73)     | 38.73      | 38.75|          |
| Entire period (days 3–73)     | 38.64      | 38.68|          |
| Nutrients digestibility, g/kg |            |     |           |
| Organic matter                | 883        | 837 |          |
| Crude protein                 | 794        | 762 |          |
| Ether extract                 | 862        | 846 |          |
| Neutral detergent fibre       | 661        | 690 |          |

1. Treatments were: (1) no soybean oil supplementation with no forage included in the starter (NSBO-NAH); (2) no soybean oil supplementation with 15% alfalfa hay included in the starter (NSBO-AH); (3) 3% soybean oil supplemented with no forage included in the starter (SBO-NAH); (4) 3% soybean oil supplemented with 15% alfalfa hay included in the starter (SBO-AH).

2. Statistical comparisons – SBO: soybean oil supplementation level in the starter (0 vs. 3%); AH: alfalfa hay inclusion level in the starter (0 vs. 15%); SBO × AH: interaction between soybean oil supplementation and alfalfa hay inclusion levels in the starter.

Means within a row with different superscript letters are different ($p < .05$).
calves, which coincided with a similar trend in BW and wither height. These changes along with the lower digestibility of NDF and CP in SBO-AH calves might reflect a less efficient tissue accretion in SBO-AH calves compared to other treatments.

In addition to the lower nutrient digestibility, the lower concentrations of VFA seem to be responsible for the lower growth performance observed in calves fed SBO-AH diet. The lower starter intake and the lower NDF and CP digestibilities in SBO-AH group may reduce substrate for ruminal microbial fermentation, and hence VFA concentration was reduced in the rumen. In ruminants, considerable energy requirement (approximately 70%) is supplied through VFA produced in the rumen (Bergman 1990); thus, the lower ruminal VFA concentration in the calves fed SBO-AH would be expectedly accompanied by a lower supply of energy for growth. The least acetate concentration (43.9 mM) but the highest propionate concentration (33.4 mM) were observed in NSBO-NAH treatment in the current study indicating the greater energy supplied in calves fed this diet compared to other experimental diets. Previous works stated that increased forage level in the starter feed can positively influence acetate concentration (Mirzaei et al. 2016). The lowest acetate concentration is found when starter feed was neither contained AH nor SBO indicating that the inclusion of AH and supplemental SBO both shift the rumen fermentation towards more acetate rather than propionate in dairy calves. As discussed earlier, more propionate is favourable than more acetate for rumen development (Bergman 1990); hence, supplemental SBO along with AH inclusion in starter feed is not recommendable from the ruminal fermentation perspective.

In the current study, supplementation of SBO along with forage inclusion reduced total PD excretion, and thus reduced estimated MPS, but increased UN excretion. This indicates lower nitrogen utilisation efficiency in SBO-AH treatments compared to other groups. It has been indicated before that the amount of total PD excreted is influenced by feed intake (Singh et al. 2007). Therefore, the lower starter intake in SBO-AH group had a substantial role in reducing total PD excretion. In addition, lower PD in the SBO-AH diet was partially due to decreased digested NDF and CP in this treatment. It has been indicated that PD excretion can be an indicator of ruminal development in

| Item                  | Treatments1                                                                 | p-Value2                                                                 |
|-----------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------|
|                       | NSBO AH                                                                     | SBO AH                                                                  | SBO x AH |
| Heart girth           |                                                                              |                                                                          |          |
| Initial (day 3)       | 80.7 81.9                                                                   | 80.4 80.6                                                                | .85      | .41      | .47      | .60      |
| Weaning (day 63)      | 102.6 103.5                                                                 | 102.2 100.4                                                             | 1.12     | .12      | .69      | .23      |
| Final (day 73)        | 106.5 107.6                                                                 | 108.7 106.4                                                             | 1.21     | .71      | .65      | .18      |
| Body length           |                                                                              |                                                                          |          |          |          |          |
| Initial (day 3)       | 48.9 48.5                                                                   | 49.6 48.5                                                                | 0.68     | .60      | .27      | .60      |
| Weaning (day 63)      | 62.5 62.6                                                                   | 62.8 61.4                                                                | 1.32     | .76      | .60      | .61      |
| Final (day 73)        | 64.2 65.5                                                                   | 65.1 65.2                                                                | 1.12     | .79      | .53      | .59      |
| Body barrel           |                                                                              |                                                                          |          |          |          |          |
| Initial (day 3)       | 80.2 81.5                                                                   | 80.4 79.0                                                                | 0.89     | .20      | .95      | .14      |
| Weaning (day 63)      | 115.5 116.3                                                                 | 115.9 114.8                                                             | 1.76     | .88      | .93      | .71      |
| Final (day 73)        | 128.2 124.8                                                                 | 128.7 125.8                                                             | 2.38     | .75      | .20      | .95      |
| Wither height         |                                                                              |                                                                          |          |          |          |          |
| Initial (day 3)       | 81.2 81.7                                                                   | 80.9 82.6                                                                | 0.81     | .71      | .46      | .18      |
| Weaning (day 63)      | 98.0ab 96.6ab                                                                | 98.6ab 93.5b                                                           | 0.86     | .12      | <.01     | .02      |
| Final (day 73)        | 100.5ab 100.6ab                                                             | 102.8b 98.3b                                                            | 0.79     | .95      | <.01     | .01      |
| Hip height            |                                                                              |                                                                          |          |          |          |          |
| Initial (day 3)       | 79.3 80.8                                                                   | 80.9 80.1                                                                | 0.80     | .57      | .66      | .19      |
| Weaning (day 63)      | 97.0 96.0                                                                   | 95.6 92.4                                                                | 0.68     | <.01     | <.01     | .12      |
| Final (day 73)        | 99.7 98.9                                                                   | 100.0 97.4                                                               | 0.71     | .40      | .02      | .21      |
| Hip width             |                                                                              |                                                                          |          |          |          |          |
| Initial (day 3)       | 15.3 15.2                                                                   | 15.2 14.6                                                                | 0.24     | .16      | .16      | .31      |
| Weaning (day 63)      | 20.6 20.8                                                                   | 20.8 19.9                                                                | 0.60     | .56      | .57      | .36      |
| Final (day 73)        | 21.1 21.4                                                                   | 21.9 21.4                                                                | 0.56     | .43      | .93      | .43      |

1Treatments were; (1) no soybean oil supplementation with no forage included in the starter (NSBO-NAH); (2) no soybean oil supplementation with 15% alfalfa hay included in the starter (NSBO-AH); (3) 3% soybean oil supplemented with no forage included in the starter (SBO-NAH); (4) 3% soybean oil supplemented with 15% alfalfa hay included in the starter (SBO-AH).  
2Statistical comparisons – SBO: soybean oil supplementation level in the starter (0 vs. 3%); AH: alfalfa hay inclusion level in the starter (0 vs. 15%); SBO x AH: interaction between soybean oil supplementation and alfalfa hay inclusion levels in the starter. Means within a row with different superscript letters are different (p < .05).
dairy calves (Terré et al. 2006). Therefore, although with no ruminal morphological measurements in the current study, our results indicate that dairy calves fed the SBO-AH diet obtained less developed rumen in comparison with other diets. With respect to the interaction of SBO supplementation and AH inclusion in starter feed, results indicate that microbial activity was the highest when neither SBO was supplemented nor AH was included ins starter feed indicating that high-fat diet along with high fibre content in starter diet cannot provide adequate energy required for microbial development. Higher UN excretion in the SBO-AH diet compared with other diets is probably related to lower utilisation of ruminal ammonia-N. Fat supplementation has a direct effect on the reduction the ruminal microbial activity (Nagaraja et al. 1997; Fiorentini et al. 2013). To the best of our knowledge, the current study is the first report regarding the interaction effect of SBO supplementation and starter forage level on urinary PD excretion and urinary nitrogen excretion in dairy calves. More research with different sources of fat and fibre contents in starter feeds may give more insight into the underlying mechanisms, which can be used to optimise fat and forage feeding strategies in dairy calves that will positively impact calf growth and future outcomes (Van De Stroet et al. 2016).

**Effect of SBO supplementation**

Reduction in starter feed intake was reported by Hill et al. (2015) when 2% SBO was included in diets for dairy calves (before 8 weeks of age). The effect of fat supplementation on starter intake in dairy calves might be related to the type of fat supplements (e.g. whole or extruded oilseeds), degree of saturation of dietary fat, supplemented level in the starter feed (Hill et al. 2011, 2015; Ghasemi et al. 2017), the fat delivery method in starter feed (Berends et al. 2018), and supplemented FA profile (Quigley et al. 2019). Our result adds to our knowledge that in addition to the items mentioned, forage level in diet also can be an important item influencing the animal response to supplemental fat. Starter intake in SBO-NAH group is 612 g/

| Item                          | NSBO       | SBO       | SEM       | p-Value   |
|-------------------------------|------------|-----------|-----------|-----------|
| Ruminal pH                    |            |           |           |           |
| Pre-weaning                   | 5.50       | 5.74      | 5.51      | 5.55      | 0.08      | .28 | .11 | .29 |
| Post-weaning                  | 5.76       | 6.05      | 5.92      | 6.03      | 0.14      | .62 | .18 | .51 |
| Volatile fatty acids, mM/L    |            |           |           |           |           |     |     |     |
| Pre-weaning                   | 93.1*      | 89.9*     | 90.4*     | 78.2*     | 2.63      | .01 | <.01 | .05 |
| Post-weaning                  | 107.0      | 99.3      | 103.5     | 98.2      | 2.37      | .34 | .01 | .63 |
| Individual short chain fatty acid, mM |           |           |           |           |           |     |     |     |
| Acetate (A)                   |            |           |           |           |           |     |     |     |
| Pre-weaning                   | 45.1       | 51.4      | 48.8      | 54.3      | 1.36      | .06 | <.01 | .79 |
| Post-weaning                  | 43.9*      | 50.0*     | 49.9*     | 49.8*     | 1.21      | .02 | .03 | .01 |
| Propionate (P)                |            |           |           |           |           |     |     |     |
| Pre-weaning                   | 35.6       | 31.6      | 30.7      | 29.3      | 1.07      | <.01 | .02 | .26 |
| Post-weaning                  | 33.4       | 31.1      | 28.7      | 31.5      | 1.39      | .13 | .84 | .08 |
| A:P                           |            |           |           |           |           |     |     |     |
| Pre-weaning                   | 1.27       | 1.65      | 1.60      | 1.87      | 0.09      | .01 | <.01 | .60 |
| Post-weaning                  | 1.32       | 1.61      | 1.74      | 1.65      | 0.10      | .04 | .35 | .08 |
| Butyrate                      |            |           |           |           |           |     |     |     |
| Pre-weaning                   | 13.6       | 11.4      | 15.9      | 11.1      | 1.01      | .36 | <.01 | .22 |
| Post-weaning                  | 17.3       | 13.4      | 16.8      | 14.1      | 1.13      | .89 | .01 | .56 |
| Valerate                      |            |           |           |           |           |     |     |     |
| Pre-weaning                   | 4.76       | 4.49      | 3.58      | 4.20      | 0.49      | .15 | .72 | .38 |
| Post-weaning                  | 4.41       | 4.38      | 3.50      | 3.56      | 0.43      | .05 | .96 | .91 |
| Isovalerate                   |            |           |           |           |           |     |     |     |
| Pre-weaning                   | 0.88       | 1.0       | 0.95      | 0.94      | 0.26      | .96 | .80 | .79 |
| Post-weaning                  | 0.95       | 1.10      | 1.02      | 0.97      | 0.22      | .89 | .82 | .64 |
| Branched-chain volatile fatty acids |           |           |           |           |           |     |     |     |
| Pre-weaning                   | 5.64       | 5.50      | 4.54      | 5.16      | 0.55      | .21 | .67 | .50 |
| Post-weaning                  | 5.36       | 5.49      | 4.52      | 4.54      | 0.48      | .07 | .88 | .90 |

1Treatments were; (1) no soybean oil supplementation with no forage included in the starter (NSBO-NAH); (2) no soybean oil supplementation with 15% alfalfa hay included in the starter (NSBO-AH); (3) 3% soybean oil supplemented with no forage included in the starter (SBO-NAH); (4) 3% soybean oil supplemented with 15% alfalfa hay included in the starter (SBO-AH).
2Statistical comparisons – SBO: soybean oil supplementation level in the starter (0 vs. 3%); AH: alfalfa hay inclusion level in the starter (0 vs. 15%); SBO × AH: interaction between soybean oil supplementation and alfalfa hay inclusion levels in the starter.
3Branched-chain volatile fatty acids (BCVFA) are the molar proportion of valerate + isovalerate.
Table 6. Least square means for purine derivative excretion and microbial protein synthesis in dairy calves supplemented with soybean oil (0 vs. 3%, DM basis) with different alfalfa hay levels (0 vs. 15%, DM basis) in starter feed (n = 10 calves per treatment).

| Item                        | NSBO | SBO | SEM | p-Value |
|-----------------------------|------|-----|-----|---------|
| Item                        | NAH  | AH  | NAH | AH      | SBO     | AH    | SBO × AH |
| Allantoin, mM/day           | 16.51| 14.60| 16.64| 13.54  | 0.79    | .01   | .02     | .08     |
| Uric acid, mM/day           | 1.54 | 1.41 | 1.40 | 1.42   | 0.08    | .38   | .44     | .41     |
| Total purine derivatives, mM/d | 18.05 | 15.9ab | 16.0ab | 14.9b  | 0.93    | <.01  | .01     | .04     |
| Microbial protein synthesis, g/day | 96.50 | 85.60ab | 85.81ab | 79.92b | 4.32    | <.01  | .01     | .04     |
| Urinary nitrogen, g/day     | 14.92b| 15.81ab| 16.12ab| 18.01a  | 0.52    | <.01  | .02     | .05     |

1Treatments were: (1) no soybean oil supplementation with no forage included in the starter (NSBO-NAH); (2) no soybean oil supplementation with 15% alfalfa hay included in the starter (NSBO-AH); (3) 3% soybean oil supplemented with no forage included in the starter (SBO-NAH); (4) 3% soybean oil supplemented with 15% alfalfa hay included in the starter (SBO-AH).

2Statistical comparisons – SBO: soybean oil supplementation level in the starter (0 vs. 3%); AH: alfalfa hay inclusion level in the starter (0 vs. 15%); SBO × AH: interaction between soybean oil supplementation and alfalfa hay inclusion levels in the starter.

Means within a row with different superscript letters are different (p < .05).

day and reduced to 494 g/day when SBO was supplemented along with AH inclusion in starter feed indicating that concurrent feeding of SBO and AH in young calves may not be favourable.

Supplementation of starter feeds with SBO reduced faecal consistency during early weeks of life. This could indicate that SBO supplementation could increase the passage rate in the gastrointestinal tract, thus causing looser faeces (Ghorbani et al. 2020). No difference in feeding different fat sources on the faecal score in dairy calves was observed in a cold environment (Ghasemi et al. 2017). The CP digestibility was influenced negatively by SBO supplementation in the current study. Hill et al. (2015) reported that calves under 2 months of age fed a starter feed supplemented with SBO had lower digestibility of OM and CP, but not in tallow supplemented calves. Interestingly and contrary to our results, Araujo et al. (2014) reported that CP digestibility was increased with supplemented fat. These inconsistent results may be related to the fat level and source (saturated vs. unsaturated) as well as the ruminal accessibility of the supplemented fatty acids. For instance, it has been stated that unsaturated FA source (linseed oil in Ikwuegbu and Sutton 1982; soybean oil in Ghorbani et al. 2020) has a detrimental effect on nutrient digestibility due to the ruminal accessibility of FA in the rumen; however, inaccessibility of FA source in the rumen (calcium salt of linseed oil in Kazemi-Bonchenari et al. 2020) had been shown to have no effect on nutrient digestibility.

Ruminal acetate concentration was increased but ruminal propionate concentration was reduced when SBO was supplemented in calves. Propionate supplies greater energy on a molar basis than acetate (Bergman 1990), and thus NSBO diet has greater potential compared to the SBO diet to improve animal performance. This may eventually cause lower hip height in calves at weaning time when supplemented with SBO. Lower CP digestibility in SBO supplemented diets may be contributed to reducing BCVFA concentration due to the lower branched amino acids available that are precursors for BCVFA in ruminal fluid (Yang 2002).

The supplemented SBO reduced purine derivatives excreted through urine indicating lower microbial activity in fat-supplemented calves. Supplemental SBO increased the UN indicating lower nitrogen efficiency in these diets (Kauffman and St-Pierre 2001). The effect of supplemental fat which is ruminally available on ruminal microbes (Nagaraja et al. 1997; Fiorentini et al. 2013) could alter nitrogen utilisation efficiency. In addition, lower OM digestibility in the SBO supplemented calves resulted in less nitrogen incorporation in ruminal protein biosynthesis and then less efficient nitrogen utilisation. The present study results confirm that from the nitrogen metabolism viewpoint, feeding low-fat starter feeds are more efficient than fat supplemented starter feeds in young calves.

Effect of AH inclusion

Forage inclusion level in the starter feed reduced BW as well as FE at final measurement. Moreover, consistent with BW results, hip height was reduced in calves fed forage in starter feed. This is partly related to reduced OM digestibility as well as reduced VFA concentration in AH included diets compared to other treatments. As stated by Bergman (1990), lower VFA concentration as the main energy source in ruminants can result in less energy towards animal growth. Furthermore, lower ruminal propionate and butyrate concentrations in forage included diets along with greater ruminal acetate concentration clarify the lower
energy supplied in forage-fed calves. It has been identified that propionate and butyrate are the two main VFA that supply greater energy on a molar basis than acetate (Bergman 1990), and thus provide greater energy to improve animal performance. Some of the beneficial effects of forage inclusion in starter feed have been identified in pre-ruminant animals such as stimulating effect of forage for the muscular layer of the rumen and promote rumination (Phillips 2004), maintain the integrity and healthiness of the rumen wall, and increase rumen motility and prevent hyperkeratinization of ruminal papillae (Suarez et al. 2007). Some discourage viewpoints also were indicated that forage may displace concentrate intake and shift rumen fermentation in favour of acetate rather than butyrate production and, thus, delay rumen papillae keratinization of ruminal papillae (Suarez et al. 2007). We found that in addition to forage feeding level and other related variables, supplemental fat also can impact the responses observed in forage-fed calves. For instance, forage inclusion per se did not have a negative impact on starter intake and NDF digestibility; however, these items were negatively influenced when forage was fed along with SBO. Thus, it can be stated here that the supplemented fat level and the source may be considered while identifying the optimum forage inclusion in dairy calves’ starter feeds.

Conclusions

Soybean oil supplementation (3%, DM basis) concomitantly with alfalfa hay inclusion (15%, DM basis) in the starter feed negatively influenced growth performance and digestibility of nutrients in young calves. Moreover, the nitrogen utilisation efficiency was negatively influenced by reducing the urinary PD excretion and increasing the urinary nitrogen excretion. In summary, considering variables evaluated in the current study, results indicated that soybean oil supplementation is not recommendable when alfalfa hay is incorporated in the starter feed of dairy calves.

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Data availability statement

Data available on request due to privacy/ethical restrictions.

References

American Society of Agricultural Engineers. 1995. Method of determining and expressing fineness of feed material by sieving. In: ASAE Standards 1995. St. Joseph (MI): ASAE; p. 461.
Aragona KM, Suarez-Mena FX, Dennis TS, Quigley JD, Hu W, Hill TM, Schlotterbeck RL. 2020. Effect of starter form, starch concentration, and amount of forage fed on Holstein calf growth from 2 to 4 months of age. J Dairy Sci. 103(3):2324–2332.
Araujo G, Terré M, Bach A. 2014. Interaction between milk allowance and fat content of the starter feed on performance of Holstein calves. J Dairy Sci. 97(10):6511–6518.
Association of Official Analytical Chemists. 2002. Official methods of analysis. 17th ed. Arlington (VA): AOAC International.
Ballou M, DePeters EJ. 2008. Supplementing milk replacer with omega-3 fatty acids from fish oil on immunocompetence and health of Jersey calves. J Dairy Sci. 91(9):3488–3500.
Beiranvand H, Ghorbani GR, Khorvash M, Kazemi-Bonchenari M. 2014. Forage and sugar in dairy calves’ starter diet and their interaction on performance, weaning age and rumen fermentation. J Anim Physiol Anim Nutr. 98(3):439–445.
Berends H, Vidal M, Terré M, Leal LN, Martín-Tereso J, Bach A. 2018. Effects of fat inclusion in starter feeds for dairy calves by mixing increasing levels of a high-fat extruded pellet with a conventional highly fermentable pellet. J Dairy Sci. 101(12):10962–10972.
Bergman EN. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev. 70(2):567–590.
Broderick GA, Kang JH. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. Journal of Dairy Science 63: 64–75.
Cersosimo LM, Radloff W, Zanton G. 2019. Microbial inoculum composition and pre-weaned dairy calf age alter the developing rumen microbial environment. Front Micr. 10: 1651.
Chen XB, Gomes MJ. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: An overview of technical details. Aberdeen (UK): Rowett Research Institute, University of Aberdeen.
Dennis TS, Suarez-Mena FX, Hill TM, Quigley JD, Schlotterbeck RL, Lascano GJ. 2018. Short communication: effect of replacing corn with beet pulp in a high concentrate diet fed to weaned Holstein calves on diet digestibility and growth. J Dairy Sci. 101(1):408–412.

Drackley JK, Grum DE, McCoy GC, Klusmeyer TH. 1994. Comparison of three methods for incorporation of liquid fat into diets for lactating cows. J Dairy Sci. 77(5):1386–1398.

Fallon RJ, Williams PEV, Innes GM. 1986. The effects of feed intake, growth and digestibility of nutrients of including calcium soaps of fat in diets for young calves. Anim Feed Sci Technol. 14(1–2):103–115.

Fiorentini G, Messana JD, Dian PHM, Reis RA, Canesin RC, Pires AV, Berchielli TT. 2013. Digestibility, fermentation and rumen microbiota of crossbred heifers fed diets with different soybean oil availabilities in the rumen. Anim Feed Sci Technol. 181(1–4):26–34.

Ghasemi E, Azad-Shahraki M, Khorvash M. 2017. Effect of different fat supplements on performance of dairy calves during cold season. J Dairy Sci. 100(7):5319–5328.

Ghorbani H, Kazemi-Bonchenari M, HosseinYazdi M, Mahjoubi E. 2020. Effects of various fat delivery methods in starter diet on growth performance, nutrients digestibility and blood metabolites of Holstein dairy calves. Anim Feed Sci Technol. 262:114429.

Gonzalez-Ronquillo M, Balcells J, Guada JA, Vicente F. 2003. Purine derivative excretion in dairy cows: endogenous excretion and the effect of exogenous nucleic acid supply. J Dairy Sci. 86(4):1282–1291.

Harvatine KJ, Allen MS. 2005. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. J Dairy Sci. 88(11):4018–4027.

Hill TM, Bateman HG, II, Aldrich JM, Quigley JD, Schlotterbeck RL. 2015. Inclusion of tallow and soybean oil to starters fed to dairy calves from birth to four months of age on calf performance and digestion. J Dairy Sci. 98:1–7.

Hill TM, Bateman IIH, Aldrich JM, Schlotterbeck RL. 2011. Effect of various fatty acids on dairy calf performance. Prof Anim Sci. 27(3):167–175.

Ikwuegbu OA, Sutton JD. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br J Nutr. 48(2):365–375.

Kauffman AJ, St-Pierre NR. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. J Dairy Sci. 84(10):2284–2294.

Kazemi-Bonchenari M, Dehghan-Banadaky M, Fatattahnia F, Saleh-Bahmanpour A, Jahani-Moghadam M, Mirzaei M. 2020. Effects of linseed oil and rumen undegradable protein:rumen degradable protein ratio on performance of Holstein dairy calves. Br J Nutr. 123(11):1247–1257.

Kazemi-Bonchenari M, Mirzaei M, Jahani-Moghadam M, Soltani A, Mahjoubi E, Patton RA. 2016. Interactions between levels of heat-treated soybean meal and prilled fat on growth, rumen fermentation, and blood metabolites of Holstein calves. J Anim Sci. 94(10):4267–4275.

Khan MA, Lee HJ, Lee WS, Kim HS, Ki KS, Hur TY, Suh GH, Kang SJ, Choi YJ. 2007. Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. J Dairy Sci. 90(7):3376–3387.

Kuehn CS, Otterby DE, Linn JG, Olson WG, Chester-Jones H, Marx GD, Barmore JA. 1994. The effect of dietary energy concentration on calf performance. J Dairy Sci. 77(9):2621–2629.

Laarman AH, Oba M. 2011. Short communication: effect of calf starter on rumen pH of Holstein dairy calves at weaning. J Dairy Sci. 94(11):5661–5664.

Lanza C, Sniffen CJ, Seo S, Tedesco LO, Fox DG. 2007. A revised CNCPS feed carbohydrates fractionation scheme for formulating rations for ruminants. Anim Feed Sci Technol. 136(3–4):167–190.

Maia MRG, Chaudhary LC, Bestwick CS, Richardson AJ, McKain N, Larson TR, Graham IA, Wallace RJ. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. BMC Microbiol. 10:52–62.

Makizadeh H, Kazemi-Bonchenari M, Mansoori-Yarahmadi M, Fakhrane J, Khankan H, Drackley JK, Ghaffari MH. 2020. Corn processing and crude protein content in calf starter: effects on growth performance, ruminal fermentation, and blood metabolites. J Dairy Sci. 103(10):9037–9053.

Miller WJ, Carmon JL, Dalton HL. 1959. Influence of high levels of plant and animal fats in calf starters on growth, feed consumption, and palatability. J Dairy Sci. 42(1):153–158.

Mirzaei M, Khorvash M, Ghorbani GR, Kazemi-Bonchenari M, Riasi A, Soltani A, Moshiri B, Ghaffari MH. 2016. Interactions between the physical form of starter (mashed versus textured) and corn silage provision on performance, rumen fermentation, and structural growth of Holstein calves. J Anim Sci. 94(2):678–686.

Nagaraja TG, Newbold CJ, Ven Nevel CJ, Demeyer DI. 1997. Manipulation of ruminal fermentation. In: Hubson PN, Stewart CS, editors. The Rumen microbial ecosystem. London (UK): Blackie Acad. and Prof., an imprint of Chapman and Hall; p. 523–632.

Nocek JE, Kesler EM. 1980. Growth and rumen characteristics of Holstein steers fed pelleted or conventional diets. J Dairy Sci. 63(2):249–254.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Washington (DC): National Academy Press.

Palmquist DL, Jenkins TC. 1980. Fat in lactation rations: review. J Dairy Sci. 63(1):1–14.

Phillips CJC. 2004. The effects of forage provision and group size on the behavior of calves. J Dairy Sci. 87(5):1380–1388.

Quigley JN, Hill TM, Hulbert LE, Dennis TS, Suarez-Mena ZF, Bortoluzzi EM. 2019. Effects of fatty acids and calf starter form on intake, growth, digestion, and selected blood metabolites in male calves from 0 to 4 months of age. J Dairy Sci. 102(9):8074–8091.

Rastgoo M, Kazemi-Bonchenari M, HosseinYazdi M, Mirzaei M. 2020. Effects of corn grain processing method (ground versus steam-flaked) with rumen undegradable to degradable protein ratio on growth performance, ruminal fermentation, and microbial protein yield in Holstein dairy calves. Anim Feed Sci Technol. 269:114646.

Singh M, Sharma K, Dutta N, Singh P, Verma AK, Mehra UR. 2007. Estimation of rumen microbial protein supply using...
urinary purine derivatives excretion in crossbred calves fed at different levels of feed intake. Asian Australas J Anim Sci. 20(10):1567–1574.
Soliva CR, Meile L, Ciešlak A, Kreuzer M, Machmüller A. 2004. Rumen simulation technique study on the interactions of dietary lauric and myristic acid supplementation in suppressing ruminal methanogenesis. Br J Nutr. 92(4):689–700.
Stewart GD, Schingoethe DJ. 1984. Evaluation of high starch and high fat rations for dairy calves. J Dairy Sci. 67(3):598–605.
Suarez BJ, Reenen CGV, Stockhofe N, Dijkstra J, Gerrits WJJ. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. J Dairy Sci. 90(5):2390–2403.
Tamate H, McGilliard AD, Jacobson NL, Getty R. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J Dairy Sci. 45(3):408–420.
Terré M, Devant M, Bach A. 2006. Performance and nitrogen metabolism of calves fed conventionally or following an enhanced-growth feeding program during the preweaning period. Liv Sci. 105(1–3):109–119.
Tsai CY, Rezamand P, Loucks WI, Scholte CM, Doumit ME. 2017. The effect of dietary fat on fatty acid composition, gene expression and vitamin status in pre-ruminant calves. Anim Feed Sci Technol. 229:32–42.
Van De Stroet DL, Calderón Díaz JA, Stalder KJ, Heinrichs AJ, Dechow CD. 2016. Association of calf growth traits with production characteristics in dairy cattle. J Dairy Sci. 99(10):8347–8355.
Van Keulen J, Young BA. 1977. Acid insoluble ash as a natural marker for digestibility studies. J Anim Sci. 44(2):282–287.
Van Soest PJ, Robertson JB, Lewi BA. 1991. Methods for dietary fiber, neutral detergent fiber nonstarch polysaccharides in relation to animal nutrition. J Anim Sci. 74(10):3583–3597.
Yang CMJ. 2002. Response of forage fibre degradation by ruminal microorganisms to branched-chain volatile fatty acids, amino acids, and dipeptides. J Dairy Sci. 85(5):1183–1190.
Yousefinejad S, Fatahnia F, Kazemi-Bonchenari M, Khanaki H, Drackley JK, Ghaffari MH. 2021. Soybean oil supplementation and starter protein content: effects on growth performance, digestibility, ruminal fermentation, and urinary purine derivatives of Holstein dairy calves. J Dairy Sci. 104(2):1630–1644.