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Original Research Article

Phosphorus has a crucial role in growth performance of calves fed starters with incorporated forage



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ABSTRACT

Forage addition (FA) to starter diets has favorable effects on ruminal development but may reduce starter intake and growth. The latter reductions may be related to an inability of the insufficiently developed ruminal microbiota to effectively use forage. Based on the crucial role of phosphorus (P) for ruminal microbial activity and the overall insufficient knowledge on the interaction of dietary fiber and P in young calves, this study hypothesized that limited availability of dietary P may contribute to the reduced intake and performance in forage-supplemented calves. Consequently, the current study evaluated the effects of forage feeding level (no alfalfa hay [NAH] vs. 100 g of chopped alfalfa hay [AH] per kg of starter) at either 0.4% P (0.4P) or 0.8% P (0.8P) on growth performance, digestibility of nutrients, ruminal fermentation, and microbial protein synthesis (MPS). Forty-eight female Holstein calves (39.2 ± 3.7 kg) were assigned randomly to the four experimental treatments including NAH-0.4P, NAH-0.8P, AH-0.4P, and AH-0.8P ($n = 12$, each) during the pre-weaning (d 3 to 53) and post-weaning periods (d 54 to 73). The P contents were 0.41%, 0.84%, 0.42%, and 0.82%, and phytate-P contents were 0.25%, 0.26%, 0.28%, and 0.29% for the experimental treatments cited above, respectively. Milk feeding schedule was identical among treatments and calves had ad libitum access to water and starters throughout the experiment. Based on FA \times P level interactions, the least and greatest starter intakes pre-weaning were observed in AH-0.4P and AH-0.8P, respectively. Compared to other groups, calves in AH-0.8P had greater average daily gain during pre-weaning and post-weaning ($P < 0.05$), greater body weight and higher withers height at weaning and the end of experiment ($P < 0.05$), higher hip height at weaning ($P = 0.021$), and greater urinary excretion of purine derivatives (PD; $P = 0.045$), the latter indicating improved microbial protein synthesis ($P = 0.045$). Feeding AH diet to calves increased ruminal acetate concentration (pre-weaning; $P = 0.014$), reduced ruminal propionate concentration (pre-weaning; $P = 0.033$), and tended to decrease ruminal butyrate concentration (pre-weaning; $P = 0.057$) and increase ruminal pH ($P = 0.074$) when compared to NAH-fed calves. A level of 0.8P vs. 0.4P increased organic matter ($P = 0.041$) and neutral detergent fiber digestibility ($P = 0.038$), increased total short chain fatty acid production in the rumen pre- and post-weaning ($P < 0.05$); whereas, ruminal ammonia nitrogen concentration and urinary nitrogen excretion were decreased by 0.8P ($P < 0.05$). It is concluded that FA to starter diets has a high potential to improve growth performance in young dairy calves. However, currently, recommended

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dietary P levels of approximately 0.45% may be insufficient to support fiber digestibility, microbial protein synthesis and growth, especially pre-weaning, when forage-containing starters are high in phytate-P.

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1. Introduction

Forage provision in calf starter feeds has been accepted as a common feeding strategy in commercial dairy farms in recent years. Favorable effects of forage incorporation in starters on the growth performance of dairy calves are mainly attributable to a more stable ruminal pH and fermentation, thickening of the muscular layer of the ruminal wall, promotion of rumination, and prevention of hyper-keratinization (Suarez et al., 2007; Beiranvand et al., 2104; Kargar et al., 2021). However, some studies reported negative effects like lower feed intake, lower nutrient digestibility, and lower daily gain when calves were supplemented with forages (Kertz et al., 1979; Hill et al., 2008). This controversy may be based on different forage feeding levels, forage particle size, forage source, starter processing method, and the access rate of calves to forage (free or limited) (Mirzaei et al., 2016; Molaei et al., 2021; Keshavarz et al., 2023). To avoid the negative effects of forage inclusion in calf starter diets, the National Academies of Science, Engineering, and Medicine (NASEM, 2021) recommended that forage (alfalfa hay [AH]) inclusion should be limited to no more than 10% (dry matter [DM] basis) in young calves (NASEM, 2021).

Previous studies indicated that the interaction of fiber feeding level with some other nutrients can alter the response of calves to forage inclusion in starters. For instance, the inclusion of a high fat level (5.9% vs. 2.9%, DM basis) elicited a negative response in calves to the forage fiber source (Karimi et al., 2021). In contrast, a greater starter protein content improved fiber digestion when forage was incorporated in starter diets (Mohammadzadeh et al., 2021). Regarding minerals, interaction with fiber is documented for phosphorus (P) in mature ruminants (Durand and Komisarczuk, 1998; Jarrett et al., 2014) where P influences fiber-digesting bacteria. In addition, it was shown that limited P supplementation in mature ruminants led to a 30% reduction of microbial protein synthesis (MPS) (Breves and Schröder, 1991), the latter being a main indicator for ruminal development in young calves (Yousefinejad et al., 2021).

The role of P is also well documented for the growth of young animals with a main role in bone development (Esser et al., 2009), cell signaling, adenosine-3-phosphate synthesis and energy metabolism (Ternouth et al., 1993). All of these can have critical roles in calves raised under accelerated growth programs. However, there is a scarcity of data about the impact of starter P level on ruminal fermentation and nutrient digestibility when starters contain different fiber contents. The interaction of fiber and P may be especially critical in young calves because the microbial community is not fully developed in the rumen (Fonty et al., 1987; Ghorbani et al., 2020). This may influence microbial phytase activity and thus availability of P supplied through starters in suckling calves. Furthermore, it should be considered that the optimum ruminal pH for maximum microbial phytase activity is 4.5 to 5.0 (Puhl et al., 2008). The greater ruminal pH upon feeding of forage-supplemented starters (Mirzaei et al., 2015) may have the potential to reduce microbial phytase activity in young calves. From another

perspective, greater reabsorption of P was observed in mature ruminants fed a high forage level (Puggaard et al., 2013), supposedly to cover the essential P needs of ruminal cell wall-degrading bacteria (Bravo et al., 2003). Increased rumination rate may contribute to ruminal P recycling in animals fed high-forage diets through enhanced salivary secretion (Jarrett et al., 2014). Accordingly, a low fiber content led to reduced P recycling to the rumen via saliva and increased urinary P excretion into the environment (Valk et al., 2002). Studies in young calves showed that bone resorption, salivary P secretion, P absorption and urinary P excretion all play a part in P homeostasis and the relative importance of each of these processes depends upon the P status of the calf (Challa and Braithwaite, 1988; Challa et al., 1989). These results collectively suggest that potential interactions between dietary forage and P contents are complex and cannot be neglected in young ruminants.

The recent recommendation by NASEM (2021) advises 0.45% P (DM basis) in starters of young calves. However, extensive ranges of total P from 0.40% (Luchini et al., 1991) up to 0.96% (Lesmeister and Heinrichs, 2004) based on DM have been used to formulate starters for dairy calves in previous studies. A high concentration of dietary P enabled dairy heifers to reach puberty and pregnancy faster (Esser et al., 2009), whereas other studies indicated that over-feeding P is not advisable because it increases P in manure and augments the expense of the producer (Wu et al., 2000; Valk et al., 2002; Bjelland et al., 2011). As such, the optimum level of P in starter diets of dairy calves reared under accelerated feeding programs in commercial farms can be considered unknown. This is especially true for forage-containing starters where increased P supply can be expected to boost cellulolytic bacterial activity (Bravo et al., 2003). Pre-weaned calves lack sufficient populations of cellulolytic bacteria as well as ciliate protozoa in the early weeks of life (Eadie, 1962; Fonty et al., 1987). Shortness of P could compromise the synthesis of microbial protein in the rumen which is a critical factor indicating rumen function and microbial development in pre-weaned calves (Kazemi-Bonchenari et al., 2022). This could possibly explain the reduced starter intake and growth performance in forage-receiving calves observed in some previous reports (Nocek and Kesler, 1980; Panahiha et al., 2022). Consequently, this study hypothesized that young dairy calves receiving forage-containing starters in some previous studies may have suffered from restricted P availability, causing reductions in digestibility, microbial activity and growth. Therefore, a two-factorial experiment was designed where the effects of two P levels (0.4% vs. 0.8%, DM basis) were examined in starter diets that were either forage-free or contained 10% (DM basis) forage as chopped AH. The lower experimental P level (i.e. 0.4% P) was chosen close to the current recommendation of NASEM (2021). The upper P level in the current experiment (i.e. 0.8% P) was well above recommendation but still lower than the high P concentrations used in some previous studies (0.96%; Lesmeister and Heinrichs, 2004) to avoid overt P excretion into the environment. Effects of interest were growth performance, nutrient digestibility, and microbial protein synthesis.

2. Materials and methods

2.1. Animal ethics statement

The present study was conducted at a commercial dairy farm (Zarrin-Khooshe Agriculture and Animal Husbandry, Arak, Iran) and was approved by the Animal Care and Use Committee of Arak University (Institutional Animal Care and Use Committee Protocol #IR22979001) according to the guidelines of the Iranian Council of Animal Care (1995).

2.2. Calves' management, treatments, and experimental diets

The study was carried out using 48 Holstein female dairy calves (3 d of age; 39.2 ± 3.7 kg of body weight [BW]). At birth, calves were separated from their dam and placed in individual pens (1.3 m \times 2.4 m) bedded with wood shavings that were refreshed every 24 h as needed. Based on the routine protocol of calf rearing at the farm, all calves were fed 2.5 L of colostrum (Brix index >22) at each of the first 2 feedings (i.e., within 1.5 h of life and at 12 h after the first feeding). On the second and third day of life, calves were fed 6 L/d of transition milk using a galvanized bucket in two equally sized meals offered at 06:00 and 18:00. Thereafter, all calves were fed 5 L/d whole milk from d 4 to 30, 7 L/d from d 31 to 45, 5 L/d from d 46 to 50 in two equal portions twice daily, and then 2 L from d 51 to 52 once daily. All calves were fully weaned at 53 d of age and remained in the trial until 73 d of age. Milk composition was calculated as (3.69 ± 0.06)% fat, (3.21 ± 0.05)% crude protein, (4.59 ± 0.06)% lactose and (12.21 ± 0.13)% total solids during the experiment. At 3 d of age, calves were allocated into four treatments (12 calves per treatment) in a completely randomized design without blinding: 1) starter with no AH and 0.4% P (NAH-0.4P); 2) starter with no AH and 0.8% P (NAH-0.8P); 3) starter with 100 g/kg AH and 0.4% P (AH-0.4P); 4) starter with 100 g/kg AH and 0.8% P (AH-0.8P). To eliminate bias by particle size distribution, the particle size of AH was similar to that of the other ingredients (geometric mean, 2.93 ± 0.14 mm). Because the interaction of calcium and P in bone metabolism and growth of young animals have become obvious in previous work (Luchini et al., 1991), calcium concentration was also modified to have relatively similar calcium to P ratio in starters to prevent possible effects of calcium deficiency on growth performance of dairy calves. The starter was mixed well with alfalfa in forage incorporated starters and then offered to calves. Starter feeds and water were offered ad libitum daily throughout the experimental period. Fresh starter feed was offered once daily at 07:00 to result in at least 10% orts over a 24-h period. The starter feeds were formulated based on the National Research Council (NRC, 2001) to have the same ingredient and nutrient composition but differed in forage and P levels (NASEM, 2021). The ingredients and chemical composition of the starter feeds is presented in Table 1.

2.3. Analysis of dietary total-P and phytate-P

The content of total-P was determined in feedstuff samples by flame atomic absorption spectrophotometry (Analyst 200, PerkinElmer Inc., Waltham, MA, USA) as described by Moreira et al. (2009). The concentration of phytate-P in experimental starters was determined as per the procedures described by Eechkhout and De Paepe (1994). The latter method relies on phytate-P extraction into 1.25% H₂SO₄ and subsequent analysis by Dionex liquid chromatography using 200 mmol/L NaOH and deionized water as eluents.

Table 1

Ingredients and chemical composition of experimental starters (g/kg of dry matter, unless otherwise indicated).

Item	Treatments ¹			
	NAH		AH	
	0.4P	0.8P	0.4P	0.8P
Ingredients				
Alfalfa hay, chopped	0	0	100	100
Barley grain	90	90	90	90
Corn grain	460	460	405	405
Soybean meal	305	305	290	290
Wheat bran	99	93	69	63
Vitamin and mineral premix ²	15	15	15	15
Di-calcium phosphate	5	14	5	14
Calcium carbonate	10	7	10	7
Sodium bicarbonate	11	11	11	11
Salt	5	5	5	5
Total	1000	1000	1000	1000
Chemical composition³				
Dry matter	879	881	882	881
Crude protein	215	210	214	215
Ether extract	28.2	28.3	28.6	28.8
Neutral detergent fiber	155	156	181	183
Metabolizable energy, Mcal/kg	3.02	3.01	2.99	2.98
Non-fiber carbohydrate	532	536	504	503
Calcium	8.70	14.11	8.90	14.40
Total-P	4.11	8.42	4.20	8.23
Phytate-P	2.51	2.63	2.80	2.91

NAH = no alfalfa hay; AH = alfalfa hay.

¹ Treatments were (1) no AH in starter with 0.4% P (NAH-0.4P); (2) no AH in starter with 0.8% P (NAH-0.8P); (3) 100 g/kg AH in starter with 0.4% P (AH-0.4P); and (4) 100 g/kg AH in starter with 0.8% P (AH-0.8P).

² Content per kilogram of vitamin and mineral premix: 600,000 IU of vitamin A, 150,000 IU of vitamin D, 600 IU of vitamin E, 7000 mg of Mn, 90 g of Ca, 8000 mg of Zn, 45 g of P, 20 g of Mg, 17 g of Na, 50 mg of Co, 2300 mg of Cu, 62 mg of I, and 45 mg of Se.

³ Metabolizable energy and non-fiber carbohydrate were calculated according to NRC (2001), while the others were analyzed values.

2.4. Starter intake, general appearance score, fecal score, and nutrient digestibility

Starter feed intake was measured daily on an individual basis by subtracting refusals from the amounts offered to calves. Body weight (using an electronic scale) was recorded at 10-d intervals. Feed efficiency (FE) was calculated by dividing kilogram BW gain by the average daily total dry matter intake (TDMI; whole milk DM + starter feed DM). Representative samples of starter feeds and refusals were collected monthly. Subsamples were mixed thoroughly, dried in a convection oven (60 °C for 48 h), ground in a mill (Ogaw Seiki Co., Ltd., Tokyo, Japan) to pass through a 1-mm screen and determined for crude protein (CP; AOAC, 2002; method 984.13) using the automatic Kjeldahl UDK 159 (VELP Scientifica, Usmate, Italy), ether extract (EE; AOAC, 2002; method 920.39) using the automatic Soxhlet extractor (AntiTeck, Huadu, Guangzhou, China), ash (AOAC, 2002; method 942.05) and neutral detergent fiber (NDF; Van Soest et al., 1991) without heat-stable α -amylase using Fiber Tech 8000 (Foss Analytical, Hillerød, Denmark). The non-fiber carbohydrate component was calculated as $100 - (CP + NDF + EE + ash)$ (NRC, 2001). Calcium and P were determined in feed samples by flame atomic absorption spectrophotometry (Analyst 200, PerkinElmer Inc., Waltham, MA, USA) as described by Moreira et al. (2009).

General appearance score and fecal score were evaluated daily according to Larson et al. (1977) and Heinrichs et al. (2007). Feces were scored as follows: 1, firm and well-formed; 2, soft and pudding-like; 3, runny and similar in consistency to pancake batter; and 4, liquid splatter and similar in consistency to pulpy orange

juice. General appearance scoring was: 1, normal and alert; 2, ears drooped; 3, head and ears drooped, dull eyes, slightly lethargic; 4, head and ears drooped, dull eyes, lethargic; and score; 5, severely lethargic.

During d 68 to 71 of the experiment, two fecal grab samples were collected daily from each animal at 06:00 and 18:00 (10 samples for each calf) as described in the previous work (Ghorbani et al., 2020). The fecal samples were dried in a forced air oven (60 °C; 72 h) and then ground in a Wiley mill through a 1-mm screen. Aliquots of all fecal samples collected for each calf were mixed to obtain one composite sample for each animal. These composite fecal samples were analyzed to determine total nitrogen, ash and NDF. The apparent total tract digestibility of nutrients was calculated using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977). Total feces output was estimated using the acid insoluble ash (AIA) marker during the digestibility trial. Similar to calcium and P in feeds, fecal samples were also determined for calcium and P by flame atomic absorption spectrophotometry (Analyst 200, PerkinElmer Inc., Waltham, MA, USA) as described by Moreira et al. (2009).

2.5. Structural growth indicators

Growth parameters, including withers height, hip height, heart girth, body length, and hip width were taken at the start of the experiment (d 3), at weaning (d 53), and on the final day of the experiment (d 73) according to the method described by Khan et al. (2007) for dairy calves.

2.6. Ruminal fluid sampling and analyses

Ruminal fluid samples (30 mL) from each calf were obtained using a stomach tube fitted to a vacuum pump 3 to 4 h after the morning feeding (at 12:00) pre-weaning (d 36 of age) and post-weaning (d 72 of age). The first 15 mL was not used for measurements for reducing saliva contamination. The samples were squeezed through four layers of cheesecloth, and then ruminal fluid pH was immediately measured with a portable pH meter (HI 8314 membrane pH meter, Hanna Instruments, Villafranca, Italy). A 10-mL sample of ruminal fluid was acidified with 0.2 mL of 50% sulfuric acid and stored at –20 °C until analyzed for the three main short chain fatty acids (SCFA), acetate, propionate, and butyrate. Ruminal concentrations of SCFA were analyzed by gas chromatography (model CP-9002, Chrompack, Middelburg, the Netherlands) according to the method described in a previous report (Dennis et al., 2018). In brief, the rumen samples were analyzed for SCFA 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, USA) after thawing at room temperature and centrifugation at 15,000 × g for 20 min. Helium was used as the carrier gas and the oven's initial and final temperatures were 55 and 195 °C, respectively. The detector and injector temperature was set at 250 °C. Crotonic acid (1:7, v/v) was used as the internal standard. Ruminal fluid subsamples were thawed at room temperature and clarified by centrifuging (15,000 × g for 20 min), then decanted and analyzed for ammonia nitrogen (NH₃-N) concentration using a modified phenol–hypochlorite reaction (Broderick and Kang, 1980).

2.7. Urinary purine derivatives and MPS

The MPS was analyzed based on purine derivatives (PD) obtained via urine as explained in young dairy calves (Kazemi-Bonchenari et al., 2020). As PD contained in bovine milk may lead to an overestimation of urinary PD in milk-fed calves as reported by Kazemi-

Bonchenari et al. (2022), urinary PD was measured only post-weaning. Daily urine volume was estimated from urinary creatinine excretion using the following model, $BW \times 26.8/\text{creatinine concentration as mg/L}$ (Kazemi-Bonchenari et al., 2017). Urine spot samples (approximately 8 mL) were collected in the mornings (between 09:30 and 11:30) and afternoons (between 14:30 and 16:30) of four consecutive days (d 68 to 71) during the post-weaning period 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. Samples were thawed and pooled per calf to analyze uric acid and allantoin concentrations as described in a previous study (Broderick and Kang, 1980). Urinary nitrogen content was analyzed according to the assay described in previous work (Chen and Gomes, 1992). Estimated daily urine output was used to calculate daily urinary excretion of allantoin, uric acid and their sum as total PD. The ruminal MPS was calculated from total daily PD excretion using the equations based on Chen and Gomes (1992). The estimated daily urine volumes were used to calculate the total P excreted through urine.

2.8. Statistical analysis

All statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA). Prior to the experiment, power analysis for sample size estimation was performed (Morris, 1999) for the primary response variables, including feed intake, BW, and average daily gain (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, TDMI, ADG, and BW. Data from all 12 calves per group were analyzed for each measurement time point or period (pre-weaning [d 3 to 53], post-weaning [d 54 to 73], and the entire period [d 3 to 73], using calf as an experimental unit without exclusion criteria. The model was as follows:

$$Y_{ijk} = \mu + AH_i + P_j + T_k + (AH \times T)_{ik} + (P \times T)_{jk} + (AH \times F)_{ij} + (AH \times P \times T)_{ijk} + \beta(X_i - \bar{X}) + \epsilon_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the overall mean; AH_i is the effect of AH addition in to starter diet; P_j is the effect of P supplementation level (0.4% vs. 0.8%); T_k is the effect of sampling time; $(AH \times T)_{ij}$ is the effect of the interaction between AH and sampling time; $(P \times T)_{jk}$ is the effect of the interaction between P and sampling time; $(AH \times P)_{jk}$ is the interaction between AH and P; $(AH \times P \times T)_{ijk}$ is the effect of AH, P and sampling time; $\beta(X_i - \bar{X})$ is the covariate variable and ϵ_{ijk} is the overall error term. Analyses started with screening for normality using the UNIVARIATE procedure of SAS 9.1. The transformation was applied before analysis if data deviated from normality; namely, data for fecal scores and general appearance scores were square root-transformed for better homogeneity of the distribution of residuals. Subsequently, statistical comparisons were performed using PROC MIXED of SAS 9.1 with fixed effects of forage addition (FA) (NAH vs. AH), P level (0.4% vs. 0.8%, DM basis) and their interactions, using calf as a random effect. Initial structural growth measurements and initial BW were considered as covariates for skeletal growth and later BW analysis. A heterogeneous autoregressive type 1 covariance structure yielded the smallest Akaike's information criterion and 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.

Table 2Least squares means for starter intake, average daily gain, and feed efficiency in dairy calves fed starters with or without forage addition (FA) as alfalfa hay (AH) and different phosphorus (P) levels ($n = 12$).

Item	Treatments ¹				SEM	P-value ²		
	NAH		AH			FA	P	FA × P
	0.4P	0.8P	0.4P	0.8P				
Starter feed intake, g/d								
Pre-weaning (d 3–53)	639 ^{ab}	593 ^b	472 ^c	698 ^a	71.2	0.663	0.203	0.047
Post-weaning (d 54–73)	2134	2111	1946	2265	157.6	0.921	0.369	0.284
Entire period (d 3–73)	1066	1026	892	1146	100.9	0.787	0.292	0.153
Milk solids intake, g/d	612	610	611	610	26.7	0.960	1.008	0.981
Total dry matter intake (milk + starter), g/d	1251 ^b	1205 ^b	1081 ^c	1309 ^a	67.5	0.615	0.145	0.025
P intake, g/d								
Pre-weaning (d 3–53)	6.63	8.82	5.95	9.66	0.418	0.850	0.001	0.070
Post-weaning (d 54–73)	8.53	15.90	7.78	18.10	1.056	0.820	0.001	0.346
Entire period (d 3–73)	7.18	11.10	6.47	12.00	0.511	0.810	0.001	0.107
Average daily gain, g/d								
Pre-weaning (d 3–53)	565 ^b	598 ^b	570 ^b	725 ^a	33.8	0.078	0.013	0.042
Post-weaning (d 54–73)	820 ^b	770 ^c	779 ^c	870 ^a	29.1	0.314	0.473	0.024
Entire period (d 3–73)	638 ^b	647 ^b	629 ^b	766 ^a	28.5	0.052	0.017	0.014
Body weight, kg								
Initial (d 3)	40.9	39.2	40.1	39.7	1.51	0.416	0.291	0.358
Weaning (d 53)	68.3 ^b	67.0 ^b	68.6 ^b	75.9 ^a	1.92	0.023	0.015	0.016
Final (d 73)	84.7 ^b	82.5 ^b	84.0 ^b	93.3 ^a	1.89	0.046	0.013	0.012
Feed efficiency³								
Pre-weaning (d 3–53)	0.45	0.51	0.52	0.56	0.045	0.066	0.092	0.703
Post-weaning (d 54–73)	0.42	0.41	0.47	0.43	0.033	0.419	0.620	0.651
Entire period (d 3–73)	0.44	0.48	0.50	0.53	0.039	0.089	0.265	0.868

NAH = no alfalfa hay.

Values in the same row with different lowercase letters indicate significant differences ($P \leq 0.05$).¹ Treatments were (1) no AH in starter with 0.4% P (NAH-0.4P); (2) no AH in starter with 0.8% P (NAH-0.8P); (3) 100 g/kg AH in starter with 0.4% P (AH-0.4P); and (4) 100 g/kg AH in starter with 0.4% P (NAH-0.4P).² Significance level for FA, P level, and interaction between FA and P level (FA × P).³ Feed efficiency = kg of body weight gain/kg of total dry matter intake.

3. Results

3.1. Nutrient intake, ADG, and FE

An interaction between FA × P level was detected for the starter intake pre-weaning with the least and greatest starter intakes for AH-0.4P and AH-0.8P, respectively ($P = 0.047$; Table 2). Milk consumption was constant based on the experimental protocol and thus no effect was found for milk solids intake among the experimental treatments ($P = 0.981$). However, TDMI (milk DMI + starter DMI) followed the differences observed for pre-weaning starter intake, evidenced by an interaction of FA × P level with the least and the greatest values for AH-0.4P and AH-0.8P, respectively ($P = 0.025$). P intake was increased during all experimental periods ($P = 0.001$) when comparing 0.4P and 0.8P diets with no effect of FA or its interaction with P levels. The ADG was influenced by a FA × P level interaction during all experimental periods with the greatest ADG found for AH-0.8P during pre-weaning ($P = 0.042$), post-weaning ($P = 0.024$) and the entire period of the experiment ($P = 0.014$). Accordingly, BW was the greatest for AH-0.8P at weaning ($P = 0.016$) and at the end of the experiment ($P = 0.012$). Feeding AH tended to increase FE during the pre-weaning ($P = 0.066$) and entire period ($P = 0.089$), whereas greater P supplementation tended to increase FE ($P = 0.092$) during the pre-weaning period only.

3.2. General appearance score, fecal score, and nutrient digestibility

Interactions between FA × P level were found for the general appearance score during the pre-weaning ($P = 0.043$) and entire period with least favorable and most favorable scores for calves receiving NAH-0.4P and AH-0.8P, respectively ($P = 0.027$; Table 3). Another interaction was found considering fecal score with the greatest fecal score detected in NAH-0.4P during pre-weaning

($P = 0.019$) and the entire period ($P = 0.032$). During the post-weaning period, calves receiving AH had more favorable fecal scores compared to calves receiving NAH ($P = 0.033$).

No interaction was found with respect to nutrient digestibility; however, 0.8P increased OM ($P = 0.041$) and NDF ($P = 0.038$) digestibility compared to the 0.4P level (Table 3).

3.3. P intake and excretion through urine and feces

P intake and excretion through urine and feces are stated in Table 4 for the feces and urine collection days (d 68–71). Intake of P was increased when calves were fed the 0.8P diet compared to the 0.4P diet ($P = 0.001$). Neither fecal P nor urinary P excretion were changed with forage feeding in the current study ($P > 0.05$). Considering the interaction between P feeding level and AH addition in the starter diet, results indicated that calves fed AH along with 0.8P had the greatest value for P intake ($P = 0.045$).

3.4. Structural growth indices

Withers height was influenced with an interaction between FA × P level with the highest withers height for AH-0.8P at weaning ($P = 0.017$) and on the final day of experiment ($P = 0.026$; Table 5). Similarly, hip height at weaning was the highest in calves receiving AH-0.8P (interaction FA × P level, $P = 0.021$). The greater P level of 0.8% increased hip height on the final day of measurements ($P = 0.034$). No effect was found for FA, P level, and their interaction with regard to body length, heart girth, and hip width ($P > 0.05$).

3.5. Ruminal fermentation characteristics

Ruminal pH tended to be greater for calves receiving AH compared to forage-free starters during the pre-weaning period ($P = 0.074$; Table 6). Ruminal ammonia nitrogen was reduced when

Table 3

Least squares means for general appearance score, fecal score, and digestibility of nutrients in dairy calves fed starters with or without forage addition (FA) as alfalfa hay (AH) and different phosphorus (P) levels ($n = 12$).

Item	Treatments ¹				SEM	P-value ²		
	NAH		AH			FA	P	FA × P
	0.4P	0.8P	0.4P	0.8P				
General appearance score								
Pre-weaning	1.38 ^a	1.13 ^b	1.20 ^{ab}	1.07 ^c	0.145	0.013	0.023	0.043
Post-weaning	1.14	1.06	1.06	1.10	0.112	0.659	0.724	0.177
Overall	1.34 ^a	1.12 ^b	1.16 ^b	1.09 ^c	0.091	0.016	0.057	0.027
Fecal score								
Pre-weaning	1.30 ^a	1.17 ^b	1.20 ^b	1.22 ^b	0.215	0.423	0.088	0.019
Post-weaning	1.18	1.11	1.02	1.08	0.097	0.033	0.484	0.243
Overall	1.28 ^a	1.16 ^b	1.17 ^b	1.18 ^b	0.133	0.069	0.072	0.032
Nutrient digestibility³, %								
Organic matter	72.9	75.7	74.0	76.8	1.43	0.466	0.041	0.991
Crude protein	69.4	70.5	71.6	70.0	1.17	0.681	0.902	0.527
Neutral detergent fiber	44.0	48.6	44.5	51.7	1.70	0.528	0.038	0.644
Ether extract	84.8	85.2	85.8	86.1	1.40	0.440	0.787	0.937
Phosphorus	43.4	46.5	45.5	48.1	0.45	0.535	0.132	0.940

NAH = no alfalfa hay.

Values in the same row with different lowercase letters indicate significant differences ($P \leq 0.05$).

¹ Treatments were (1) no AH in starter with 0.4% P (NAH-0.4P); (2) no AH in starter with 0.8% P (NAH-0.8P); (3) 100 g/kg AH in starter with 0.4% P (AH-0.4P); and (4) 100 g/kg AH in starter with 0.4% P (NAH-0.4P).

² Significance level for FA, P level, and interaction between FA and P level (FA × P).

³ Determined once over the last 5 d of the experiment.

Table 4

Least squares means for phosphorus (P) intake, and fecal and urinary P excretions (g/d) in dairy calves fed starters with or without forage addition (FA) as alfalfa hay (AH) and different P levels on feces and urine collection days (d 68–71) ($n = 12$).

Item	Treatments ¹				SEM	P-value ²		
	NAH		AH			FA	P	FA × P
	0.4P	0.8P	0.4P	0.8P				
P intake	9.44 ^b	15.80 ^{ab}	8.47 ^c	17.30 ^a	0.630	0.704	0.001	0.045
Fecal P	4.81	8.24	4.99	7.98	0.561	0.947	0.001	0.712
Urine P	0.79	0.82	0.90	0.78	0.135	0.764	0.806	0.576

NAH = no alfalfa hay.

Values in the same row with different lowercase letters indicate significant differences ($P \leq 0.05$).

¹ Treatments were (1) no AH in starter with 0.4% P (NAH-0.4P); (2) no AH in starter with 0.8% P (NAH-0.8P); (3) 100 g/kg AH in starter with 0.4% P (AH-0.4P); and (4) 100 g/kg AH in starter with 0.4% P (NAH-0.4P).

² Significance level for FA, P level, and interaction between FA and P level (FA × P).

calves were supplemented with 0.8P both pre-weaning ($P = 0.023$) and post-weaning ($P = 0.048$). The addition of AH increased ruminal acetate concentration ($P = 0.014$) but decreased ruminal propionate concentration pre-weaning ($P = 0.033$) with a resulting increase in the acetate: propionate ratio pre-weaning ($P = 0.001$). Ruminal butyrate concentrations also tended to be lower in the AH-receiving groups ($P = 0.057$). Despite having no effect on the molar proportions of individual SCFA, 0.8P increased total SCFA concentration in the rumen compared to 0.4P pre-weaning ($P = 0.016$) and post-weaning ($P = 0.014$). A trend for a FA × P level interaction ($P = 0.094$) indicated that total SCFA concentration during pre-weaning was especially low in the rumen of calves fed NAH-0.4P.

3.6. Urinary purine derivatives, MPS, and urinary nitrogen excretion

The concentrations of allantoin and total PD excreted through urine were influenced by an interaction between FA × P level in starters with the greatest urinary allantoin ($P = 0.034$) and total PD concentrations ($P = 0.045$) for AH-0.8P (Table 7). Accordingly, the greatest value for MPS was also found for AH-0.8P (FA × P interaction, $P = 0.045$). Urinary nitrogen excretion was lower when

Table 5

Least squares means for growth indices (cm) in dairy calves fed starters with or without forage addition (FA) as alfalfa hay (AH) and different phosphorus (P) levels ($n = 12$).

Item	Treatments ¹				SEM	P-value ²		
	NAH		AH			FA	P	FA × P
	0.4P	0.8P	0.4P	0.8P				
Body length								
Initial	47.5	46.7	47.8	46.7	0.71	0.839	0.157	0.847
Weaning	60.8	58.1	58.9	61.4	0.82	0.667	0.947	0.087
Final	62.8	62.4	63.4	64.0	0.74	0.455	0.903	0.725
Heart girth								
Initial	78.4	78.6	79.8	80.1	0.53	0.137	0.784	0.950
Weaning	101.0	99.7	100.0	101.0	1.10	0.896	0.806	0.115
Final	108	106	105	108	1.4	0.886	0.738	0.065
Withers height								
Initial	80.1	78.9	79.3	80.2	0.92	0.770	0.763	0.142
Weaning	97.3 ^{ab}	93.0 ^c	93.9 ^b	98.7 ^a	1.47	0.227	0.567	0.017
Final	101.0 ^b	99.2 ^c	100.0 ^{bc}	102.0 ^a	1.94	0.353	0.472	0.026
Hip height								
Initial	78.9	78.1	78.3	78.5	0.69	0.907	0.717	0.535
Weaning	90.8 ^b	90.4 ^b	90.9 ^b	93.9 ^a	1.23	0.014	0.053	0.021
Final	94.7	96.3	95.6	96.9	1.28	0.196	0.034	0.866
Hip width								
Initial	14.8	14.6	14.7	15.3	0.58	0.383	0.550	0.236
Weaning	19.8	18.7	20.0	20.5	0.79	0.136	0.737	0.238
Final	20.9	20.3	21.0	21.9	0.83	0.143	0.744	0.203

NAH = no alfalfa hay.

Values in the same row with different lowercase letters indicate significant differences ($P \leq 0.05$).

¹ Treatments were (1) no AH in starter with 0.4% P (NAH-0.4P); (2) no AH in starter with 0.8% P (NAH-0.8P); (3) 100 g/kg AH in starter with 0.4% P (AH-0.4P); and (4) 100 g/kg AH in starter with 0.4% P (NAH-0.4P).

² Significance level for FA, P level, and interaction between FA and P level (FA × P).

calves received 0.8P diets compared to 0.4P diets ($P = 0.029$), indicating a lower urinary nitrogen waste when calves received the greater supplemental P level in the starter diet (Table 7).

4. Discussion

The present study addressed the interaction effect of FA (0 vs. 100 g/kg) and P supplementation level (0.4% vs. 0.8%) of starter

Table 6

Least squares means for ruminal fermentation profile in dairy calves fed starters with or without forage addition (FA) as alfalfa hay (AH) and different phosphorus (P) levels ($n = 12$).

Item	Treatments ¹				SEM	P-value ²		
	NAH		AH			FA	P	FA × P
	0.4P	0.8P	0.4P	0.8P				
Ruminal pH								
d 36	5.79	5.77	5.90	5.85	0.061	0.074	0.533	0.856
d 72	6.03	6.08	6.10	6.14	0.088	0.478	0.576	0.962
Ruminal ammonia nitrogen, mg/dL								
d 36	10.8	9.55	10.2	8.98	0.693	0.265	0.023	0.948
d 72	14.1	12.2	13.5	12.8	0.75	0.885	0.048	0.426
Total short chain fatty acids, mmol/L								
d 36	76.9	86.4	81.7	84.9	1.80	0.482	0.016	0.094
d 72	102	107	104	108	2.4	0.245	0.014	0.656
Acetate, mol/100 mol								
d 36	48.4	50.5	53.4	54.0	1.29	0.014	0.328	0.598
d 72	48.5	48.2	48.6	49.7	1.31	0.205	0.556	0.267
Propionate, mol/100 mol								
d 36	37.6	36.2	35.0	33.5	1.14	0.033	0.215	0.988
d 72	31.9	32.1	32.3	31.3	0.98	0.558	0.612	0.547
Acetate: propionate ratio								
d 36	1.29	1.42	1.55	1.64	0.081	0.001	0.246	0.805
d 72	1.51	1.53	1.52	1.59	0.065	0.370	0.450	0.413
Butyrate, mol/100 mol								
d 36	13.9	13.3	11.5	12.4	0.78	0.057	0.858	0.406
d 72	20.0	19.6	19.2	18.9	0.94	0.265	0.566	0.893

NAH = no alfalfa hay.

Values in the same row with different lowercase letters indicate significant differences ($P \leq 0.05$).

¹ Treatments were (1) no AH in starter with 0.4% P (NAH-0.4P); (2) no AH in starter with 0.8% P (NAH-0.8P); (3) 100 g/kg AH in starter with 0.4% P (AH-0.4P); and (4) 100 g/kg AH in starter with 0.4% P (NAH-0.4P).

² Significance level for FA, P level, and interaction between FA and P level (FA × P).

feeds in young dairy calves. The main focus was on growth performance, FE and ruminal metabolism. Treatments were chosen with recognition of NASEM (2021) recommendations to explore no forage inclusion vs. the maximum recommended AH inclusion on the one hand and marginal P supplementation with practically relevant over-supplementation on the other hand. The diets were designed with commonly used ingredients. Retrospectively, however, the diets contained a relatively high phytate-P content with slightly higher values for AH-containing starters. This was not intended but greatly supported the proof of the hypothesis of the present study, which postulated that the P level currently recommended for calf starters may become inadequate when forage is included in the diet.

Table 7

Least squares means for urinary purine derivatives (PD) and microbial protein synthesis (MPS) in dairy calves fed starters with or without forage addition (FA) as alfalfa hay (AH) and different phosphorus (P) levels ($n = 12$).

Item	Treatments ¹				SEM	P-value ²		
	NAH		AH			FA	P	FA × P
	0.4P	0.8P	0.4P	0.8P				
Allantoin, mmol/d	12.6 ^c	13.1 ^b	12.8 ^c	15.9 ^a	0.89	0.019	0.025	0.034
Uric acid, mmol/d	0.95	0.97	1.03	0.98	0.064	0.517	0.849	0.562
Total purine derivatives, mmol/d	13.5 ^b	14.0 ^b	13.9 ^b	16.9 ^a	1.37	0.012	0.018	0.045
Microbial protein synthesis ³ , g/d	72.6 ^b	75.2 ^b	74.3 ^b	90.4 ^a	4.47	0.012	0.018	0.045
Urinary nitrogen, g/d	17.5	15.0	16.9	14.6	1.17	0.624	0.029	0.916

NAH = no alfalfa hay.

Values in the same row with different lowercase letters indicate significant differences ($P \leq 0.05$).

¹ Treatments were (1) no AH in starter with 0.4% P (NAH-0.4P); (2) no AH in starter with 0.8% P (NAH-0.8P); (3) 100 g/kg AH in starter with 0.4% P (AH-0.4P); and (4) 100 g/kg AH in starter with 0.4% P (NAH-0.4P).

² Significance level for FA, P level, and interaction between FA and P level (FA × P).

³ MPS was estimated from urinary PD excretion based on Chen and Gomes (1992) as $MPS (g/d) = 70 \times PD (mmol/d) / (0.85 \times 0.116 \times 0.83 \times 1000) \times 6.25$.

Interestingly, both the least and the greatest starter intakes during the pre-weaning period were found in calves receiving starters containing AH. The greatest starter intake and TDMI during the pre-weaning period was observed in calves fed starters with 10% AH and 0.8% P. As a similar interaction was not observed for forage-free starters, this can be taken as strong evidence that the higher P level of 0.8% was causative for greater starter intake during the pre-weaning period in AH-supplemented calves. Because the requirement for P in intermediate metabolism should be rather similar in both AH groups, the reason for this interaction has to be assumed in the gastrointestinal tract, primarily the rumen. It may be related, at least to some extent, to a positive effect of surplus P on microbial activity, leading to enhanced fiber digestion in this critical period when calves have insufficiently developed ruminal microbiota (Makizadeh et al., 2020; Panahiha et al., 2022). The present study verified the positive effect of P on fiber digestion only during the post-weaning period; however, a similarly positive effect can be assumed for the pre-weaning period. This can be expected to decrease ruminal retention time and provide more capacity for feed intake.

The superior starter intake of the AH-0.8P group can be considered as an important factor that selectively promoted the growth of these animals. Because the superior growth performance of group AH-0.8P continued post-weaning without noticeable differences in starter intake among groups, other factors should also be considered as contributors. Of these, the present study identified a promoting effect of 0.8% P on OM and NDF digestibility with a link to increased ruminal SCFA concentrations, as well as an interaction effect of AH and 0.8% P on ruminal MPS. Together with a possibly improved ruminal development, these factors plausibly explain the superior growth performance of group AH-0.8P. The enhanced growth performance was evidenced by the greatest values for ADG in all periods, increased BW and withers height at weaning and the final day of the experiment, as well as by increased hip height at weaning. The addition of forage and the provision of supplemental P above currently recommended levels independently tended to increase FE. However, only in their combination, they elicited superior growth performance.

With regard to feed intake, previous studies had observed a high variation of starter intake when adding forage in young calves. Greater starter intake (Mirzaei et al., 2015), lower starter intake (Nocek and Kesler, 1980; Phillips, 2004), or unchanged starter intake (Karimi et al., 2021; Panahiha et al., 2022) have all been reported when forage-inclusive starter diets were compared to forage-free starter diets. As the current study identified that the effect of FA on starter intake is opposite with marginal-P vs. high-P

diets, it may be speculated that the different results obtained in previous studies may, at least in part, reflect different P available from the used diets; however, P content and its phytate-P portion is mostly not reported in these studies (Nocek and Kesler, 1980; Phillips, 2004; Mirzaei et al., 2015).

The fact that supplemental P promoted the intake of starters with a greater fiber content selectively in the pre-weaning period may be, at least in part, linked to the limited ability of the immature rumen to degrade fiber. In other words, a greater P availability in the pre-weaning period could have the potential to stimulate fiber-digesting bacteria. The microbial composition was not directly analyzed in the current study. However, MPS can be a mirror for overall microbial activity in ruminants. It is not only a critical descriptor of microbial activity in dairy calves but has also been considered as a suitable indicator for the establishment and development of the microbial community in young calves (Kazemi-Bonchenari et al., 2022). A concept where higher P supplementation favors fiber digestibility in young calves would be compatible with the already known role of P for fiber digestion in dairy cows (Puggaard et al., 2013; Jarrett et al., 2014) and other mature ruminants (Durand and Komisarczuk, 1998).

Among the available health indicators, the general appearance and fecal consistency were scored in the current study. Calves receiving AH-0.8P had the most favorable appearance score pre-weaning. The opposite group NAH-0.4P had the least favorable general appearance score and, additionally, the least favorable fecal score pre-weaning. It has been reported before that an optimum level of forage incorporation in starters optimizes passage rate, prevents looser feces and at the same time keeps the digestibility of nutrients in a favorable range (Molaei et al., 2021). The results of the current study extend these previous observations by showing that this health benefit of forage is maximized when P availability is increased in starter diets.

When considering that feeding AH with 0.8P and 0.4P led to the highest and lowest starter intakes pre-weaning, respectively, these groups also had the highest and lowest absolute P intakes. The also performed determination of P excretion through urine and feces from spot samples is not as accurate as balance trials. Nonetheless, it indicated that FA to the diet had no influence on P excretion through urine and feces. The latter was further supported by the measurement of P digestibility. These results suggest that altered P excretion is likely not a cause of why forage-supplemented starters may benefit from increased P addition in young calves.

The promoting effect of P on OM and NDF digestibility logically coincided with a greater concentration of total SCFA in the ruminal fluid of calves receiving 0.8P at the end of both the pre-weaning and the post-weaning periods. As indicated before, greater OM digested in the rumen has a pivotal role in the fermentation rate and stimulates SCFA production in dairy calves (Makizadeh et al., 2020). The intake of AH vs. NAH, on the other hand, increased the concentration of acetate at the expense of propionate in the pre-weaning period, resulting in an increased acetate: propionate ratio. The increase in acetate concentration can be explained by a greater substrate provision to fiber-digesting bacteria (Beiranvand et al., 2014; Mirzaei et al., 2015); whereas, the lower ruminal propionate concentration can be attributed to lower substrate utilization by amylolytic bacteria that digest starch (Kazemi-Bonchenari et al., 2017; Rastgoo et al., 2020). A fermentation pattern with less propionate concentration in the rumen has repeatedly been associated with greater ruminal pH (Khan et al., 2008; Makizadeh et al., 2020; Molaei et al., 2021), which was also observed as a trend in the present study in AH-receiving calves pre-weaning. Moreover, it has been indicated that feeding forage to lambs fed high concentrate diets can modulate the microbiome of the rumen that eventually may contribute to the fermentation pattern (Gebeyew et al., 2025).

From the nitrogen metabolism perspective, a reduced ammonia nitrogen concentration was observed in the rumen of calves fed starters with a greater P level both pre- and post-weaning. This conformed with lower urinary nitrogen excretion in the 0.8P groups and suggests that P can improve nitrogen utilization in the rumen. When additionally considering urinary PD excretion through urine, however, it became clear that N efficiency with high-P diets was only translated to increased microbial protein production when starters additionally contained AH. This likely suggests that the improvement of MPS by high-P and forage-containing diets should be primarily attributable to fiber-degrading bacteria. The latter suggestion should be substantiated in further studies by more detailed analyses of the ruminal microbiota and microbial nitrogen turnover. However, the current data already allow the conclusion that lower ruminal ammonia nitrogen concentration, lower urinary wastage of nitrogen and greater MPS can be regarded as beneficial for both the animal and the environment. They collectively indicate greater nitrogen utilization efficiency in the rumen (Kohn et al., 2005; Kazemi-Bonchenari et al., 2022). From an environmental perspective, this implies the unfortunate conflict that a reduction of environmental pollution with nitrogen is achieved by increasing environmental pollution with P. A potential solution to this conflict could be dietary supplementation with phytase to improve ruminal availability of P without surplus P addition to the diet (Humer and Zebeli, 2015).

5. Conclusions

It can be concluded that currently recommended P levels of calf starter diets (derived from experiments without forage inclusion) can be limiting for nitrogen efficiency, microbial fermentation, and digestibility of nutrients when forage is included in those diets. Thus, P limitation can explain the negative results of some previous studies where forage inclusion in starter diets reduced growth performance. With an optimized P nutrition, however, FA has great potential to promote the development of ruminal microbial activity and calf performance. In the present study, optimized P nutrition was achieved by high supplemental P. This is not generally recommendable for feeding practice because P is a limited resource with a great environmental impact. As an alternative, further studies should explore the potential of dietary phytase addition to optimize P nutrition in forage-receiving young calves. Limited phytase activity of the immature ruminal community might be an important factor contributing to inadequate P availability in calf starter diets. Because of the high growth potential of young calves during the suckling period, the interaction of P and nitrogen in this critical period should be explored in more detail to achieve maximum growth while avoiding P and nitrogen wastage in the environment.

Credit Author Statement

Masoumeh Eghtedari: Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Amin Khezri:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Mehdi Kazemi-Bonchenari:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Mohammadreza Mohammadabadi:** Validation, Resources, Formal analysis. **Saeed Esmaeili Mahani:** Validation, Methodology, Formal analysis. **Jörg R. Aschenbach:** Writing – review & editing, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

- AOAC. Official methods of analysis. 17th ed. Arlington, VA: AOAC International; 2002.
- Beiranvand H, Ghorbani GR, Khorvash M, Kazemi-Bonchenari M. Forage and sugar in dairy calves' starter diet and their interaction on performance, weaning age and rumen fermentation. *J Anim Physiol Anim Nutr* 2014;98:439–45.
- Bjelland DW, Weigel KA, Hoffman PC, Esser NM, Coblenz WK. The effect of feeding dairy heifers diets with and without supplemental phosphorus on growth, reproductive efficiency, health, and lactation performance. *J Dairy Sci* 2011;94:6233–42.
- Bravo D, Sauvant D, Bogaert C, Meschy F. Quantitative aspects of phosphorus absorption in ruminants. *Reprod Nutr Dev* 2003;43(3):271–84.
- Breves G, Schröder B. Comparative aspects of gastrointestinal phosphorus metabolism. *Nutr Res Rev* 1991;4:125–40.
- Broderick GA, Kang JH. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J Dairy Sci* 1980;63:64–75.
- Challa J, Braithwaite GD, Phanoa MS. Phosphorus homeostasis in growing calves. *J Agric Sci Camb* 1989;112:217–26.
- Challa J, Braithwaite GD. Phosphorus and calcium in growing calves with especial emphasis on phosphorus homeostasis. 3. Studies of the effect of continuous intravenous infusion of different levels of phosphorus in ruminating calves receiving adequate dietary phosphorus. *J Agric Sci Camb* 1988;110:591–5.
- Chen XB, Gomes MJ. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of technical details. Aberdeen, UK: International Feed Research Unit, Rowett Research Institute; 1992. Occasional Publication.
- Dennis TS, Suarez-Mena FX, Hill TM, Quigley JD, Schlotterbeck RL, Lascano GJ. 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* 2018;101:408–12.
- Durand M, Komisarczuk S. Influence of major minerals on rumen microbiota. *J Nutr* 1998;118:249–60.
- Eadie JM. The development of rumen microbial populations in lambs and calves under various conditions of management. *J Gen Microbiol* 1962;29:563–78.
- Eechkhout W, De Paepe M. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Anim Feed Sci Technol* 1994;47:19–19.
- Esser NM, Hoffman PC, Coblenz WK, Orth MW, Weigel KA. The effect of dietary phosphorus on bone development in dairy heifers. *J Dairy Sci* 2009;92:1741–9.
- Fonty G, Gouet P, Jouany J, Senaud J. Establishment of the microflora and anaerobic fungi in the rumen of lambs. *Microbiol* 1987;133:1835–43.
- Gebeyew K, Mi H, Du R, Gao M, Diba D, Tang Sh, He Zh, Tan Z. Wheat straw and alfalfa hay alone or combined in a high-concentrate diet alters microbial-host interaction in the rumen of lambs. *Anim Nut* 2025;20:444–57.
- Ghorbani H, Kazemi-Bonchenari M, HosseinYazdi M, Mahjoubi E. 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* 2020;262:114429.
- Heinrichs AJ, Erb HN, Rogers GW, Cooper JB, Jones CM. Variability in Holstein heifer heart-girth measurements and comparison of prediction equations for live weight. *Prev Vet Med* 2007;78:333–8.
- Hill TM, Bateman HG, Aldrich JM, Schlotterbeck RL. Effects of the amount of chopped hay or cottonseed hulls in a textured calf starter on young calf performance. *J Dairy Sci* 2008;91:2684–93.
- Humer E, Zebeli Q. Phytate in feed ingredients and potentials for improving the utilization of phosphorus in ruminant nutrition. *Anim Feed Sci Technol* 2015;209:1–15.
- Jarrett JP, Wilson JW, Ray PP, Knowlton KF. The effects of forage particle length and exogenous phytase inclusion on phosphorus digestion and absorption in lactating cows. *J Dairy Sci* 2014;97:411–8.
- Kargar S, Kowsar Z, Poorhamdollah M, Kanani M, Ansari K, Ghaffari MH. Effects of replacing steam-flaked corn with shredded sugar beet pulp on feed sorting, behavior, blood metabolites, and growth performance of dairy calves. *Anim Nut* 2021;7:917–26.
- Karimi A, Alijoo YA, Kazemi-Bonchenari M, Mirzaei M, Sadri H. 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* 2021;20:1817–28.
- Kazemi-Bonchenari M, Dehghan-Banadaky M, Fattahnia F, Saleh-Bahmanpour A, Jahani-Moghadam M, Mirzaei M. Effects of linseed oil and rumen undegradable protein:rumen-degradable protein ratio on performance of Holstein dairy calves. *Br J Nutr* 2020;123:1247–57.
- Kazemi-Bonchenari M, Khanaki H, Jafari A, Eghbali M, Poorhamdollah M, Ghaffari MH. Milk feeding level and starter protein content: effects on growth performance, blood metabolites, and urinary purine derivatives of Holstein dairy calves. *J Dairy Sci* 2022;105:1115–30.
- Kazemi-Bonchenari M, Salem AZM, Lopez S. Influence of barley grain particle size and treatment with citric acid on digestibility, ruminal fermentation and microbial protein synthesis in Holstein calves. *Animal* 2017;11:1295–302.
- Kertz AF, Prewitt LR, Jr Everett JP. An early weaning calf program: summarization and review. *J Dairy Sci* 1979;62:1835–43.
- Keshavarz V, Dehghan-Banadaky M, Ganjkanlou M, Kazemi-Bonchenari M. Effects of deeding wheat straw or beet pulp in starters supplemented with either soybean oil or palm fatty acids on growth performance and urinary purine derivatives in dairy calves. *Anim Feed Sci Technol* 2023:115569.
- Khan MA, Lee HJ, Lee WS, Kim HS, Ki KS, Hur TY, Suh GH, Kang SJ, Choi YJ. Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. *J Dairy Sci* 2007;90:3376–87.
- Kohn RA, Dinneen MM, Russek-Cohen E. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *J Anim Sci* 2005;83:879–89.
- Larson LL, Owen FG, Albright JL, Appleman RD, Lamb RC, Muller JD. Guidelines toward more uniformity in measuring and reporting calf experimental data. *J Dairy Sci* 1977;60:989–93.
- Lesmeister KE, Heinrichs AJ. Effects of corn processing on growth characteristics, rumen development, and rumen parameters in neonatal dairy calves. *J Dairy Sci* 2004;87:3439–50.
- Luchini ND, Lane SF, Combs DK. Evaluation of starter diet crude protein level and feeding regime for calves weaned at 26 days of age. *J Dairy Sci* 1991;74:3949–55.
- Makizadeh H, Kazemi-Bonchenari M, Mansoori-Yarahmadi H, Fakhraei J, Khanaki H, Drackley JK, Ghaffari MH. Corn-processing and crude protein content in calf starter: effects on growth performance, ruminal fermentation, and blood metabolites. *J Dairy Sci* 2020;103:9037–53.
- Miller-Cushon EK, DeVries TJ. Effect of early feed type exposure on diet-selection behavior of dairy calves. *J Dairy Sci* 2011;94:342–50.
- Mirzaei M, Khorvash M, Ghorbani GR, Kazemi-Bonchenari M, Riasi A, Soltani A, Moshiri B, Ghaffari MH. 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* 2016;94:678–86.
- Mirzaei M, Khorvash M, Ghorbani GR, Kazemi-Bonchenari M, Riasi A, Nabipour A, van der Drone JJGC. Effects of supplementation level and particle size of alfalfa hay on growth characteristics and rumen development in dairy calves. *J Anim Physiol Anim Nutr* 2015;99:553–64.
- Mohammadzadeh M, Kazemi-Bonchenari M, HosseinYazdi M, Mirzaei M. Forage source (alfalfa hay vs wheat straw) and rumen undegradable to degradable protein ratio: effects on growth performance, microbial protein yield, digestibility, blood metabolites, and behavior of Holstein dairy calves. *Spanish J Agric Res* 2021;19:e610.
- Molaei M, Kazemi-Bonchenari M, Mirzaei M, Esmaeili HR. 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* 2021;279:115031.
- Moreira VR, Zeringue LK, Williams CC, Leonardi C, McCormick ME. Influence of calcium and phosphorus feeding on markers of bone metabolism in transition cows. *J Dairy Sci* 2009;92:5189–98.
- Morris TR. Experimental design and analysis in animal sciences. CABI Publishing; 1999.
- NASEM. National academies of science, engineering, and medicine. Nutrient requirements of dairy cattle: 8th revised edition. Washington, DC, USA: National Academies Press; 2021.
- Nocek JE, Kesler EM. Growth and rumen characteristics of Holstein steers fed pelleted or conventional diets. *J Dairy Sci* 1980;63:249–54.
- NRC. National Research Council. Nutrient requirements of dairy cattle. 7th Revised Edition. Washington, DC, USA: National Academies Press; 2001.
- Panahiha P, Mirzaei-Alamouti H, Kazemi-Bonchenari M, Aschenbach JR. Growth performance, nutrient digestibility, and ruminal fermentation of dairy calves fed starter diets with alfalfa hay versus corn silage. *J Dairy Sci* 2022;105:9597–609.
- Phillips CJC. The effects of forage provision and group size on the behavior of calves. *J Dairy Sci* 2004;87:1380–8.

- Puggaard L, Lund P, Sehested J. Effect of feed forage particle size and dietary urea on excretion of phosphorus in lactating dairy cows. *Livest Sci* 2013;158:50–6.
- Puhl AA, Greiner R, Selinger LB. A protein tyrosine phosphatase-like inositol polyphosphatase from *Selenomonas ruminantium* subsp. *lactilytica* has specificity for the 5-phosphate of myo-inositol hexakisphosphate. *Int J Biochem Cell Biol* 2008;40:2053–64.
- Rastgoo M, Kazemi-Bonchenari M, HosseinYazdi M, Mirzaei M. 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* 2020;269:114646.
- Ternouth JH, McLachlan BP, Clark JM, Thomas BJ. Effects of dietary phosphorus and nitrogen deficiencies on the intake, growth and metabolism of lambs. *J Agric Sci* 1993;121:409–19.
- Valk H, Sebek LBJ, Beynen AC. Influence of phosphorus intake on excretion and blood plasma and saliva concentrations of phosphorus in dairy cows. *J Dairy Sci* 2002;85:2642–9.
- Van Keulen J, Young BA. Acid insoluble ash as a natural marker for digestibility studies. *J Dairy Sci* 1977;44:282–7.
- Van Soest PJ, Roberts JB, Lewis BA. Methods of dietary fiber, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* 1991;74:3583–97.
- Wu Z, Satter LD, Sojo R. Milk production, reproductive performance, and fecal excretion of phosphorus by dairy cows fed three amount of phosphorus. *J Dairy Sci* 2000;83:1028–41.
- Yousefinejad S, Fattahnia F, Kazemi-Bonchenari M, Khanaki H, Drackley JK, Ghaffari MH. 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* 2021;104:1630–44.
- Zhang YQ, He DC, Meng QX. Effect of a mixture of steam-flaked corn and soybeans on health, growth, and selected blood metabolism of Holstein calves. *J Dairy Sci* 2010;93:2271–9.