Spanish Journal of Agricultural Research
19 (3), e0610, 12 pages (2021)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2021193-16733
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

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

Milad Mohammadzadeh, Mehdi Kazemi-Bonchenari, Mehdi HosseinYazdi and Mehdi Mirzaei

Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349 Arak, Iran

Abstract

Aim of study: The effects of forage source (alfalfa hay; AH *vs*. wheat straw; WS) with rumen undegradable to degradable protein ratio [low ratio (LR) = 28:72; high ratio (HR) = 36:64] were evaluated in young dairy calves.

Area of study: Arak, Iran.

Material and methods: Forty-eight 3-d old female Holstein dairy calves (44.5 kg of BW) were allocated in four treatments: (1) AH with low dietary RUP:RDP ratio (AH-LR); (2) AH with high dietary RUP:RDP ratio (AH-HR); (3) WS with low dietary RUP:RDP ratio (WS-LR); and (4) WS with high dietary RUP:RDP ratio (WS-HR), being RDP and RUP rumen degradable and undegradable proteins, respectively. The calves weaned on d 53 of the experiment and remained in the study until d 73 of age.

Main results: The average daily gain and feed efficiency were improved in dairy calves receiving HR diets compared to LR diets during the post-weaning period ($p<0.05$). The fecal score ($p=0.03$) and neutral detergent fiber digestibility ($p=0.04$) were improved when calves fed WS diets compared to AH diets. Feeding HR diets increased allantoin ($p=0.04$) and microbial protein yield, and reduced blood urea nitrogen concentration ($p=0.03$) compared to LR diets. Assessing the interaction effects of the experimental factors resulted that the greatest BW, wither height, and blood beta-hydroxybutyrate, and the lowest urinary N concentration were observed in the WS-HR treatment ($p<0.05$).

Research highlights: Feeding WS with high RUP:RDP ratio is recommendable in dairy calves due to the improvement in gain and N efficiency.

Additional key words: urea recycling; fiber; nitrogen efficiency; calf growth

Abbreviations used: ADG (average daily gain); AH (alfalfa hay); ALT (alanine aminotransferase); AST (aspartate aminotransferase); BHB (beta-hydroxybutyrate); BW (body weight); CP (crude protein); DM (dry matter); DMI (DM intake); EE (ether extract); FE (feed efficiency); HR (high ratio); LQF (low-quality forage); LR (low ratio); MPY (microbial protein yield); NDF (neutral detergent fiber); PD (purine derivatives); RDP (rumen degradable protein); RUP (rumen undegradable protein); UN (urinary nitrogen); WS (wheat straw).

Authors' contributions: Conceptualization: MKB, MMo, and MHY. Methodology, writing, review and editing: MKB and MMo. Formal analysis: MYH and MMo. Investigation and data curation: MM, MHY and MMi. Writing, original draft preparation: MMo. Supervision, and project administration: MKB.

Citation: Mohammadzadeh, M; Kazemi-Bonchenari, M; HosseinYazdi, M; Mirzaei, M (2021). 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 Journal of Agricultural Research, Volume 19, Issue 3, e0610. https://doi.org/10.5424/sjar/2021193-16733

Received: 24 Mar 2020. **Accepted:** 27 Jul 2021.

Funding agencies/institutions	Project / Grant
Deputy of Research and Technology of Arak University	95-3108

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Mehdi Kazemi-Bonchenari: m-kazemibonchenari@araku.ac.ir; mehdi_kazemi59@yahoo.com

Introduction

Feeding forage to dairy calves are still controversial from the perspectives such as the forage feeding levels, sources, and particle sizes (Suárez *et al.*, 2007; Khan *et al.*, 2012). Some studies recommended to include forage in the starter diet (Suárez *et al.*, 2007; Mirzaei *et al.*, 2017) because of its favorable effects such as improvement of muscular layer of rumen, promotion of rumination, prevention of hyper-keratinization, and the increased

nutrients absorption (Beiranvand *et al.*, 2014; Mirzaei *et al.*, 2017). In addition, Suárez *et al.* (2007) stated that ruminal pH (< 5.2) is reduced, when calves received forage-free starter diets. Forage intake can reduce the incidence of poorly developed mucosa and plaque formation observed in concentrate-fed calves (Suárez *et al.*, 2006). However, some others reported unfavorable effects of forage inclusion in starter diets such as the reduced intake through rumen fullness, the reduced nutrients digestibility, and the weakened of ruminal papillae development (Movahedi *et al.*, 2016; Soltani *et al.*, 2017). These researchers believe that forage is also less digestible, which may be especially problematic for the young calf with an immature rumen and inadequate ruminal conditions for cellulose and hemicellulose fermentation. Thus, forage inclusion is generally thought to lead to poorer average daily gain (Kertz *et al.*, 1979; Hill *et al.*, 2008). However, in recent years, it has been proposed that a minimum content of 5% forage in starter diet (DM basis) is necessary to avoid pH decline in dairy calves (Aragona *et al.*, 2020).

Regardless of advantages and disadvantages of forge inclusion in starter diet of dairy calves, wheat straw (WS), as an agricultural by-product, is easily available, while it is considered as a cheap feedstuff for animal nutrition. WS contains high physical effective fiber content suitable to stimulate rumination (NRC, 2001) and producing adequate buffer, in order to aid ruminal health (Maekawa *et al.*, 2002). It can be known as a valuable characteristic of WS in dairy calves which are more susceptible for lower ruminal pH than mature ruminants (Laarman & Oba, 2011). However, it was mostly considered a low-quality forage (LQF) because of its low protein content as well as its lower nutrient digestibility compared to other forage sources in ruminant nutrition (Chegini *et al.*, 2019). This feedstuff has been shown to reduce intake in dairy calves when offered as a free choice in the starter diet (Movahedi *et al.*, 2016). However, Hill *et al.* (2010) reported that the intake of chopped WS in calves responded in a curvilinear manner relative to the provision of forage neutral detergent fiber (NDF). Thus, they concluded that WS has the potential to be included in limited amounts in dairy calf starter diets. In dairy calf nutrition, there are scarce documents which compare WS feeding with other forage sources, especially when forages are fed as limited access and not as free-choice. We hypothesized whether limited amount of WS incorporated in their starter diets is useful. Moreover, animal growth performance can be compared with starter diets supplemented with alfalfa hay (AH) as a word-wide forage source used in dairy calves. Therefore, the first aim was comparing limited feeding amount (7% of starter diet, DM basis) of forage source (alfalfa hay *vs* wheat straw; AH *vs.* WS) in dairy calves during the pre-weaning period.

Regardless the high-fiber content of WS and its low N content, previous works indicated that different strategies may be applied to improve fiber digestibility when LQF is fed to ruminants. Chemical treatment (Antongiovanni *et al.*, 1991) or providing high ruminally available N for fiber degrading bacteria (Chegini *et al.*, 2019) in the rumen have been evaluated as applicable strategies. Chemical processing may not be suitable in animal nutrition due to the potential poisonous impacts of chemical residuals on animal health (Chegini *et al.*, 2019). This has to be more attended in young dairy calves because of its higher susceptibility in early weeks of life (Kazemi-Bonchenari *et al.*, 2020). Instead, as an another strategy, the greater ruminal undegradable to degradable protein ratio (RUP:RDP) has resulted in improved N efficiency and animal performance in mature ruminants when fed LQF in diet (Wickersham *et al.*, 2008; Koch *et al.*, 2017; Valizadeh *et al.*, 2021). For instance, Koch *et al.* (2017) showed improved growth performance in heifers fed high fiber levels when diets contained greater RUP:RDP ratio. They postulated that additional dietary fiber depressed microbial protein yield (MPY) and N utilization, but greater RUP supplement was instrumental in reestablishing N balance mostly through increasing the N recycling in to the rumen and provide greater ruminal available N for microbial protein synthesis (Wickersham *et al.*, 2008; Dorri *et al.*, 2021). The effects of different dietary RUP:RDP ratios in dairy calves on animal growth performance has not been well documented when calves received LQF in starter diet. In fact, it is not well understood whether inclusion the WS as low quality forage in starter diet of dairy calves can influence N metabolism and efficiency. Two levels of dietary RUP:RDP (21 and 34% of crude protein (CP), DM basis) ratios were evaluated in dairy calves (Kazemi-Bonchenari *et al.*, 2016); however, no forage source was incorporated in starter diets of that study. To the best of author's knowledge, there is no document evaluating feeding different forage sources with different RUP:RDP ratios in dairy calves. We postulated that different RUP:RDP ratios may affect dairy calves' performance as a result of receiving different forage sources through modifying the ruminal N metabolism. Thus, the present study aimed to evaluate the effects of forage sources (AH *vs*. WS) and different RUP:RDP ratios (28:72 *vs*. 36:64 as the LR and HR diets, respectively) on growth performance, nutrients digestibility, behavior, MPY, excretion of urinary nitrogen (UN), blood metabolites, and liver enzymes in Holstein dairy calves.

Materials and methods

Location of experiment and approval

The present study was carried out at commercial dairy farm (Zarrin-Khoosheh Dairy Farm), Arak, Iran. All the animal procedures were approved by the Animal Care

and Use Committee of Arak University (IACUC Protocol #IR95-3108) outlined by the Iranian Council of Animal Care (1995).

Experimental design, treatments, and management

Forty-eight 3-day-old female Holstein dairy calves with 44.5 ± 1.8 kg of initial BW were randomly assigned to experimental starter diets (12 calves per treatment) in a 2 × 2 factorial arrangements with the factors of forage source (AH *vs.* WS) and RUP:RDP ratio [low ratio (LR)=28:72; high ratio (HR)=36:64]. The crude protein content was constant across the treatments (20%, DM basis), and the figures for the RUP:RDP ratios were based on the crude protein content (Table 1). Immediately after birth, calves were separated from their dams, weighed, and moved to individual pens bedded with sand. Calves received 4 L/d whole milk in galvanized tin buckets twice a day at 0730 and 1630 h from d 3 to 10 and 7 L/d from d 11 to 48 of the study followed by 3 L/d from d 49 to d 52 of the study. Whole milk samples were weekly taken and analyzed for fat, CP, lactose and total solids using an infrared spectrophotometer (FOSS milk-o-scan; FOSS Electric, HillerØd, Denmark). The average composition of offered milk was 3.16 ± 0.09% fat, 3.03 ± 0.07% CP, 4.83 ± 0.04% lactose and 11.8 ± 0.16% total solids. All the calves were

Table 1. The ingredients and chemical composition of experimental starter diet (g/kg of DM, unless otherwise stated)

Item	Treatments[1]			
	AH		WS	
	LR	HR	LR	HR
Ingredients				
Alfalfa hay, chopped	70	70	0	0
Wheat straw, chopped	0	0	70	70
Barley grain, finely ground	40	40	40	40
Corn grain, finely ground	520	520	520	520
Soybean meal	245	136	270	161
Corn gluten meal	0	80	0	80
Wheat bran	77	106	51	80
Vitamin and mineral mix[2]	23	23	23	23
Calcium carbonate	6	6	7	7
Di-calcium phosphate	4	4	4	4
Sodium bicarbonate	10	10	10	10
Salt	5	5	5	5
Chemical composition				
Metabolizable energy,[3] (Mcal/kg)	2.99	2.93	2.95	2.90
Crude protein	20.0	20.0	20.0	20.0
Rumen undegradable protein , % of CP[3]	28.1	36.3	28.8	36.1
RUP:RDP ratio	28:72	36:64	28:72	36:64
Non-fibrous carbohydrate[4]	50.8	51.1	49.2	50.1
Neutral detergent fiber	21.1	20.6	23.7	23.2
Ether extract	2.92	2.84	2.83	2.81
Calcium	7	7	7	7
Phosphorus	4	4	4	4

[1]Treatments were: (1) alfalfa hay with low dietary RUP:RDP ratio (AH-LR); (2) alfalfa hay with high dietary RUP:RDP ratio (AH-HR); (3) wheat straw with low dietary RUP:RDP ratio (WS-LR); (4) wheat straw with high dietary RUP:RDP ratio (WS-HR). [2]Contained per kilogram of supplement: 700,000 IU vitamin A, 150,000 IU vitamin D, 1000 IU vitamin E, 1 g Mn, 100 g Ca, 2g Zn, 60 g P, 12 g Mg, 10 g Na, 2 g Fe, 45 mg Co, 150 mg Cu, 30 mg I, and 3 mg Se. [3]Calculated from NRC (2001) [4]Non-fiber-carbohydrate was calculated as [DM- (NDF + CP + ether extract + ash)] (NRC, 2001).

weaned on d 53 of the study but the experiment continued 20 days thereafter. Experimental treatments were: (1) AH with low RUP:RDP ratio (AH-LR); (2) AH with high RUP:RDP ratio (AH-HR); (3) WS with low RUP:RDP ratio (WS-LR); and (4) WS with high RUP:RDP ratio (WS-HR). Experimental starter concentrates were formulated to meet the current NRC (2001) nutrients requirements. Ingredients and chemical composition of experimental diets are presented in Table 1. Starter concentrates were formulated to be iso-nitrogenous but different in RUP:RDP ratio by replacing corn gluten meal by soybean meal as stated by Amirabadi Farahani *et al.* (2017). The level of AH and WS was 70 g/kg of the basal diet. Because particle size could be a variation source of forage inclusion in starter diet of dairy calves (Mirzaei *et al.*, 2015), similar geometric particle size has been considered for treatments when chopping AH and WS to prevent this variation (geometric mean particle size was 2.92 ± 0.13 and 2.95 ± 0.12 mm, for AH and WS, respectively). The concentrate feed was mixed well with forages and the diet was offered as total mixed ration to dairy calves throughout the study. Starter diet was fed *ad libitum* to permit at least 10% orts in a day. The calves had free access to water throughout the experimental period.

Data recording for intake, gain, and efficiency

Starter feed refusals were collected and recorded daily at 0730 h and fresh starter feed was fed at 0800 h. Body weight (BW) was recorded every 10 d using an electronic balance which was calibrated before initiation of the study and every month thereafter. Calves were weighed before the morning meal to eliminate the effects of the empty/full status of the gastrointestinal tract on BW. Average daily gain (ADG, kg of BW/d) was calculated as the difference between BW taken every 10 d apart divided by 10. Feed efficiency (FE) was computed as g of ADG/kg of total DM intake (DMI; liquid feed DMI + starter feed DMI).

Chemical composition of diets and digestibility trial

At 10-d intervals, samples from feeds and orts were dried in a convection oven (60C° for 48 h). Subsamples of dried feeds and orts were mixed thoroughly and then ground through a mill (Ogaw Seiki Co., Ltd., Tokyo, Japan) to pass a 1-mm screen and stored at –20°C until chemical analysis. Diet samples were analyzed for CP (method 988.05; AOAC, 2002), ether extract (EE, method 920.39; AOAC, 2002), and NDF without sodium sulfite, but with the inclusion of α-amylase adopted by Van Soest *et al.* (1991). The non-fibrous carbohydrate component was computed as 100 – (CP + NDF + EE + ash) (NRC, 2001).

In the last five days of the experiment, fecal samples were collected from the rectum of each calf initiated on 08:00 h of first sampling day and continued by 12 h intervals (10 samples per each calf). Collected fecal samples were dried in a forced dried oven (60°C; 72 h), and then ground in a Wiley mill through a 1-mm screen. Aliquots of fecal samples were analyzed to determine total N, ash and NDF. Apparent total tract digestibility of nutrients (DM, NDF, CP, and EE) was measured by using acid insoluble ash as an internal marker based on Van Keulen & Young (1977).

Fecal scoring and behavioral data recording

Fecal scoring was 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 (Heinrichs *et al.*, 2007).

Behavioral data (standing, lying, eating, ruminating, and non-nutritional behavior were monitored by direct observations of all calves over the total time (min) devoted to each monitored feeding behavior twice on pre-weaning period (d 45 and 52 of the trial) as well as twice after weaning (d 66 and 73 of the trial). On behavioral recording days, calves were taken under observation 1 h after the solid feed offering and 3 h immediately after the morning milk feeding during the pre-weaning period. During the post-weaning recording times, calves were observed 1 h before and 3 h after the solid feed was offered at 08:00 h. Therefore, the total time for each calf's behavior recording length was equal to 16 h (8 h before and 8 h after weaning).

Growth indices recording

Growth variables, including heart girth (circumference of the chest), body length (distance between the points of shoulder and rump), body girth (circumference of the belly before feeding), wither height (distance from the base of the front feet to wither) and hip height (length from the base of the rear feet to the hook bones) were taken at the start of the experiment (d 3), weaning (d 53) and last day of experiment (d 73).

Microbial protein yield measurement

The MPY was estimated based on the purine derivatives (PD) excretion obtained via spot sampling technique explained by Valadares *et al.* (1999). Because feeding milk into dairy calves could interfere with PD excretion during the pre-weaning period due to its PD content (Gonzalez-Ronquillo *et al.*, 2003), the spot urine sampling

technique was used for MPY estimation only in the post-weaning period as reported by Makizadeh *et al.* (2020) in the post-weaned calves. This technique was used for the estimation of daily urine output from creatinine concentration as explained in detail by Valadares *et al.* (1999). Urine volumes were estimated as BW × 26.8 / creatinine concentration (mg/L) in post-weaned dairy calves as reported by Dennis *et al.* (2017). Spot urine samples were collected on 3 sampling days during the post-weaning period from each animal during the morning (between 09:00 and 11:00 h) and during the afternoon (between 15:00 and 17:00 h) as explained in a recent work on dairy calves (Kazemi-Bonchenari *et al.*, 2020). Samples were collected when calves urinated spontaneously (≈ 10 mL). An aliquot of 5 mL of each sample was diluted immediately with 45 mL of 0.036 N sulfuric acid then stored at –20°C for analysis. Later, urine samples were thawed at room temperature and analyzed to determine the creatinine (Kit No. 555-A; Sigma Chemical Co.), UN (using the assay described by Broderick & Kang, 1980), uric acid (Kit No. 685-50; Sigma Chemical Co). Allantoin was measured using the high-performance liquid chromatography method described by Chen & Gomes (1992). Total excretion of allantoin and acid uric was calculated from estimated daily urine output and determined metabolite concentrations. The ruminal microbial N synthesis was calculated from daily urinary PD output using the following equation described by Chen & Gomes (1992): Microbial N (g N / d) = X (mmol/d) × 70 / (0.116 × 0.83 × 1000); where X is microbial purine absorbed (mmol /d), 70 is the N content of purines coefficient (mg N / mmol), 0.116 is the ratio of purine-N to total N in mixed ruminal microbes which is 11.6:100, and 0.83 is average digestibility of microbial purines (Chen & Gomes, 1992).

Blood sampling

The blood samples for the experimental period were collected on d 35 (pre-weaning) and 70 (post-weaning) 4 h after morning feeding (at 1200 h) from the jugular vein into 10 mL tubes, placed on ice and centrifuged at 3,000 × g for 20 min at 4°C. Serum subsamples stored at –20°C and subsequently were analyzed to determine concentrations of glucose (Kit No. 93008), albumin (Kit No. 9307), total protein (Kit No. 9304), and blood urea N (BUN) (Kit No. 93013) using commercial kits in accordance to the manufacturer's instruction protocols (Pars Azmoon Co., Tehran, Iran). Beta-hydroxybutyrate (BHB) was measured with a commercial kit (Abbott Diabetes Care Ltd., Oxin, UK). Liver function indicator enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were measured using ELISA (Auto Analyzer Hitachi 717 Instruments, Inc., Tokyo, Japan) and commercial laboratory kits (Kits No. 92005 and 92004, for AST and ALT, respectively).

Statistical analysis

Data were subjected to ANOVA using the MIXED procedure of SAS (SAS 9.1, SAS Inst. Inc., Cary, NC, USA) during the pre-weaning (d 3 to 53), post-weaning (d 54 to 73) and overall (d 3 to 73 of the study) periods with time as repeated measures for starter intake, total DMI, ADG, and FE. The model included fixed effects of the time (sampling date), forage source (AH *vs.* WS), the RUP:RDP ratio (28:72 as LR and 36:64 as HR), and their interactions and calf as a random effect. Before analyses, all data were screened for normality using the UNIVARIATE procedure of SAS. Two variance-covariance structures (auto-regressive type 1 and compound symmetry) were tested and the covariance structure that minimized the Schwarz's Bayesian information criterion was chosen. The fecal score was square-root transformed for better homogeneity of the distribution of residuals. The structural growth, digestibility, behavior, UN, and PD excretion, microbial protein yield, and blood metabolite variables were analyzed using a similar model, but without the effect of time. Initial BW was used as a covariate for weaning weight and final weight. All reported values are least square means, with the PDIFF procedure of SAS used to separate treatment means. The threshold of significance was set at $p \leq 0.05$; trends were declared at $0.05 < p \leq 0.10$.

Results

Starter intake and feed efficiency

The results for starter intake, ADG, BW, and FE are presented in Table 2. The ADG in the post-weaning period was improved in HR diets compared to the LR diets (p=0.05). Accordingly, the FE was improved in the HR diets during the post-weaning period (p=0.04). The greatest BW was observed for WS-HR treatment among the experimental treatments at final measurement (p=0.05). Starter intake did not differ among experimental treatments neither pre-weaning nor post-weaning.

Fecal score, nutrients digestibility, and feeding behavior

Results showed that feeding WS improved fecal score during the pre-weaning period (p=0.03) and the

Table 2. Least square means for starter intake, ADG, FE, fecal score, nutrients digestibility and feeding behavior in calves fed different forage source (AH *vs.* WS) and different rumen undegradable to degradable protein ratios (28:72 *vs*. 36:64).

Item	Treatments[1]				SEM	*p*-value[2]		
	AH		WS			Forage	RUP	Forage × RUP
	LR	HR	LR	HR				
Starter feed intake (g/day)								
Pre-weaning	395	403	300	385	36.2	0.12	0.19	0.28
Post-weaning	1859	1825	2020	1754	105.9	0.67	0.15	0.27
Entire period	813	809	792	776	84.1	0.74	0.90	0.94
Milk intake (g DM/d)	666	669	666	667	14.7	0.94	0.82	0.42
Average daily gain (g/day)								
Pre-weaning	560	526	535	580	47.6	0.76	0.90	0.41
Post-weaning	693	772	664	868	71.4	0.63	0.05	0.38
Entire period	598	596	572	665	40.9	0.60	0.26	0.24
Body weight (kg)								
Pre-weaning	0.52	0.49	0.53	0.54	0.04	0.45	0.85	0.56
Post-weaning	0.36	0.42	0.37	0.49	0.04	0.35	0.04	0.49
Entire period	0.47	0.47	0.48	0.53	0.03	0.28	0.50	0.41
Fecal score								
Pre-weaning	1.24	1.26	1.19	1.18	0.04	0.03	0.88	0.58
Post-weaning	1.08	1.16	1.05	1.05	0.05	0.14	0.38	0.38
Entire period	1.22	1.25	1.17	1.16	0.03	0.02	0.73	0.44
Nutrients digestibility (g/kg)								
Dry matter	634	648	660	642	15.8	0.54	0.89	0.31
Crude protein	707	701	705	692	22.2	0.93	0.85	0.45
Neutral detergent fiber	511	546	562	597	27.7	0.04	0.22	0.98
Ether extract	756	750	745	742	23.8	0.68	0.85	0.92
Behavior min/16 h								
Standing	337	328	330	332	7.25	0.71	0.22	0.17
Lying	308	316	309	299	6.67	0.15	0.77	0.11
Eating	115	111	118	113	5.63	0.43	0.33	0.84
Ruminating	70	71	72	83	4.92	0.06	0.12	0.20
Non-nutritional behavior	130	134	131	133	5.12	0.94	0.53	0.62

[1]Treatments: see Table 1. [2]Statistical comparisons: Forage = forage sources (AH *vs.* WS); RUP; different ratios of RUP:RDP [low ratio (LR) = 28:72; high ratio (HR) = 36:64]; Forage × RUP= interaction between forage source and dietary RUP:RDP ratios. [3] kg of body weight gain/kg of total dry matter intake Means within a row with different superscript letters are statistically different ($p<0.05$).

entire period ($p=0.02$) compared to feeding AH diets (Table 2). Regarding the nutrients digestibility, results revealed that digestibility of NDF was greater when WS was fed to dairy calves in comparison with AH diets ($p=0.04$). However, the digestibility of DM, CP, and EE were not influenced with experimental factors or their interaction.

Regarding the feeding behavior results showed that the time spent for rumination tended to be greater ($p=0.06$) in WS fed calves in comparison with AH diets. The time spent for standing, lying, eating, and non-nutritional behaviors were not influenced with different treatments. No interaction was found for forage source and RUP:RDP ratios regarding the feeding behavior results in the present study.

Skeletal growth

With respect to the skeletal growth indices results showed that the greatest wither height was found for the WS-HR treatment among experimental treatments ($p=0.05$; Table 3). Hip height was greater in final

Table 3. Least square means for growth variables in dairy calves fed different forage source (AH *vs*. WS) and different rumen undegradable (RUP) to degradable protein (RUD) ratios (28:72 *vs*. 36:64).

Item	Treatments[1]				SEM	*p*-value[2]		
	AH		WS			Forage	RUP	Forage × RUP
	LR	HR	LR	HR				
Heart girth								
Initial (d 3)	79.0	79.7	80.6	80.0	0.73	0.36	0.96	0.54
Weaning (d 53)	98.6	98.4	98.0	98.2	0.87	0.73	0.99	0.84
Final (d 73)	99.5	99.6	100.5	102.0	0.86	0.17	0.54	0.54
Body length								
Initial (d 3)	45.0	45.0	45.4	45.1	0.49	0.68	0.85	0.85
Weaning (d 53)	55.6	55.1	55.1	54.5	0.62	0.26	0.99	0.45
Final (d 73)	56.5	56.5	57.6	57.4	0.60	0.26	0.88	0.88
Body girth								
Initial (d 3)	82.7	80.8	82.6	83.0	0.83	0.34	0.55	0.34
Weaning (d 53)	108.1	106.9	107.1	107.0	1.39	0.81	0.72	0.78
Final (d 73)	107.7	108.8	111.6	111.4	1.63	0.16	0.85	0.77
Wither height								
Initial (d 3)	76.8	76.5	76.6	77.5	0.95	0.66	0.78	0.55
Weaning (d 53)	86.9^{ab}	85.1^{b}	85.3b	87.1a	0.80	0.85	0.97	0.05
Final (d 73)	91.6	92.8	91.0	94.1	0.86	0.78	0.08	0.41
Hip height								
Initial (d 3)	80.0	80.5	80.8	80.7	0.80	0.66	0.82	0.86
Weaning (d 53)	93.2	92.0	91.7	93.7	0.81	0.91	0.74	0.15
Final (d 73)	94.6	95.2	94.7	99.0	0.94	0.21	0.04	0.24

[1]Treatments: see Table 1. [2]Statistical comparisons: Forage = forage sources (AH *vs*. WS); RUP; different ratios of RUP:RDP [low ratio (LR) = 28:72; high ratio (HR) = 36:64]; Forage × RUP= interaction between forage source and dietary RUP:RDP ratios. Means within a row with different superscript letters are statistically different ($p<0.05$)

measurement when the HR diets were fed to dairy calves compared to the LR diets ($p=0.04$). Wither height at final measurement tended to be greater also for the HR diets compared with the LR diets ($p=0.08$). Heart girth, body length, and body girth were not influenced with different experimental treatments.

Purine derivatives, microbial protein yield, and urinary nitrogen

The results for purine derivatives, microbial protein synthesis, and urinary nitrogen excretion are presented in Table 4. The urinary allantoin concentration was greater in the calves received the HR diets compared to the calves fed LR diets ($p=0.04$). The purine derivatives and subsequently microbial protein yield were increased when calves were fed the HR diets in comparison with the LR diets ($p=0.05$). The lowest UN concentration was observed for the WS-HR treatment among other experimental treatments ($p=0.05$). Allantoin excretion through urine tended to be greater in calves fed WS compared with AH fed calves ($p=0.09$). Uric acid was influenced neither with the forage source nor with RUP:RDP ratio.

Blood metabolites and liver enzymes

The greatest concentration of BHB among experimental treatments was found in the blood of calves fed WS-HR treatment ($p=0.04$; Table 5). The concentration of BUN was greater in the LR diets in comparison with the HR diets ($p=0.03$). The concentrations of glucose, total protein, and albumin in the blood of experimental animals was not affected by the different treatments ($p > 0.05$).

Regarding the liver enzymes activity in the current study, results revealed that no changes were found for liver enzymes concentrations in the blood of calves among experimental treatments.

Discussion

Effect of forage source

The intake of starters did not differ among experimental treatments in the current study. However, previous works identified different starter intakes when calves were fed various forage sources (Movahedi *et al.*, 2016; Mirzaei *et al.*, 2017). Mirzaei *et al.* (2017) stated that feeding corn silage increased intake in dairy calves compared to AH feeding due to the greater palatability of the starter with increased moisture in corn silage fed calves. AH and WS diets had constant moisture content in the current study, and similar intake was observed during the pre-and post-weaning periods. Movahedi *et al.* (2016) reported lower intake in dairy calves fed WS in comparison with AH fed calves while forage was available as free choice. The forage to concentrate ratio was 9.1:90.9 and 28.6:71.4 for WS and AH diets, respectively, in free choice feeding (Movahedi *et al.*, 2016). Those results contrast with the constant forage to concentrate ratio considered for AH and WS diets (7:93; Table 1). Recently, Poczynek *et al.* (2020) stated that the increase of NDF content in the starter feed up to 310 g/kg DM with replacement of corn by soybean hull or hay supplementation did not influence the starter intake and performance of calves at weaning. In general, the effect of forage feeding on starter intake in dairy calves may be influenced with different variables such as inclusion level, particle size, starter feed texture, total dietary moisture, and grain source (Hill *et al.*, 2010; Mirzaei *et al.*, 2015). ADG and FE were similar in calves fed either AH or WS diets in the current study mostly due to the similar starter intake among diets.

The fecal score was improved in the current study during pre-weaning period and the entire period of experiment when calves were fed with WS in contrast to feeding AH diets. The ruminal variables were not measured; however, previous works stated that ruminal conditions such as high passage rate could reduce fecal consistency (Kazemi-Bonchenari *et al.*, 2017; Mirzaei *et al.*, 2017). Thus, it may be assumed that WS can provide more a favorable ruminal condition than AH to have better fecal score. Minimum forage content (5%, DM basis) was proposed to be included in starter feed that is necessary to avoid pH decline in dairy calves (Aragona *et al.*, 2020). Assessing the behavioral results show that the time spent on rumination was greater in WS fed calves which is due to the greater NDF content supplied in this diet (20.85 *vs.* 23.45% NDF for AH and WS diets, respectively). The greater content of NDF could increase the time spent for rumination in the pre-weaning dairy calves (Poczynek *et al.*, 2020). Greater NDF digestibility was also obtained in calves fed WS in the current study, which suggests that the ruminal environment was more favorable for cellulolytic bacteria activity in WS diets. It could be supposed that greater saliva secretion could be induced by the greater rumination time when calves fed WS compared to AH feeding which can improve rumen function and subsequently improve animal gastrointestinal health status (Maekawa *et al.*, 2002; Aragona *et al.*, 2020). Our results indicate that feeding WS compared to AH in limited amount (7% of starter diet, DM basis) is supposed to provide more stable ruminal fermentation which consequently caused better fecal score, more ruminating time, and greater NDF digestibility.

Effect of RUP:RDP ratio

Regardless the similar intake in the calves fed different dietary RUP:RDP ratios, the ADG and FE improved in

Table 4. Least square means for purine derivatives, microbial protein yield, and urinary nitrogen in dairy calves fed different forage source (AH *vs*. WS) and different rumen undegradable (RUP) to degradable protein (RUD) ratios (28:72 *vs*. 36:64).

Item	Treatments[1]				SEM	*p*-value[2]		
	AH		WS			Forage	RUP	Forage × RUP
	LR	HR	LR	HR				
Allantoin (mmol/d)	11.72	13.98	13.59	15.48	1.09	0.09	0.04	0.85
Uric acid (mmol/d)	0.64	0.70	0.78	0.71	0.08	0.38	0.98	0.46
Total purine derivatives (mmol/d)	12.37	14.69	14.37	16.19	1.05	0.08	0.05	0.81
Purine derivatives (mmol/kg BW0.75)	0.52	0.54	0.53	0.57	0.02	0.10	0.04	0.72
Microbial protein yield (g/d)	66.1	78.5	76.8	86.5	5.63	0.08	0.05	0.81
Urinary nitrogen (g/d)	15.27^{a}	14.81^{ab}	14.77^{ab}	12.49^{b}	1.04	0.09	0.25	0.05

[1]Treatments: see Table 1. [2]Statistical comparisons: Forage = forage sources (AH *vs.* WS); RUP; different ratios of RUP:RDP [low ratio (LR) = 28:72; high ratio (HR) = 36:64]; Forage × RUP= interaction between forage source and dietary RUP:RDP ratios. Means within a row with different superscript letters are statistically different ($p<0.05$)

Table 5. Least square means for blood metabolites and liver enzymes in dairy calves fed different forage source (AH *vs.* WS) and different rumen undegradable to degradable protein ratios (28:72 *vs.* 36:64).

Item	Treatments[1]				SEM	*p*-value[2]		
	AH		WS					
	LR	HR	LR	HR		Forage	RUP	Forage × RUP
Glucose, mg/dL								
d 35	87.2	91.0	85.7	84.8	3.63	0.47	0.79	0.65
d 70	68.1	73.6	72.8	75.3	3.87	0.57	0.48	0.75
Beta-hydroxybutyrate (mmol/L)								
d 35	0.09^{ab}	0.06^{ab}	0.07^{ab}	0.13^{a}	0.01	0.52	0.61	0.04
d 70	0.18	0.19	0.20	0.21	0.02	0.54	0.77	0.93
Total protein (g/dL)								
d 35	7.08	6.84	7.07	7.02	0.12	0.62	0.44	0.62
d 70	7.11	7.05	7.23	7.36	0.13	0.28	0.87	0.63
Albumin (g/dL)								
d 35	3.27	3.15	3.32	3.28	0.06	0.35	0.42	0.68
d 70	3.07	3.20	3.21	3.26	0.07	0.26	0.32	0.63
Blood urea nitrogen (mg/dL)								
d 35	18.0	15.8	18.2	17.1	0.15	0.64	0.31	0.75
d 70	21.2	18.2	21.7	16.1	1.77	0.73	0.03	0.60
Aspartate aminotransferase (IU/L)								
d 35	38.20	40.89	41.23	36.42	2.63	0.85	0.62	0.21
d 70	51.32	59.33	50.03	57.45	6.12	0.54	0.63	0.47
Alanine aminotransferase (IU/L)								
d 35	7.13	8.31	9.63	7.15	0.60	0.46	0.29	0.12
d 70	14.63	13.46	13.08	11.36	1.89	0.54	0.23	0.87

[1]Treatments: see Table 1. [2]Statistical comparisons: Forage = forage sources (AH *vs*. WS); RUP; different ratios of RUP:RDP [low ratio (LR) = 28:72; high ratio (HR) = 36:64]; Forage × RUP= interaction between forage source and dietary RUP:RDP ratios. Means within a row with different superscript letters are statistically different (p<0.05)

HR diets in the current study. The higher levels of available amino acids reaching into small intestine could positively influence the growth rate in young animals (Kazemi-Bonchenari *et al.*, 2018). Furthermore, the greater microbial protein yield which was observed in the HR diets in the current study could also contribute to a greater availability of amino acid in the small intestine, which positively influenced growth rate of young calves. Accordingly, the HR diets increased hip height at final measurement which is in line with improved ADG and FE in this diet. Heinrichs *et al.* (2007) showed a positive correlation between growth rate and skeletal growth indices in growing dairy calves.

The urinary excretion of allantoin was greater in the HR diets compared to LR diets. Accordingly, total urinary PD excretion was greater in HR diets than in the LR diets. According to the greater PD excreted through urine in the HR diets compared with the LR diets, the MPY also was greater in this diet. The higher MPY obtained in HR diets can be attributed to the more efficient N metabolism which indicates higher capture of N toward microbial protein synthesis. It should be further discussed here that in addition to the RUP:RDP ratio in dairy calves, the incorporated RUP source in the starter diet can also influence animal performance. However, some discrepancies among previous studies (Hill *et al.*, 2007; Kazemi-Bonchenari *et al.*, 2016) for the effects of RUP:RDP ratios on dairy calves' growth rate might be attributed to the differences in the source and level of RUP feeding and different amino acid profiles of experimental feedstuffs included in the starter diet.

The concentration of BUN was greater in the LR diets compared to the HR diets during the post-weaning period probably due to the greater ruminal NH3-N concentration found in these diets. Blood urea N may be a promising indicator of N capturing in the rumen and blood urea nitrogen level is positively correlated with ruminal NH3-N concentration (DePeters & Fergusen, 1992). The MPY was improved in the calves received HR diet compared to calves fed LR diets indicating the more efficient capturing

of the ruminal NH3-N produced in the rumen toward microbial utilization rather than converting to urea and excrete through urine. Hence, our results show that from the N perspective, HR diets were more efficient rather than LR diets in the current study because of reduction in BUN and then reduced urinary N excretion through urine. It is notable here that lower growth performance in animals fed LR diets compared to HR diets may be related to higher urinary N excretion through which can increase energy wastage through urea synthesis in the liver and kidneys that has been shown to reduce energy partitioning toward production in ruminants (Lapierre & Lobley, 2001; Kohn *et al.*, 2005).

Interaction effects of forage source and RUP:RDP ratio

The greatest BW and the greatest wither height at final measurement were obtained for WS-HR treatment which can be partly due to the greater N efficiency in this diet. Our results suggest that feeding WS with high RUP:RDP ratio has potential to improve gain and efficiency which is in line with the previous finding in heifers (Koch *et al.*, 2017), growing lambs (Dorri *et al.*, 2021) or steers (Wickersham *et al.*, 2008). Our results confirm previous works (Wickersham *et al.*, 2008; Koch *et al.*, 2017) which indicated that partial incorporation of LQF in mature ruminant's diet can improve ruminal N metabolism probably through increased urea recycling in to the rumen. Partial dietary inclusion of WS as a LQF in the starter diet of young calves would be more efficient when greater dietary RUP:RDP ratio was supplied for dairy calves. Koch *et al.* (2017) postulated that additional fiber in the heifers' diet depressed microbial protein flow and N utilization, but providing greater undegradable protein in the diet was instrumental in reestablishing N balance. The suggested mechanism for the positive impact of greater RUP level on microbial protein yield in animals fed with LQF is providing the greater available N in the rumen through higher rate of urea recycling (Wickersham *et al.*, 2008).

Blood glucose concentration was constant among experimental treatments that is mostly because of similar intake among experimental treatments. However, blood BHB concentration was influenced with the interaction of forage source and RUP:RDP ratio with the greatest blood BHB concentration was found for WS-HR treatment. It has been stated before that the blood BHB in young ruminants is an indicator of more developed rumen in pre-ruminant animals which could positively influence animal growth performance (Soltani *et al.*, 2017). Therefore, in addition to the more efficient N metabolism in WS-HR treatment, the greater blood BHB concentration in this diet can also be an indicator of higher developed gastrointestinal activity. Total protein and albumin concentrations were not differed across experimental treatments probably because of the adequate crude protein supplied in all experimental treatments. Similar liver enzymes concentrations among the experimental treatments in the current study indicate that liver function was not negatively influenced with different forage sources or dietary RUP:RDP ratios in dairy calves.

Conclusions

Considering the interaction effects between forage source (alfalfa hay *vs.* wheat straw) and RUP:RDP ratios in the starter diet of dairy calves, results clarify that concurrent feeding of WS with high RUP:RDP ratio increased weight gain, wither height, and blood BHB concentration, and also reduced urinary N excretion indicating higher N efficiency. Regarding the forage source in the starter diet, limited amount of wheat straw incorporation in diet (7%, DM basis) increased the time spent for rumination, and improved fecal score, and fiber digestibility in contrast to feeding AH in dairy calves. Greater dietary RUP:RDP ratio increased ADG, FE, and hip height, and improved microbial protein yield in young calves. In conclusion, higher RUP:RDP ratio can have more benefits when WS was incorporated as low quality forage in the starter diet of dairy calves which probably is related to improved nitrogen efficiency in dairy calves.

Acknowledgements

The authors thank Dr. Moshayedi, manager in-chief and Mr. Sadeghi, technical manager of the Zarrin-Khoosheh commercial dairy farm. Great thanks to Mr. Namdari (calf rearing technician) for valuable helping throughout the experiment. Thanks to Amin Mirjafari, Sahar Avakh, and other students (Arak University) for helping in urine sampling times in this experiment.

References

Amirabadi Farahani T, Amanlou H, Kazemi-Bonchenari M, 2017. Effects of shortening the close-up period length coupled with increased supply of metabolizable protein on performance and metabolic status of multiparous Holstein cows. J Dairy Sci 100: 6199-6217. https://doi.org/10.3168/jds.2016-12263

Antongiovanni M, Acciaoli A, Grifoni F, Martini A, Ponzetta P, 1991. Effects of wheat straw treated with ammonia from urea hydrolysis in lamb diets. Small Rum Res 6: 39-47. https://doi.org/10.1016/0921-4488(91)90006-C

AOAC, 2002. Official methods of analysis, 17th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Aragona KM, Suarez-Mena FX, Dennis TS, Quigley JD, Hu W, Hill TM, Schlotterbeck RL, 2020. Effect of starter form, starch concentration, and amount of forage fed on Holstein calf growth from 2 to 4 months of age. J Dairy Sci 103: 2019-17474. https://doi.org/10.3168/jds.2019-17474

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

Broderick GA, Kang JH, 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

Chegini R, Kazemi-Bonchenari M, Khaltabadi-Farahani AH, Khodaei-Motlagh M, Salem AZM, 2019. Effects of liquid protein feed on growth performance and ruminal metabolism of growing lambs fed low-quality forage and compared to conventional protein sources. J Agric Sci (Cambridge) 157: 272-280. https://doi.org/10.1017/S0021859619000595

Chen XB, Gomes MJ, 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: An overview of technical details. Rowett Res Inst, Univ of Aberdeen, UK.

Dennis TS, Suarez-Mena FX, Hill TM, Quigley JD, Schlotterbeck RLL, Lascano GJ, 2017. 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

DePeters EJ, Ferguson JD, 1992. Nonprotein nitrogen and protein distribution in the milk of cows. J Dairy Sci 75: 3192- 3209. https://doi.org/10.3168/jds.S0022-0302(92)78085-0

Dorri T, Kazemi-Bonchenri M, HosseinYazdi M, Mirzaei M, 2021. Effects of inclusion different level of low-quality forage and ruminal undegradable protein to degradable protein ratio in starter diet on growth performance, ruminal fermentation, and urinary purine derivatives in young lambs. Liv Sci 248: 104507. https://doi.org/10.1016/j.livsci.2021.104507

Gonzalez-Ronquillo M, Balcells J, Guada JA, Vicente F 2003. Purine derivative excretion in dairy cows: endogenous excretion and the effect of exogenous nucleic acid supply. J Dairy Sci 86: 1282-1291. https://doi.org/10.3168/jds.S0022-0302(03)73712-6

Heinrichs AJ, Erb HN, Rogers GW, Cooper JB, Jones CM, 2007. Variability in Holstein heifer heart-girth measurements and comparison of prediction equations for live weight. Prev Vet Med 78: 333-338. https://doi.org/10.1016/j.prevetmed.2006.11.002

Hill TM, Aldrich JM, Schlotterbeck RL Bateman HG, 2007. Protein concentrations for starters fed to transported neonatal calves. The Prof Anim Sci 23: 123-134. https://doi.org/10.15232/S1080-7446(15)30952-9

Hill TM, Bateman H.G, Aldrich JM, Schlotterbeck RL, 2008. Effects of the amount of chopped hay or cottonseed hulls in a textured calf starter on young calf performance. J Dairy Sci 91: 2684-2693. https://doi.org/10.3168/jds.2007-0935

Hill TM, Bateman HG, Aldrich JM, Schlotterbeck RL, 2010. Roughage amount, source, and processing for diets fed to weaned dairy calves. The Prof Anim Sci 26: 181-187. https://doi.org/10.15232/S1080-7446(15)30578-7

Iranian Council of Animal Care (ICAC), 1995. Guide to the care and use of experimental animals, Vol. 1. Isfahan University of Technology, Isfahan, Iran.

Kazemi-Bonchenari M, Mirzaei M, Jahani-Moghadam M, Soltani A, Mahjoubi E, Patton RA, 2016. Interactions between levels of heat-treated soybean meal and prilled fat on growth, rumen fermentation, and blood metabolites of Holstein calves. J Anim Sci 94: 4267-4275. https://doi.org/10.2527/jas.2016-0514

Kazemi-Bonchenari M, Salem AZM, Lopez S, 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

Kazemi-Bonchenari M, Falahati R, Poorhamdollah M, Heidari SR, Pezeshki A, 2018. Essential oils improved weight gain, growth and feed efficiency of young dairy calves fed 18 or 20% crude protein starter diets. J Anim Physiol Anim Nutr 102: 652-661. https://doi.org/10.1111/jpn.12867

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

Kertz AF, Prewitt LR, Everett Jr JP, 1979. An early weaning calf program: Summarization and review. J Dairy Sci 62:1835-1843. https://doi.org/10.3168/jds.S0022-0302(79)83508-0

Khan MA, Weary DM, Veira DM, von Keyserlingk MAG, 2012. Post-weaning performance of heifers provided hay during the milk feeding period. J Dairy Sci 95: 3970-3976. https://doi.org/10.3168/jds.2011-5027

Koch LE, Gomez NA, Bowyer A, Lascano GJ, 2017. Precision-feeding dairy heifers a high rumen-undegradable protein diet with different proportions of dietary fiber and forage-to-concentrate ratios. J Anim Sci 95: 5617-5628. https://doi.org/10.2527/jas2017.1974

Kohn RA, Dinneen MM, Russek-Cohen E, 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: 79-889. https://doi.org/10.2527/2005.834879x

Laarman AH, Oba M, 2011. Short communication: Effect of calf starter on rumen pH of Holstein dairy calves at weaning. J Dairy Sci 94: 5661-5664. https://doi.org/10.3168/jds.2011-4273

Lapierre H, Lobley GE, 2001. Nitrogen recycling in the ruminant: A review. J Dairy Sci 84 (E Suppl):E223-E236. https://doi.org/10.3168/jds.S0022-0302(01)70222-6

Maekawa, M, Beauchemin KA, Christensen DA, 2002. Chewing activity, saliva production, and ruminal pH of primiparous and multiparous lactating dairy cows. J Dairy Sci 85: 1176-1182. https://doi.org/10.3168/jds.S0022-0302(02)74180-5

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

Mirzaei M, Khorvash M, Ghorbani GR, Kazemi-Bonchenari M, Riasi A, Nabipour A, van den Borne JJGC, 2015. Effects of supplementation level and particle size of alfalfa hay on growth characteristics and rumen development in dairy calves. J Anim Physiol Anim Nutr 99: 553-564. https://doi.org/10.1111/jpn.12229

Mirzaei M, Khorvash M, Ghorbani GR, Kazemi-Bonchenari M, Ghaffari MH, 2017. Growth performance, feeding behavior, and selected blood metabolites of Holstein dairy calves fed restricted amounts of milk: No interactions between sources of finely ground grain and forage provision. J Dairy Sci 100: 1086-1094. https://doi.org/10.3168/jds.2016-11592

Movahedi B, Foroozandeh AD, Shakeri P, 2016. Effects of different forage sources as a free-choice provision on the performance, nutrient digestibility, selected blood metabolites and structural growth of Holstein dairy calves. J Anim Physiol Anim Nutr 101: 293-301. https://doi.org/10.1111/jpn.12527

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

Poczynek M, Toledo AF, Silva AP, Silva MD, Oliveira GB, Coelho MG, *et al.*, 2020. Partial corn replacement by soybean hull, or hay supplementation: Effects of increased NDF in diet on performance, metabolism and behavior of pre-weaned calves. Livest Sci 231: 103858. https://doi.org/10.1016/j.livsci.2019.103858

Soltani M, Kazemi-Bonchenari M, Khaltabadi-Farahani AH, Afsarian O, 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 Tech 230: 77-86. https://doi.org/10.1016/j.anifeedsci.2017.06.002

Suárez BJ, Van Reenen CG, Gerrits WJJ, Stockhofe N, van Vuuren AM, Dijkstra J, 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: II. Rumen development. J Dairy Sci 89: 4376-4386. https://doi.org/10.3168/jds.S0022-0302(06)72484-5

Suárez BJ, Van Reenen CG, Stockhofe N, Dijkstra J, Gerrits WJJ, 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. J Dairy Sci 90: 2390-2403. https://doi.org/10.3168/jds.2006-524

Valadares RFD, Broderick GA, Valadaresfilho SS, Clayton MK, 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J Dairy Sci 82: 2686-2696. https://doi.org/10.3168/jds.S0022-0302(99)75525-6

Valizadeh A, Kazemi-Bonchenari M, Khodaei-Motlagh M, Moradi MH, Salem AZM, 2021. Effects of different rumen undegradable to rumen degradable protein ratios on performance, ruminal fermentation, urinary purine derivatives, and carcass characteristics of growing lambs fed a high wheat straw-based diets. Small Rum Res 197: 106330. https://doi.org/10.1016/j.smallrumres.2021.106330

Van Keulen J, Young BA, 1977. Acid insoluble ash as a natural marker for digestibility studies. J Anim Sci 44: 282-287. https://doi.org/10.2527/jas1977.442282x

Van Soest PJ, Robertson JB, Lewis BA, 1991. Methods for dietary fiber, neutral detergent fiber nonstarch polysaccharide in relation to animal nutrition. J Dairy Sci 74: 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Wickersham TA, Titgemeyer EC, Cochran RC, Wickersham EE, 2008. Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. Br J Nut 101: 225-232. https://doi.org/10.1017/S0007114508995672