J. Dairy Sci. 105:9597–9609
https://doi.org/10.3168/jds.2022-22165
© 2022, The Authors. Published by Elsevier Inc. and Fass Inc. on behalf of the American Dairy Science Association®.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

# Growth performance, nutrient digestibility, and ruminal fermentation of dairy calves fed starter diets with alfalfa hay versus corn silage as forage and soybean oil versus palm fatty acids as fat source

**Pedram Panahiha,[1] Hamidreza Mirzaei-Alamouti,[1]* Mehdi Kazemi-Bonchenari,[2] and Jörg R. Aschenbach[3]***
[1]Department of Animal Science, University of Zanjan, Zanjan, 45371-38791, Iran
[2]Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, Arak, 38156-8-8349, Iran
[3]Institute of Veterinary Physiology, Freie Universität Berlin, Oertzenweg 19b, 14163 Berlin, Germany

## ABSTRACT

The present study was intended to evaluate the effect of forage source (alfalfa hay; ALF vs. corn silage; CS) along with a supplemental fat source (soybean oil; SO vs. rumen-inert palm fatty acids; PF) on growth performance, nutrient digestibility, and ruminal fermentation in dairy calves. Forty-eight new-born Holstein female calves (3 d old) were assigned to one of 4 treatments: (1) alfalfa hay with soybean oil (ALF–SO); (2) alfalfa hay with palm fatty acids (ALF–PF); (3) corn silage with soybean oil (CS–SO); (4) corn silage with palm fatty acids (CS–PF). Starter diets had equal amounts of forage (100 g/kg dry matter; DM) and fat source (30 g/kg DM). Calves were fed a constant amount of milk (d 1 to 63) and had ad libitum access to water and starters (d 1 to 83). The lowest and greatest starter intakes during the preweaning period occurred in ALF–SO and CS–PF, respectively. This coincided with forage × fat source interaction for average daily gain (ADG) during preweaning. The forage source affected total DM intake and ADG over the entire period, body weight (BW) at weaning, and final BW with greater values in calves that received CS compared with ALF. The concentrations of total short-chain fatty acids and butyrate were increased, whereas concentration of acetate and acetate:propionate ratio were decreased in the rumen of calves fed CS compared with ALF. Feeding CS increased urinary excretion of allantoin and, as a trend, total purine derivatives (PD) and estimated microbial protein synthesis in comparison with ALF. The fat source affected starter intake, ADG, and BW postweaning with the highest values in PF. The digestibility of neutral detergent fiber, crude protein and, as a trend, organic matter were higher in calves fed PF compared with SO. Calves fed PF had lower ruminal ammonia-N concentration and urinary N excretion and greater urinary excretion of allantoin and total PD. Calves receiving SO had a lower ruminal protozoa population. In conclusion, supplementing starter diets with CS and PF is superior to ALF and SO. Interaction of the positive effects of CS and PF on performance underlines that concurrent supplementation of CS with PF is especially recommendable in young calves before weaning.

**Key words:** calf growth, starter diet, ruminal fermentation

## INTRODUCTION

Providing forage in the starter diets of dairy calves is a common practice in commercial dairy farms. However, recommendation for forage incorporation level in starter diets of dairy calves may be influenced by forage source, forage particle size, starter physical form, milk feeding volume, and other factors (Phillips, 2004; Beiranvand et al., 2014; Mirzaei et al., 2016; Hosseini et al., 2019). Reduced total DMI may be a negative side effect of forage inclusion in starter diets of young calves (Phillips, 2004; Hosseini et al., 2019), attributable to their NDF filling effect in the rumen (Allen and Piantoni, 2014). Moreover, the lower energy content of forage compared with concentrate can reduce the energy density per unit of starter feed when forage is incorporated in starters compared with forage-free starters (Molaei et al., 2021). This can finally limit the total metabolizable energy provided to young dairy calves and may become an obstacle especially for early weaning programs where adequate energy content in starter feed is essential.

Fat supplementation can compensate for the low energy density per unit of feed when forage is included in starters (Ghasemi et al., 2017; Karimi et al., 2021). However, negative effects of supplemental fat on fiber and other nutrients' digestibility remain a concern

Received April 6, 2022.
Accepted July 23, 2022.
*Corresponding authors: alamoutih@gmail.com and joerg.aschenbach@fu-berlin.de

(Ghorbani et al., 2020; Karimi et al., 2021). Furthermore, supplemental fat level and source and method of its delivery can have an influence on the extent of these negative effects on ruminal fermentation (Berends et al., 2018; Ghorbani et al., 2020; Kazemi-Bonchenari et al., 2020). The unfavorable effects of fat on ruminal fermentation are related to toxic effects of lipid metabolites on ruminal microbes, the physical coating effect of fat on fiber, and alterations of ruminal microbes through fat supplementation (Ikwuegbu and Sutton, 1982; Soliva et al., 2004). Young calves lack sufficient populations of cellulolytic bacteria in the early weeks of life; therefore, forage inclusion in starters should be done with caution in this critical period (Nocek and Kesler 1980). Moreover, supplemental fat has a potential to deteriorate the nutrient digestibility of the forage portion of starters due to negative effects on the development of the microbial community (Ikwuegbu and Sutton, 1982). Although establishment of ciliate protozoa occurs later than bacteria in young ruminants (Eadie, 1962), the negative effect of some fatty acids (**FA**) on protozoa numbers in the rumen may be partly responsible for reduced NDF digestibility in fat-supplemented diets. This can be due to the pivotal role of protozoa in fiber digestibility in ruminants (Sutton et al., 1983). However, data availability on the effect of supplemental fat on ruminal protozoa in young calves is rather scarce.

Inclusion of forage in starter diets of dairy calves has favorable effects on the stability of the ruminal environment, microbial development, and the health of animals (NASEM, 2021). In a recent recommendation, the National Academies of Science, Engineering, and Medicine (NASEM, 2021) stated that alfalfa hay (**ALF**) should be limited to no more than 10% of total DMI in young calves. Therefore, it seems vital to evaluate strategies to feed forage and fat concurrently in dairy calves to support the high energy demand for accelerated growth and, at the same time, keep the animal in a healthy condition.

Recently, Karimi et al. (2021) evaluated soybean oil (**SO**) in starter diets containing ALF as the sole source of forage. They concluded that feeding ALF with a fat supplement may not be recommended in early weeks of life. However, the amount of ALF included in that study was 150 g/kg DM, which was higher than the maximum level recommended by NASEM (2021). These results suggest a limitation of feed intake by gut fill when including too much forage in calf starter diets (Allen and Piantoni, 2014). The latter conforms with Aragona et al. (2020), who stated that dairy calves can consume substantial amounts of forage with concurrent suppression of total DMI. In a comparison of different forages, ad libitum intake for ALF was higher than ad libitum intake of oat hay (Castells et al., 2013). Consequently, limiting the amount of forage, especially of ALF, is deemed appropriate for young calves to keep their health and optimum growth performance.

In addition to suppressing total starter feed intake (Phillips, 2004) and decreasing BW gain (Leibholz, 1975), forage addition to young calves has also been blamed for decreasing nutrient digestibility (Leibholz, 1975) and shifting rumen fermentation in favor of acetate rather than butyrate production, thus, delaying rumen papillae development (Tamate et al., 1962; Nocek and Kesler, 1980). Moreover, the digestibility of NDF may be more negatively influenced when fat is included with an ALF diet (Karimi et al., 2021).

In contrast to ALF, corn silage (**CS**) has been shown to have favorable effects on young dairy calves at dietary inclusion proportions of 15%, which can be due to reduced dustiness of starters, increased moisture, and higher palatability (Mirzaei et al., 2016). Nonetheless, application of CS as a forage source in young dairy calves is also seen to be critical based on the general concerns about forage addition described above. Additionally, to the knowledge of the authors, no study exists that evaluates fat supplementation in calves receiving CS as forage source in starter.

It can be supposed that an optimum forage level along with a suitable source of fat may have favorable effects on the energy content of starter feed to support maximum growth of young calves. Furthermore, it might be proposed that using an optimum content of fat and forage in starter diets of dairy calves may create the opportunity to eliminate or at least reduce negative effects of fat on nutrient digestibility. Although SO, as unsaturated fat source rich in linoleic acid, has been used extensively in dairy calves (Hill et al., 2015; Ghasemi et al., 2017; Ghorbani et al., 2020; Karimi et al., 2021; Yousefinejad et al., 2021), its effects in starter diets containing CS are unknown. Moreover, we additionally evaluated rumen-inert palm fatty acids (**PF**) rich in palmitic acid in the current study. A previous study on steers fed a high level of forage showed that supplementation of 40 g/kg of hydrogenated palm oil showed no negative effects on feed intake and growth performance but modified the FA profile and some quality traits of meat (Castro et al., 2016). The response of young calves to different fat sources has not been documented when calves received different forage sources. Thus, the aim of the present study was to assess the effects of 2 forage sources (ALF vs. CS) along with 2 fat sources (SO vs. PF) on growth performance, nutrient digestibility, ruminal fermentation, protozoa population, and urinary purine derivatives in young dairy calves. We hypothesized that rumen-inert PF would provide extra energy for growth with less suppressive effects on ru-

minal fermentation and that a combination of PF with CS would affect the target variables most favorably to allow for maximum growth.

## MATERIALS AND METHODS

The present study was conducted in a commercial dairy farm (Zarrin-Khooshe Agriculture and Animal Husbandry, Arak, Iran). Experimental design, management protocols, and procedures were approved by the Animal Care Committee at the University of Zanjan, Iran (ID 1353).

### Animals, Experimental Treatments, and Management

A total of forty-eight 3-d-old female Holstein dairy calves with 40.7 ± 2.1 kg of initial BW were randomly assigned to experimental diets (n = 12 calves per treatment) in a 2 × 2 factorial arrangement with the factors forage source (ALF vs. CS) and fat source (SO vs. PF) of starter diets without blinding to investigators. Calves were separated from their dams immediately after birth, weighed, and moved to individual pens (1.2 × 2.5 m) bedded with sand, the latter being renewed every 24 h. Based on the routine protocol of calf rearing at the farm, all calves were fed 2.5 L of colostrum at each of the first 2 feedings (i.e., within 1.5 h of life and at 12 h after the first feeding). Colostrum feeding was continued for the first 2 d of life. The quality of colostrum was measured with a digital Brix refractometer (PAL-1, Atago Co. Ltd.); colostrum was discarded if the Brix scale was below 22. Calves received whole milk in amounts of 4.5 L/d from d 3 to 10, 7 L/d from d 11 to 40, 3.5 L/d from d 41 to 50, 2.5 L/d from d 51 to 60 and 1.5 L/d on d 61 and 62. Calves were weaned on d 63 and remained in the study until d 83 of age. Whole milk was sampled weekly and analyzed for fat, CP, lactose, and TS using an infrared spectrophotometer (Foss Milk-O-Scan, Foss Electric). The average composition of offered milk was 31.9 ± 0.10 g/kg fat, 30.9 ± 0.4 g/kg CP, 48.8 ± 0.4 g/kg lactose, and 119.1 ± 6.9 g/kg total solids.

Experimental diets were isoenergetic, isonitrogenous, and formulated to meet National Research Council (NRC, 2001) requirements. We evaluated 4 treatments in the current study: (1) starter diet containing 10% ALF with 3% SO (**ALF**–**SO**); (2) starter diet containing 10% ALF with 3% PF (**ALF**–**PF**); (3) starter diet containing 10% CS with 3% SO (**CS**–**SO**); and (4) starter diet containing 10% CS with 3% PF supplementation (**CS**–**PF**). To eliminate bias by particle size distribution (Mirzaei et al., 2016), geometric mean particle size was similar in ALF (2.88 ± 0.11) and CS (2.93 ± 0.12 mm). The content of CP was 16.8 and 9.1% and the content of NDF was 49.3 and 47.8% for ALF and CS, respectively. The concentrate feed was mixed well with forages and the diet was offered as TMR throughout the study. The SO source (Naz Industrial Vegetable Oil Co.) had the following FA composition: C16:0 = 12.1%, C18:0 = 5.2%, C18:1 = 21.8%, C18:2 = 51.2%, C18:3 = 8.1%, and other FA = 1.6%. The PF source (rumen-inert; Energizer RP-10, IFFCO) contained: C12:0 = 2.3%, C14:0 = 4.2%, C16:0 = 86.0%, C18:0 = 2.0%, C18:1 = 4.1%, and other FA = 1.4%. Ingredients and chemical composition of the experimental calf starters are presented in Table 1. Starter feed was fed ad libitum to permit at least 10% waste or uneaten feed. The calves had free access to water throughout the experimental period.

### Intake, Daily Gain, Feed Efficiency, and Nutrient Digestibility

Starter feed refusals were collected and recorded daily at 0730 h and fresh starter was fed at 0800 h. Measurements of BW were taken at 10-d intervals through the study starting from d 3 until d 83. Calves were weighed before the morning meal to minimize the effects of gastrointestinal tract fill on recorded BW. Starter feed offered and refused was weighed daily to determine the total starter DMI for each calf throughout the study. Average daily gain and feed efficiency (**FE**), defined as kg of BW gain/kg of total DMI (starter DMI + milk DMI), were calculated pre- and postweaning, and over the whole experimental period. Feed and leftover feed samples were combined over 21-d intervals and dried in a convection oven (60 C° for 48 h). Subsamples of dried feeds and uneaten feeds were mixed thoroughly and ground in a mill (Ogaw Seiki Co. Ltd.) to pass a 1-mm screen before chemical analysis. Standard methods of AOAC International (2002) were used for the determination of DM (method 2001.12), ash (method, 942.05), CP (method 991.20), and ether extract (method 920.39). The method of Van Soest et al. (1991) was used to determine NDF. For NDF analysis, samples were treated with a heat-stable α-amylase in the absence of sodium sulfite and not corrected for residual ash and protein. To determine apparent digestibility, fecal samples were collected manually from the rectum during 5 consecutive days (from d 78 to 82) at 6, 12, and 18 h after the morning meal. The collected fecal samples were dried at 72°C for 48 h in a forced-air oven, ground to pass a 1 mm screen in a Wiley mill (Ogaw Seiki Co. Ltd.), and then mixed thoroughly. Acid insoluble ash was used as an internal marker to estimate apparent total-tract digestibility coefficients of OM, CP, ether extract, and NDF according to Van Keulen and Young (1977).

**Table 1.** Experimental starter diet ingredients and chemical composition

| | Treatment[1] | | | |
|---|---|---|---|---|
| | ALF | | CS | |
| Item | SO | PF | SO | PF |
| Ingredient, g/kg of DM | | | | |
| Alfalfa hay, chopped | 100 | 100 | 0 | 0 |
| Corn silage, chopped | 0 | 0 | 100 | 100 |
| Barley grain, ground | 100 | 100 | 100 | 100 |
| Corn grain, coarsely ground | 460 | 430 | 460 | 430 |
| Soybean meal | 260 | 290 | 260 | 290 |
| Soybean oil | 30 | 0 | 30 | 0 |
| Palm fatty acids | 0 | 30 | 0 | 30 |
| Calcium carbonate | 10 | 10 | 10 | 10 |
| Dicalcium phosphate | 5 | 5 | 5 | 5 |
| Sodium bicarbonate | 12 | 12 | 12 | 12 |
| Salt | 5 | 5 | 5 | 5 |
| Vitamin and mineral mix[2] | 18 | 18 | 18 | 18 |
| Chemical composition, g/kg of DM, unless stated otherwise | | | | |
| ME,[3] Mcal/kg | 2.87 | 2.88 | 2.91 | 2.92 |
| CP | 202 | 203 | 201 | 202 |
| NDF | 179 | 182 | 178 | 180 |
| Ether extract (EE) | 54.2 | 54.3 | 54.2 | 54.3 |
| NFC[4] | 504 | 499 | 506 | 502 |
| Ca | 87 | 87 | 87 | 87 |
| P | 43 | 43 | 43 | 43 |

[1]Treatments were alfalfa hay-containing starter diet supplemented with soybean oil (ALF–SO); alfalfa hay-containing starter diet supplemented with palm fatty acids (ALF–PF); corn silage-containing starter diet supplemented with soybean oil (CS–SO); corn silage-containing starter diet supplemented with palm fatty acids (CS–PF).

[2]Contained per kilogram: vitamin A (IU) = 1,200,000, vitamin D (IU) = 150,000, vitamin E (IU) = 1,300, Ca (g) = 110, P (g) = 30, Mg (g) = 40, Zn (mg) = 2,200, Cu (mg) = 600, I (mg) = 120, Co (mg) = 100, Mn (mg) = 1,700, Se (mg) = 120.

[3]Calculated according to NRC (2001).

[4]Calculated as DM − (NDF + CP + EE + ash) (NRC, 2001).

### Structural Growth Indicators

Structural growth measurements were taken at the start of the experiment (d 3), at weaning (d 63) and at the end of the study (d 83) according to the method described by Heinrichs et al. (2003) for dairy calves. They included withers height (distance from the base of the front feet to withers), hip height (distance from the base of the rear feet to the hook bones), heart girth (circumference of the chest), body length (distance between the points of shoulder and rump), and body girth (circumference of the belly before feeding).

### Ruminal Sampling and Protozoa Counting

Ruminal fluid (approximately 20 mL) was collected (3 to 4 h after morning feeding) on d 34 (preweaning) and d 79 (postweaning) of the experiment using a stomach tube fitted to a vacuum pump. The first 10 mL was discarded to eliminate the potential saliva contamination and thereafter, ruminal pH was measured immediately (HI 8314 membrane pH meter, Hanna Instruments). Ruminal fluid samples were squeezed through 4 layers of cheesecloth. An aliquot (10 mL) was preserved with 2 mL of 25% meta-phosphoric acid and frozen at −20°C. After thawing at room temperature, experimental samples were analyzed for short-chain fatty acids (**SCFA**) concentrations using gas chromatography (model CP-9002, Chrompack) as explained in detail previously (Kazemi-Bonchenari et al., 2016). A ruminal fluid subsample was thawed at room temperature and clarified by centrifuging (15,000 × *g* for 20 min), then decanted and analyzed for $NH_3$–N concentration using a modified phenol-hypochlorite reaction (Broderick and Kang, 1980).

Rumen protozoa were counted by use of a Neubauer counting chamber (0.1 mm depth; Hausser Scientific Co.) from aliquots of ruminal contents conserved in a solution of methyl green–formalin–saline according to Dehority (1993).

### Urinary Purine Derivatives and Urinary Nitrogen Excretion

The spot sampling technique was used to estimate microbial protein synthesis (**MPS**) in the rumen. This technique is based on the purine derivatives (**PD**) excretion obtained via urine in dairy calves during the postweaning period as explained by Kazemi-Bonchenari et al. (2022). Urine volume excretion rate per day was estimated by urinary creatinine excretion using the following model; BW × 26.8/creatinine concentration (mg/L) as reported by Dennis et al. (2018). Spot urine samples were collected on 3 consecutive days during the postweaning period from each calf in the morning (between 0900 and 1100 h) and afternoon (between 1500 and 1700 h). Samples (approximately 10 mL) were collected when calves urinated spontaneously. An aliquot of 5 mL of each sample was diluted immediately with 45 mL of 0.036 *N* sulfuric acid and stored at −20°C for analysis. The concentrations of creatinine, urea-N, uric acid, and allantoin were measured in thawed urine samples as described previously (Kazemi-Bonchenari et al., 2017). Estimated daily urine output was used to calculate daily urinary excretion of allantoin + uric acid as total daily PD. The ruminal MPS was estimated from total daily PD output based on an equation of Chen and Gomes (1992): MPS (g/d) = 70 × PD (mmol/d)/(0.85 × 0.116 × 0.83 × 1,000) × 6.25; where 70 is the N content of purines coefficient (mg N/mmol), 0.85 is the efficiency of PD absorption, 0.116 is the ratio of purine-N to total N in mixed ruminal microbes, 0.83 is the average digestibility of microbial purines, and 6.25 is the N content of protein.

### Statistical Analysis

All data points without any exclusion and calf as experimental unit were analyzed as a completely randomized design with a 2 × 2 factorial arrangement of treatments, with the factors forage source (ALF vs. CS) and fat source (SO vs. PF). The MIXED procedure of SAS (SAS 9.1, SAS Institute Inc.) was used with fixed effects of time, forage source, fat source, and their interactions and calf as a random effect. Power analysis for sample size estimation was performed (Morris, 1999) for the primary response variables, including intake, BW, and ADG, based on previously published values (Zhang et al., 2010; Miller-Cushon and DeVries, 2011). From the power test analysis, using $\alpha = 0.05$ and power = 0.80, the projected sample size was 12 calves per treatment for starter intake, total DMI, ADG, and BW. Repeated measures analysis was used for intakes of starter feed, milk, and total DMI (recorded daily), ADG, FE, BW (recorded every 10 d), skeletal growth (d 3, 63, and 83), ruminal pH, urinary PD excretion, MPS, and protozoa population over time. Initial structural growth measurements were considered as covariates for skeletal growth analysis. Before analyses, all data were screened for normality using the UNIVARIATE procedure of SAS. A heterogeneous autoregressive type 1 covariance structure was used in the mixed model. If the primary test indicated statistical significance, differences among treatment means were determined using Tukey's multiple range tests. Effects were considered significant when $P \leq 0.05$, and a tendency was considered when $0.05 < P \leq 0.10$. All data are contained within the manuscript.

## RESULTS

### Starter Intake, Daily Gain, Feed Efficiency, and Nutrient Digestibility

Starter intake during the preweaning period was affected by forage source ($P = 0.033$) and fat source ($P < 0.01$) with a trend for interaction between forage × fat source ($P = 0.054$) with CS showing greater intake than ALF and PF showing greater intake than SO (Table 2). An effect of fat source (PF > SO) was also evident in the postweaning period ($P = 0.056$) and, as a trend, over the entire period ($P = 0.094$). Because milk intake was not different among groups, total DMI (i.e., intake of milk DM + starter DM) also showed an effect of forage source ($P = 0.021$) and fat source ($P = 0.016$), with trend for an interaction between forage × fat source that pointed to highest DMI in CS–PF ($P = 0.060$).

The changes in starter intake were almost mirrored by changes in ADG, which tended to be affected by forage source ($P = 0.063$) and was affected by fat source ($P = 0.011$), with an interaction between forage × fat source in the preweaning period with greatest ADG in CS–PF and lowest ADG in ALF–SO ($P = 0.047$). An effect of fat source (PF > SO) on ADG was also evident in the postweaning period ($P < 0.01$). Over the entire period, ADG was affected by forage ($P = 0.045$) and fat source ($P = 0.017$), with a trend for an interaction between forage × fat source ($P = 0.075$), pointing to greatest ADG in CS–PF. Given the positive effect of CS and PF on starter intake and ADG, BW at weaning and final BW were greater in calves fed CS versus ALF ($P = 0.059$ and $P = 0.031$, respectively) and greater for PF versus SO ($P = 0.014$ and $P < 0.01$, respectively). Feed efficiency (**FE**) was not different among groups, except for a trend for higher FE in calves receiving PF compared with SO in the preweaning period ($P = 0.098$; Table 2).

**Table 2.** Least squares means for starter intake, ADG, and feed efficiency in dairy calves fed alfalfa hay (ALF) or corn silage (CS) as forage (For) sources and soybean oil (SO) or palm fatty acids (PF) as fat sources (n = 12 calves per treatment)

| | Treatment | | | | | *P*-value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ALF | | CS | | | For | Fat | For × Fat | Time | Time × For | Time × Fat | Time × For × Fat |
| Item | SO | PF | SO | PF | SEM | | | | | | | |
| Starter feed intake, g/d | | | | | | | | | | | | |
| Preweaning (d 3–63) | 376 | 434 | 415 | 581 | 31.3 | 0.033 | <0.01 | 0.054 | | | | |
| Postweaning (d 64–83) | 2,088 | 2,219 | 2,221 | 2,390 | 83.5 | 0.127 | 0.056 | 0.796 | | | | |
| Entire period (d 3–83) | 804 | 881 | 865 | 1,033 | 94.9 | 0.209 | 0.094 | 0.588 | 0.002 | 0.327 | 0.379 | 0.642 |
| Milk intake, g/d | 649 | 647 | 646 | 649 | 21.3 | 0.974 | 0.982 | 0.938 | | | | |
| Total DMI (milk + starter), g/d | 1,082 | 1,025 | 1,065 | 1,231 | 47.4 | 0.021 | 0.016 | 0.060 | 0.007 | 0.210 | 0.258 | 0.567 |
| ADG, g/d | | | | | | | | | | | | |
| Preweaning (d 3–63) | 493[c] | 549[b] | 512[c] | 678[a] | 34.2 | 0.063 | 0.011 | 0.047 | | | | |
| Postweaning (d 64–83) | 821 | 872 | 847 | 959 | 46.8 | 0.114 | <0.01 | 0.311 | | | | |
| Entire period (d 3–83) | 575 | 629 | 596 | 748 | 34.6 | 0.045 | 0.017 | 0.075 | 0.010 | 0.007 | 0.838 | 0.162 |
| BW, kg | | | | | | | | | | | | |
| Initial (d 3) | 40.4 | 40.6 | 41.9 | 39.5 | 1.43 | 0.757 | 0.283 | 0.246 | | | | |
| Weaning (d 63) | 69.6 | 73.1 | 72.9 | 80.2 | 1.98 | 0.059 | 0.014 | 0.252 | | | | |
| Final (d 83) | 86.0 | 90.4 | 90.9 | 99.1 | 1.81 | 0.031 | <0.01 | 0.176 | | | | |
| Feed efficiency[1] | | | | | | | | | | | | |
| Preweaning (d 3–63) | 0.48 | 0.49 | 0.47 | 0.54 | 0.02 | 0.368 | 0.098 | 0.241 | | | | |
| Postweaning (d 64–83) | 0.40 | 0.39 | 0.39 | 0.42 | 0.03 | 0.776 | 0.965 | 0.527 | | | | |
| Entire period (d 3–83) | 0.45 | 0.46 | 0.46 | 0.50 | 0.03 | 0.449 | 0.121 | 0.198 | 0.083 | 0.091 | 0.185 | 0.368 |

[a–c]Values not sharing a common letter differ ($P < 0.05$).

[1]Kg of BW gain/kg of total DMI.

**Table 3.** Least squares means for nutrient digestibility in dairy calves fed alfalfa hay (ALF) or corn silage (CS) as forage (For) sources and soybean oil (SO) or palm fatty acids (PF) as fat sources (n = 12 calves per treatment)

| | Treatment | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | ALF | | CS | | | | | |
| Item | SO | PF | SO | PF | SEM | For | Fat | For × Fat |
| OM, % | 64.5 | 69.4 | 68.9 | 71.8 | 1.44 | 0.125 | 0.052 | 0.691 |
| Ether extract, % | 80.9 | 83.5 | 82.8 | 83.7 | 1.37 | 0.493 | 0.276 | 0.578 |
| NDF, % | 41.0 | 48.8 | 43.8 | 52.8 | 1.67 | 0.351 | 0.015 | 0.865 |
| CP, % | 63.3 | 70.9 | 62.6 | 72.3 | 1.19 | 0.918 | 0.022 | 0.675 |

Nutrient digestibility was not influenced by different forage types. The digestibility of NDF ($P = 0.015$), CP ($P = 0.022$) and, as a trend, OM ($P = 0.052$) was higher when calves were fed PF compared with SO (Table 3).

Regarding the time effect and its interaction with variables, results showed that time effect was significant for starter intake (Figure 1), total DMI, ADG ($P < 0.01$), and, as a trend, FE ($P = 0.083$). Interaction of time and forage was significant ($P = 0.007$) when ADG changes were considered over the entire period of the experiment (Table 2).

## Growth Indices

Withers height and heart girth were or tended to be greatest at weaning in CS–PF as supported by forage × fat source interaction ($P = 0.013$ and $P = 0.052$, respectively; Table 4). A greater withers height in CS–PF was still measurable at the end of the experiment (forage × fat source interaction, $P = 0.032$). Feeding CS compared with ALF tended to increase hip height at weaning ($P = 0.077$). Supplemental PF in comparison to SO increased hip height during preweaning ($P = 0.042$) and the entire period ($P = 0.036$; Table 4).

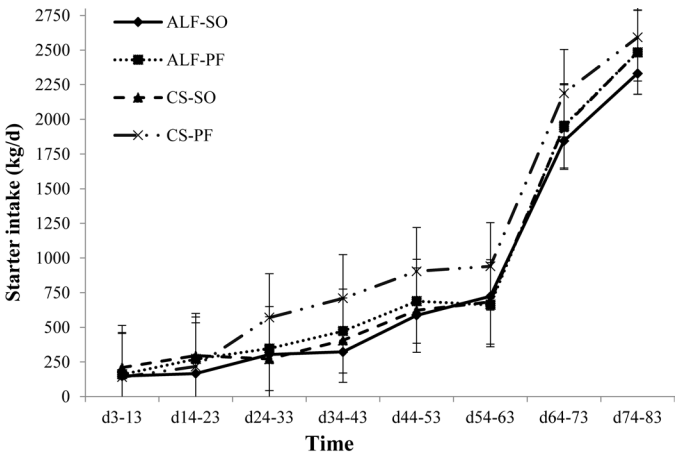


**Figure 1.** Starter intake in calves receiving different starter diets. (♦) Alfalfa hay with soybean oil (ALF–SO); (■) alfalfa hay with palm fatty acids (ALF–PF); (▲) corn silage with soybean oil (CS–PF); and (×) corn silage with palm fatty acids (CS–PF). Factor and interaction *P*-values are presented in Table 2. Error bars represent SEM.

## Ruminal Fermentation Profile and Protozoa Population

Ruminal pH tended to be lower in calves supplemented with PF compared with SO during the preweaning period ($P = 0.091$; Table 5). This coincided with lower ruminal ammonia-N concentration in calves fed PF compared with SO during preweaning and the entire period ($P < 0.05$). Furthermore, ruminal ammonia-N concentration tended to be affected by forage source in the preweaning period (CS < ALF; $P = 0.051$; Table 5).

Regarding total SCFA concentration, effect of forage source during pre- ($P = 0.038$) and postweaning ($P = 0.014$) and an effect of fat source during preweaning were accompanied by forage × fat source interactions during both periods ($P = 0.048$) with greatest SCFA concentration in the CS–PF group (Table 5). No interactions were found for individual SCFA between forage source × fat source. However, the concentration of ruminal butyrate was greater and that of acetate was smaller in calves fed CS compared with ALF during pre- and postweaning ($P < 0.05$ each), which coincided with a lower ratio of acetate:propionate in calves fed CS. The concentration of branched-chain (**BC**)-SCFA was greater in the ruminal fluid of calves supplemented with PF compared with SO during the preweaning period ($P = 0.023$; Table 5).

Protozoa populations were higher when calves were supplemented with PF diets compared with SO diets ($P = 0.027$). Forage level or its interaction with fat source did not have any effect on protozoa population (Table 6).

## Urinary Purine Derivatives and Nitrogen Efficiency

Feeding PF compared with SO increased the urinary excretion of allantoin ($P = 0.023$) and PD ($P = 0.038$)

**Table 4.** Least squares means for growth indices in dairy calves fed alfalfa hay (ALF) or corn silage (CS) as forage (For) sources and soybean oil (SO) or palm fatty acids (PF) as fat sources (n = 12 calves per treatment)

| | Treatment | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | ALF | | CS | | | | | |
| Item | SO | PF | SO | PF | SEM | For | Fat | For × Fat |
| Heart girth, cm | | | | | | | | |
| d 3 | 79.6 | 79.8 | 80.4 | 79.9 | 0.56 | 0.214 | 0.604 | 0.917 |
| d 63 | 99.8 | 98.1 | 98.8 | 100 | 1.02 | 0.753 | 0.931 | 0.052 |
| d 83 | 106 | 105 | 104 | 108 | 1.34 | 0.797 | 0.137 | 0.144 |
| Body length, cm | | | | | | | | |
| d 3 | 48.3 | 47.6 | 48.2 | 47.5 | 0.65 | 0.788 | 0.127 | 0.577 |
| d 63 | 61.0 | 59.8 | 60.5 | 61.3 | 0.79 | 0.639 | 0.412 | 0.401 |
| d 83 | 63.2 | 63.6 | 64.7 | 64.5 | 0.80 | 0.410 | 0.920 | 0.848 |
| Withers height, cm | | | | | | | | |
| d 3 | 81.0 | 80.3 | 80.2 | 81.3 | 0.98 | 0.853 | 0.837 | 0.269 |
| d 63 | 95.7$^{ab}$ | 93.5$^{b}$ | 94.6$^{ab}$ | 97.3$^{a}$ | 1.34 | 0.093 | 0.862 | 0.013 |
| d 83 | 99.8$^{ab}$ | 97.6$^{b}$ | 99.3$^{ab}$ | 101$^{a}$ | 1.89 | 0.136 | 0.886 | 0.032 |
| Hip height, cm | | | | | | | | |
| d 3 | 70.6 | 79.2 | 78.9 | 79.5 | 0.86 | 0.761 | 0.958 | 0.506 |
| d 63 | 91.7 | 92.0 | 92.1 | 94.3 | 1.06 | 0.077 | 0.042 | 0.182 |
| d 83 | 95.8 | 97.6 | 96.8 | 97.6 | 1.19 | 0.438 | 0.036 | 0.356 |

$^{a,b}$Values within a row not sharing a common letter differ ($P < 0.05$).

and, hence, the estimated MPS ($P = 0.038$; Table 6). Feeding CS in comparison with ALF also increased the urinary excretion of allantoin ($P = 0.042$) and tended to increase PD ($P = 0.054$) and estimated MPS ($P = 0.054$). Urinary N-excretion was decreased by supplemental PF compared with SO ($P = 0.044$; Table 6).

## DISCUSSION

The present study evaluated the inclusion of 2 forage sources (ALF vs. CS) in combination with 2 fat sources (SO vs. PF) in starter diets of young dairy calves on feed intake, nutrient utilization, and growth performance. Starter intake during the preweaning period was enhanced by CS versus ALF and PF versus SO. A trend for interaction of forage × fat source even indicated that both factors can superimpose on each other and cause a 50% change in starter feed intake (581 vs. 376 g/d) in the critical preweaning period. Effects of forage source, as well as supplemental fat source, in starter diets of young calves have been evaluated separately in previous studies. However, limited work is published on the interaction effect of forage and fat in dairy calves during the preweaning period. Regarding forage source, CS may be especially favorable due to lower dustiness of the finely ground grains in the starter feeds containing CS together with a greater palatability and increased moisture (Beiranvand et al., 2016). In dairy cows, increased feed intake in CS versus ALF-based diets was suggested to result from higher ruminal buffering capacity (Erdman, 1988), which has not yet been verified in dairy calves. Regarding different fat sources, UFA as contained in SO have a greater hypophagic effect than saturated FA (Drackley et al., 1992; Harvatine and Allen, 2006), which can be explained by enhanced oxidation of unsaturated FA in the liver (Mashek et al., 2002) with subsequent negative effects on appetite (Allen et al., 2009) or by reduced nutrient digestibility (Ghorbani et al., 2020). In addition, Tsai et al. (2017) stated that lineoleic acid contained in SO has some inflammatory effect in young ruminants that can modulate intake and performance in preweaning calves. Several studies indicated that supplemental FA generally have a negative influence on the starter feed intake in dairy calves under 2 mo of age (Hill et al., 2015; Ghorbani et al., 2020). However, no negative effects on the feed intake of steers was found when supplementing diets using 4% hydrogenated palm oil; the only findings were a modified FA profile and some alterations of meat quality traits (Castro et al., 2016). From this, it appears that separate positive effects of PF versus SO on ruminal fermentation and nutrient digestibility, on one hand, and higher palatability of CS versus ALF, on the other hand, interacted to potently increase the starter intake of the CS–PF diet during the preweaning period of the present study. In addition, individual FA effects can also be considered as an explanation for the different responses when supplemental fat is added to starter diets of young calves (Hill et al., 2011).

According to the greater feed intake, the greatest ADG (678 g/d) during the preweaning period was found in calves receiving CS–PF, coinciding with greatest heart girth (100.3 cm) and highest withers height

**Table 5.** Least squares means for ruminal fermentation profile in dairy calves fed alfalfa hay (ALF) or corn silage (CS) as forage (For) sources and soybean oil (SO) or palm fatty acids (PF) as fat sources (n = 12 calves per treatment)

| | Treatment | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | ALF | | CS | | | | | |
| Item | SO | PF | SO | PF | SEM | For | Fat | For × Fat |
| Ruminal pH | | | | | | | | |
| Preweaning | 5.88 | 5.77 | 6.02 | 5.81 | 0.05 | 0.254 | 0.091 | 0.473 |
| Postweaning | 6.0 | 6.12 | 6.07 | 5.96 | 0.07 | 0.513 | 0.885 | 0.154 |
| Ruminal ammonia-N, mmol/L | | | | | | | | |
| Preweaning | 11.6 | 10.1 | 9.93 | 8.65 | 0.48 | 0.051 | 0.036 | 0.847 |
| Postweaning | 15.4 | 12.5 | 14.0 | 13.1 | 0.61 | 0.546 | 0.027 | 0.195 |
| Total short-chain fatty acids, mmol/L | | | | | | | | |
| Preweaning | 72.4[c] | 86.6[b] | 90.3[b] | 95.7[a] | 1.69 | 0.038 | 0.017 | 0.048 |
| Postweaning | 110.6[b] | 106.7[b] | 114.5[b] | 121.6[a] | 2.19 | 0.014 | 0.422 | 0.024 |
| Acetate, mmol/L | | | | | | | | |
| Preweaning | 51.4 | 51.2 | 45.9 | 46.2 | 1.17 | 0.013 | 0.929 | 0.883 |
| Postweaning | 49.3 | 49.8 | 44.7 | 47.8 | 1.28 | 0.011 | 0.107 | 0.118 |
| Propionate, mmol/L | | | | | | | | |
| Preweaning | 34.4 | 32.9 | 35.3 | 33.2 | 1.06 | 0.575 | 0.184 | 0.778 |
| Postweaning | 28.1 | 28.9 | 31.3 | 29.5 | 1.01 | 0.137 | 0.694 | 0.295 |
| Acetate:propionate ratio | | | | | | | | |
| Preweaning | 1.51 | 1.58 | 1.30 | 1.39 | 0.07 | 0.028 | 0.342 | 0.913 |
| Postweaning | 1.77 | 1.73 | 1.44 | 1.64 | 0.06 | 0.019 | 0.348 | 0.174 |
| Butyrate, mmol/L | | | | | | | | |
| Preweaning | 9.28 | 9.62 | 13.4 | 14.1 | 0.61 | 0.019 | 0.569 | 0.873 |
| Postweaning | 16.1 | 14.4 | 17.6 | 16.9 | 0.75 | 0.013 | 0.134 | 0.557 |
| Branched short-chain fatty acids, mmol/L | | | | | | | | |
| Preweaning | 4.91 | 6.04 | 5.22 | 6.44 | 0.32 | 0.471 | 0.023 | 0.922 |
| Postweaning | 6.29 | 6.70 | 6.14 | 5.63 | 0.40 | 0.155 | 0.891 | 0.279 |

[a–c]Values within a row not sharing a common letter differ ($P < 0.05$).

(97.3 cm) at weaning. These results show that feeding starters containing CS with PF supplementation can provide favorable nutritional conditions for acquiring better gain in young dairy calves. This is partly related to an improved ruminal fermentation profile achieved by CS–PF (Table 4) which provided a high energy and microbial protein output for the growth of calves. Our result clarified that feeding CS along with PF provided the greatest ruminal SCFA concentration during the preweaning (95.7 mmol) and postweaning periods (121.6 mmol/L). Bergman (1990) stated that nearly 70% of required energy in ruminants can be supplied through SCFA produced in the rumen; thus, higher SCFA production with CS–PF diets has greater potential to improve growth performance in dairy calves. Because SCFA production depends strongly on fermentable OM in the rumen (Clark et al., 1992), the higher ruminal SCFA concentration in CS–PF indicated that more OM is provided to be fermented when calves received CS along with PF.

**Table 6.** Least squares means for urinary purine derivatives (PD) and protozoa population in dairy calves fed alfalfa hay (ALF) or corn silage (CS) as forage (For) sources and soybean oil (SO) or palm fatty acids (PF) as fat sources (n = 12 calves per treatment)

| | Treatment | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | ALF | | CS | | | | | |
| Item | SO | PF | SO | PF | SEM | For | Fat | For × Fat |
| Allantoin, mmol/d | 12.2 | 14.6 | 14.3 | 16.1 | 0.94 | 0.042 | 0.023 | 0.754 |
| Uric acid, mmol/d | 0.90 | 0.94 | 0.99 | 0.91 | 0.05 | 0.675 | 0.697 | 0.327 |
| Total PD, mmol/d | 13.2 | 15.5 | 15.3 | 17.0 | 1.19 | 0.054 | 0.038 | 0.713 |
| Microbial protein synthesis,[1] g/d | 70.5 | 83.1 | 82.0 | 90.9 | 4.13 | 0.054 | 0.038 | 0.713 |
| Urinary N, g/d | 17.3 | 15.5 | 16.7 | 13.6 | 1.08 | 0.256 | 0.044 | 0.547 |
| Protozoa population × $10^4$/mL | 9.64 | 11.59 | 10.26 | 10.84 | 0.34 | 0.898 | 0.027 | 0.198 |

[1]Microbial protein synthesis (MPS) was estimated from urinary PD excretion based on Chen and Gomes (1992) as MPS (g/d) = 70 × PD (mmol/d)/(0.85 × 0.116 × 0.83 × 1,000) × 6.25.

In addition, a lower digestibility of OM, NDF, and CP in calves supplemented with SO may further explain the lower growth performance of SO diets. The lower protein digestibility was additionally supported by lower ruminal concentrations of BC-SCFA because digested protein is the most likely source of BC-SCFA (Wang et al., 2017). The lower fiber digestibility found for SO-supplemented diets likely plays a crucial role, affecting the digestibility of OM and CP as well, and may result from toxic effects of fat on fiber-digesting bacteria (Soliva et al., 2004). Karimi et al. (2021) recently also identified that unsaturated FA supplementation was associated with reduced digestibility of OM and NDF in dairy calves, which was exacerbated when forage was included in the starter. This suggests that depression of NDF digestibility by fat supplementation may become an issue, especially, upon forage inclusion in dairy calf starters because cellulolytic bacteria are not fully developed in the early weeks of life (Ghorbani et al., 2020). Although we did not characterize the bacterial community in the current study, reduced activity of cellulolytic bacteria such as *Fibrobacter succinogenes* (Maia et al., 2010) and *Ruminococcus flavefaciens* (Maczulak et al., 1981) has been reported previously regarding supplemental fat inclusion in ruminant diets.

Lower protozoa numbers could also contribute to the reduced nutrient digestibility in SO-supplemented starter diets. Ruminal protozoa have a high ability for fiber digestion (Orpin, 1986; Newbold et al., 2015) and their aminopeptidase activity contributes to protein digestion (Forsberg et al., 1984). Protozoal numbers were lower when supplying SO-supplemented diets, which conforms to previous studies where ruminally unprotected unsaturated FA from SO reduced (Machmüller et al., 1998) or eliminated protozoa (Sutton et al., 1983) and altered ruminal fermentation (Machmüller et al., 1998).

The establishment of the ciliate protozoal community occurs later in young ruminants (Eadie, 1962) compared with cellulolytic bacteria, fungi, and archaea (Fonty et al., 1987) and is impeded by low ruminal pH (Hristov et al., 2001). Such pH effects on protozoa abundance can be excluded for the present study because ruminal pH tended to be higher in SO-supplemented diets (5.95) versus PF diets (5.79) preweaning. This also eliminates the possibility that the reduced intake of SO-supplemented diets had a link to ruminal pH, because reductions in feed intake are commonly due to severe reductions of pH (Khan et al., 2008; Kazemi-Bonchenari et al., 2017).

The feeding of CS versus ALF changed the ruminal SCFA pattern toward less acetate (46.0 vs. 51.3 mmol/L for CS vs. ALF) and more butyrate (13.7 vs. 9.4 mmol/L for CS vs. ALF). Butyrate, in turn, is considered favorable for ruminal development (Sakata and Tamate, 1978; Mentschel et al., 2001; Soltani et al., 2017). Greater starch content in CS rather than ALF may be partly responsible for the higher butyrate concentrations in the ruminal fluid of calves receiving the CS diets (Khan et al., 2008; Mirzaei et al., 2016; Rastgoo et al., 2020). This may be partly related to different microbial species fermenting the different substrate provided in starters (Vital et al., 2013; Wang et al., 2017). As such, it is well accepted that starch is the main energy source for ruminal development in young ruminants (Hu et al., 2018) because its higher availability can improve dairy calf growth performance (Makizadeh et al., 2020).

The higher starch content of CS diets may also explain the greater urinary excretion of allantoin and total PD in CS compared with ALF (Kmicikewycz and Heinrichs, 2015), which is indicative of higher MPS in the rumen (Chen and Gomes, 1992). A reduction of allantoin and total PD excretion through urine was detected, on the other hand, in calves fed supplemental SO, which suggests lower microbial activity when supplementing SO compared with PF. The provided OM to ruminal microbes is a key factor that affects MPS (Clark et al., 1992). As discussed earlier, PF versus SO and CS versus ALF increased total DMI in the current study, which, as expected, provided higher OM to ruminal microbes. These facts collectively indicate that the potential to increase MPS in young calves is greater for CS compared with ALF and PF compared with SO. This is in line with total SCFA production in the current study, which was greatest in calves fed CS with PF. In turn, the lower protozoa number in calves receiving SO-supplemented diets does not seem to have a direct link to lower estimated MPS in those calves. Although ciliate protozoa may contribute up to 50% to the bio-mass in the adult rumen (Newbold et al., 2015), it is well known that elimination of protozoa increases microbial protein supply by up to 30% due to decreased bacterial protein turn-over in the rumen. This is because protozoa prey on bacteria as their main protein source (Williams and Coleman, 1992) and, as a result, defaunation makes the rumen more efficient in terms of protein synthesis (Newbold et al., 2015).

Urinary nitrogen excretion did not change with forage source; however, supplemental SO increased urea-N compared with PF diets. Greater urinary nitrogen excretion is indicative of lower nitrogen efficiency in ruminants (Kohn et al., 2005). This indicates that SO supplementation not only had negative effects on ruminal fermentation and nutrient digestibility but also increased urinary N concentration, indicating lower nitrogen utilization efficiency. This means that more nitrogen was directed toward urinary excretion in SO-

supplemented calves instead of being used for MPS. This might be due to lower NDF and OM digestibility caused by supplemental SO, which reduced nutrient availability for ruminal microbes and, hence, less nitrogen was captured in microbial protein.

## CONCLUSIONS

The present study provides unique insight into the effects and interaction of 2 different forages and fat sources on zootechnical traits and readouts of digestibility and ruminal fermentation in starter diets of young dairy calves. Feeding CS proved superior to ALF because it was associated with higher ruminal production of microbial protein and butyrate and enhanced total DMI and BW at weaning and final measurement. Feeding rumen-inert PF proved superior to SO because the latter impaired digestibility of NDF, OM, and CP; decreased estimated MPS; increased ruminal ammonia-N and urinary-N excretion; and was associated with lower DMI and BW at weaning and final measurement. Most importantly, the effects of forage × fat source interacted for ruminal SCFA concentration, ADG and, as a trend, for starter intake in the critical preweaning period with highest values in CS–PF. The latter indicates that concurrent supplementation of CS with PF at the applied dosages is particularly favorable in young calves before weaning.

## ACKNOWLEDGMENTS

The manuscript was extracted from the first author thesis (Grant No. 95-6102) that was financially supported by University of Zanjan, Iran. The authors appreciate the support of A. Sadeghi, technical manager of the Zarrin-Khoosheh commercial dairy farm (Arak, Iran). We further acknowledge support by the Open Access Publication Fund of the Freie Universität Berlin (Germany). The authors have not stated any conflicts of interest.

## REFERENCES

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. https://doi.org/10.2527/jas.2009-1779.

Allen, M. S., and P. Piantoni. 2014. Carbohydrate nutrition: Managing energy intake and partitioning through lactation. Vet. Clin. North Am. Food Anim. Pract. 30:577–597. https://doi.org/10.1016/j.cvfa.2014.07.004.

AOAC International. 2002. Official Methods of Analysis. 17th ed. AOAC International.

Aragona, K. M., F. X. Suarez-Mena, T. S. Dennis, J. D. Quigley, W. Hu, T. M. Hill, and R. L. Schlotterbeck. 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. 103:2324–2332. https://doi.org/10.3168/jds.2019-17474.

Beiranvand, H., G. R. Ghorbani, M. Khorvash, and M. Kazemi-Bonchenari. 2014. Forage and sugar in dairy calves' starter diet and their interaction on performance, weaning age and rumen fermentation. J. Anim. Physiol. Anim. Nutr. (Berl.) 98:439–445. https://doi.org/10.1111/jpn.12089.

Beiranvand, H., M. Khani, S. Omidian, M. Ariana, R. Rezvani, and M. H. Ghaffari. 2016. Does adding water to dry calf starter improve performance during summer? J. Dairy Sci. 99:1903–1911. https://doi.org/10.3168/jds.2015-10004.

Berends, H., M. Vidal, M. Terre, L. N. Leal, J. Martin-Tereso, and A. Bach. 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. 101:10962–10972. https://doi.org/10.3168/jds.2018-15116.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567–590. https://doi.org/10.1152/physrev.1990.70.2.567.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 63:64–75. https://doi.org/10.3168/jds.S0022-0302(80)82888-8.

Castells, L., A. Bach, A. Arís, and M. Terré. 2013. Effects of forage provision to young calves on rumen fermentation and development of the gastrointestinal tract. J. Dairy Sci. 96:5226–5236. https://doi.org/10.3168/jds.2012-6419.

Castro, T., A. Cabezas, J. De LaFuente, B. Isabel, T. Manso, and V. Jimeno. 2016. Animal performance and meat characteristics in steers reared in intensive conditions fed with different vegetable oils. Animal 10:520–530. https://doi.org/10.1017/S1751731115002554.

Chen, X. B., and M. J. Gomes. 1992. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives—An Overview of Technical Details. International Feed Research Unit, Occasional Publ. Rowett Research Institute, Aberdeen, United Kingdom. Accessed Aug. 31, 2022. https://www.researchgate.net/publication/265323654.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304–2323. https://doi.org/10.3168/jds.S0022-0302(92)77992-2.

Dehority, B. A. 1993. Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa. CRC Press.

Dennis, T. S., F. X. Suarez-Mena, T. M. Hill, J. D. Quigley, R. L. Schlotterbeck, and G. J. Lascano. 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. 101:408–412. https://doi.org/10.3168/jds.2017-13292.

Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into abomasum of lactating dairy cows. J. Dairy Sci. 75:1517–1526. https://doi.org/10.3168/jds.S0022-0302(92)77908-9.

Eadie, J. M. 1962. The development of rumen microbial populations in lambs and calves under various conditions of management. J. Gen. Microbiol. 29:563–578. https://doi.org/10.1099/00221287-29-4-563.

Erdman, R. A. 1988. Dietary buffering requirement of lactating dairy cows: A review. J. Dairy Sci. 71:3246–3266. https://doi.org/10.3168/jds.S0022-0302(88)79930-0.

Fonty, G., P. Gouet, J. Jouany, and J. Senaud. 1987. Establishment of the microflora and anaerobic fungi in the rumen of lambs. Microbiology (Reading) 133:1835–1843. https://doi.org/10.1099/00221287-133-7-1835.

Forsberg, C. W., J. K. Lovelock, L. Krumholz, and J. G. Buchanan-Smith. 1984. Protease activities of rumen protozoa. Appl. Environ. Microbiol. 47:101–110. https://doi.org/10.1128/aem.47.1.101-110.1984.

Ghasemi, E., M. Azad-Shahraki, and M. Khorvash. 2017. Effect of different fat supplements on performance of dairy calves during

cold season. J. Dairy Sci. 100:5319–5328. https://doi.org/10.3168/jds.2016-11827.

Ghorbani, H., M. Kazemi-Bonchenari, M. HosseinYazdi, and E. Mahjoubi. 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. https://doi.org/10.1016/j.anifeedsci.2020.114429.

Harvatine, K. J., and M. S. Allen. 2006. Effects of fatty acid supplements on feed intake, and feeding and chewing behavior of lactating dairy cows. J. Dairy Sci. 89:1104–1112. https://doi.org/10.3168/jds.S0022-0302(06)72178-6.

Heinrichs, A. J., C. M. Jones, L. R. Van Roekel, and M. A. Fowler. 2003. Calf Track: A system of dairy calf workforce management, training, and evaluation and health evaluation. J. Dairy Sci. 86(Suppl. 1):115–123.

Hill, T. M., H. G. Bateman II, J. M. Aldrich, J. D. Quigley, and R. L. Schlotterbeck. 2015. Inclusion of tallow and soybean oil to calf starters fed to dairy calves from birth to four months of age on calf performance and digestion. J. Dairy Sci. 98:4882–4888. https://doi.org/10.3168/jds.2015-9376.

Hill, T. M., H. G. Bateman II, J. M. Aldrich, and R. L. Schlotterbeck. 2011. Effect of various fatty acids on dairy calf performance. Prof. Anim. Sci. 27:167–175. https://doi.org/10.15232/S1080-7446(15)30470-8.

Hosseini, H., H. Mirzaei-Alamouti, M. Vazirigohar, E. Mahjoubi, and P. Rezamand. 2019. Effects of whole milk feeding rate and straw level of starter feed on performance, rumen fermentation, blood metabolites, structural growth, and feeding behavior of Holstein calves. Anim. Feed Sci. Technol. 255:114238. https://doi.org/10.1016/j.anifeedsci.2019.114238.

Hristov, A. N., M. Ivan, L. Rode, and T. A. McAllister. 2001. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. J. Anim. Sci. 79:515–524. https://doi.org/10.2527/2001.792515x.

Hu, W., T. M. Hill, T. S. Dennis, F. X. Suarez-Mena, J. D. Quigley, J. R. Knapp, and R. L. Schlotterbeck. 2018. Relationships between starch concentration of dry feed, diet digestibility, and growth of dairy calves up to 16 weeks of age. J. Dairy Sci. 101:7073–7081. https://doi.org/10.3168/jds.2018-14583.

Ikwuegbu, O. A., and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr. 48:365–375. https://doi.org/10.1079/BJN19820120.

Karimi, A., Y. A. Alijoo, M. Kazemi-Bonchenari, M. Mirzaei, and H. Sadri. 2021. Soybean oil supplementation and alfalfa hay inclusion in starter feed of Holstein dairy calves: Growth performance, digestibility, ruminal fermentation and urinary purine derivatives. Ital. J. Anim. Sci. 20:1817–1828. https://doi.org/10.1080/1828051X.2021.1955626.

Kazemi-Bonchenari, M., M. Dehghan-Banadaky, F. Fattahnia, A. Saleh-Bahmanpour, M. Jahani-Moghadam, and M. Mirzaei. 2020. Effects of linseed oil and rumen-undegradable protein: rumen-degradable protein ratio on performance of Holstein dairy calves. Br. J. Nutr. 123:1247–1257. https://doi.org/10.1017/S0007114520000586.

Kazemi-Bonchenari, M., H. Khanaki, A. Jafari, M. Eghbali, M. Poorhamdollah, and M. H. Ghaffari. 2022. Milk feeding level and starter protein content: Effects on growth performance, blood metabolites, and urinary purine derivatives of Holstein dairy calves. J. Dairy Sci. 105:1115–1130. https://doi.org/10.3168/jds.2021-21208.

Kazemi-Bonchenari, M., M. Mirzaei, M. Jahani-Moghadam, A. Soltani, E. Mahjoubi, and R. A. Patton. 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. 94:4267–4275. https://doi.org/10.2527/jas.2016-0514.

Kazemi-Bonchenari, M., A. Z. M. Salem, and S. Lopez. 2017. Influence of barley grain particle size and treatment with citric acid on digestibility, ruminal fermentation and microbial protein synthesis in Holstein calves. Animal 11:1295–1302. https://doi.org/10.1017/S1751731116002810.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, S. B. Park, K. S. Baek, J. K. Ha, and Y. J. Choi. 2008. Starch source evaluation in calf starter: II. Ruminal parameters, rumen development, nutrient digestibilities, and nitrogen utilization in Holstein calves. J. Dairy Sci. 91:1140–1149. https://doi.org/10.3168/jds.2007-0337.

Kmicikewycz, A. D., and A. J. Heinrichs. 2015. Effect of corn silage particle size and supplemental hay on rumen pH and feed preference by dairy cows fed high-starch diets. J. Dairy Sci. 98:373–385. https://doi.org/10.3168/jds.2014-8103.

Kohn, R. A., M. M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim. Sci. 83:879–889. https://doi.org/10.2527/2005.834879x.

Leibholz, J. 1975. Ground roughage in the diet of the early-weaned calf. Anim. Sci. 20:93–100. https://doi.org/10.1017/S0003356100035042.

Machmüller, A., D. A. Ossowski, M. Wanner, and M. Kreuzer. 1998. Potential of various fatty feeds to reduce methane release from rumen fermentation in vitro (Rusitec). Anim. Feed Sci. Technol. 71:117–130. https://doi.org/10.1016/S0377-8401(97)00126-0.

Maczulak, A. E., B. A. Dehority, and D. L. Palmquist. 1981. Effects of long-chain fatty acids on growth of rumen bacteria. Appl. Environ. Microbiol. 42:856–862. https://doi.org/10.1128/aem.42.5.856-862.1981.

Maia, M. R. G., L. C. Chaudhary, C. S. Bestwick, A. J. Richardson, N. McKain, T. R. Larson, I. A. Graham, and R. J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. BMC Microbiol. 10:52. https://doi.org/10.1186/1471-2180-10-52.

Makizadeh, H., M. Kazemi-Bonchenari, H. Mansoori-Yarahmadi, J. Fakhraei, H. Khanaki, J. K. Drackley, and M. H. Ghaffari. 2020. Corn-processing and crude protein content in calf starter: Effects on growth performance, ruminal fermentation, and blood metabolites. J. Dairy Sci. 103:9037–9053. https://doi.org/10.3168/jds.2020-18578.

Mashek, D. G., S. J. Bertics, and R. R. Grummer. 2002. Metabolic fate of long-chain unsaturated fatty acids and their effects on palmitic acid metabolism and gluconeogenesis in bovine hepatocytes. J. Dairy Sci. 85:2283–2289. https://doi.org/10.3168/jds.S0022-0302(02)74308-7.

Mentschel, J., R. Leiser, C. Mulling, C. Pfarrer, and R. Claus. 2001. Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. Arch. Tierernahr. 55:85–102. https://doi.org/10.1080/17450390109386185.

Miller-Cushon, E. K., and T. J. DeVries. 2011. Effect of early feed type exposure on diet-selection behavior of dairy calves. J. Dairy Sci. 94:342–350. https://doi.org/10.3168/jds.2010-3382.

Mirzaei, M., M. Khorvash, G. R. Ghorbani, M. Kazemi-Bonchenari, A. Riasi, A. Soltani, B. Moshiri, and M. H. Ghaffari. 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. 94:678–686. https://doi.org/10.2527/jas.2015-9670.

Molaei, M., M. Kazemi-Bonchenari, M. Mirzaei, and H. R. Esmaeili. 2021. The physical form of starter (finely ground versus pelleted) and alfalfa hay (chopped versus pelleted) in Holstein dairy calves: Effects on growth performance, feeding behaviour, ruminal fermentation, and urinary purine derivatives. Anim. Feed Sci. Technol. 279:115031. https://doi.org/10.1016/j.anifeedsci.2021.115031.

Morris, T. R. 1999. Experimental Design and Analysis in Animal Sciences. CABI Publishing.

NASEM (National Academies of Science, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. National Academy of Sciences.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press.

Newbold, C. J., G. de la Fuente, A. Belanche, E. Ramos-Morales, and N. R. McEwan. 2015. The role of ciliate protozoa in the rumen. Front. Microbiol. 6:1313. https://doi.org/10.3389/fmicb.2015.01313.

Nocek, J. E., and E. M. Kesler. 1980. Growth and rumen characteristics of Holstein steers fed pelleted or conventional diets. J. Dairy Sci. 63:249–254. https://doi.org/10.3168/jds.S0022-0302(80)82921-3.

Orpin, C. G. 1984. The role of ciliate protozoa and fungi in the rumen digestion of plant cell walls. Anim. Feed Sci. Technol. 10:121–143. https://doi.org/10.1016/0377-8401(84)90003-8.

Phillips, C. J. C. 2004. The effects of forage provision and group size on the behavior of calves. J. Dairy Sci. 87:1380–1388. https://doi.org/10.3168/jds.S0022-0302(04)73287-7.

Rastgoo, M., M. Kazemi-Bonchenari, M. HosseinYazdi, and M. Mirzaei. 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. https://doi.org/10.1016/j.anifeedsci.2020.114646.

Sakata, T., and H. Tamate. 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. J. Dairy Sci. 61:1109–1113. https://doi.org/10.3168/jds.S0022-0302(78)83694-7.

Soliva, C. R., L. Meile, A. Cieslak, M. Kreuzer, and A. Machmüller. 2004. Rumen simulation technique study on the interaction of dietary lauric and mysrustic acid supplementation in suppressing ruminal methanogenesis. Br. J. Nutr. 92:689–700. https://doi.org/10.1079/BJN20041250.

Soltani, M., M. Kazemi-Bonchenari, A. H. Khaltabadi-Farahani, and O. Afsarian. 2017. Interaction of forage provision (alfalfa hay) and sodium butyrate supplementation on performance, structural growth, blood metabolites and rumen fermentation characteristics of lambs during pre-weaning period. Anim. Feed Sci. Technol. 230:77–86. https://doi.org/10.1016/j.anifeedsci.2017.06.002.

Sutton, J. D., R. Knight, A. B. McAllan, and R. H. Smith. 1983. Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. Br. J. Nutr. 49:419–432. https://doi.org/10.1079/BJN19830051.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408–420. https://doi.org/10.3168/jds.S0022-0302(62)89406-5.

Tsai, C. Y., P. Rezamand, W. I. Loucks, C. M. Scholte, and M. E. Doumit. 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. https://doi.org/10.1016/j.anifeedsci.2017.05.004.

Van Keulen, J., and B. A. Young. 1977. Acid insoluble ash as a natural marker for digestibility studies. J. Dairy Sci. 44:282–287.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Vital, M., C. R. Penton, Q. Wang, V. B. Young, D. A. Antonopoulos, M. L. Sogin, H. G. Morrison, L. Raffals, E. B. Chang, G. B. Huffnagle, T. M. Schmidt, J. R. Cole, and J. M. Tiedje. 2013. A gene-targeted approach to investigate the intestinal butyrate-producing bacterial community. Microbiome 1:8. https://doi.org/10.1186/2049-2618-1-8.

Wang, Z., Y. Cui, P. Liu, Y. Zhao, L. Wang, Y. Liu, and J. Xie. 2017. Small peptides isolated from enzymatic hydrolyzate of fermented soybean meal promote endothelium-independent vasorelaxation and ACE inhibition. J. Agric. Food Chem. 65:10844–10850. https://doi.org/10.1021/acs.jafc.7b05026.

Williams, A. G., and G. S. Coleman. 1992. The Rumen Protozoa. Springer-Verlag.

Yousefinejad, S., F. Fattahnia, M. Kazemi-Bonchenari, H. Khanaki, J. K. Drackley, and M. H. Ghaffari. 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. 104:1630–1644. https://doi.org/10.3168/jds.2020-18823.

Zhang, Y. Q., D. Ch. He, and Q. X. Meng. 2010. Effect of a mixture of steam-flaked corn and soybeans on health, growth, and selected blood metabolism of Holstein calves. J. Dairy Sci. 93:2271–2279. https://doi.org/10.3168/jds.2009-2522.

## ORCIDS

Hamidreza Mirzaei-Alamouti https://orcid.org/0000-0003-2200-6449

Jörg R. Aschenbach https://orcid.org/0000-0001-5080-1007