



## Effects of starter protein content and alkali processing of wheat straw on growth, ruminal fermentation, and behavior in Holstein calves

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### ABSTRACT

The present study was conducted to investigate the effects of crude protein (CP) content of starter feed and wheat straw (WS) processing on growth performance, digestibility, ruminal fermentation, and behavior of Holstein calves. Sixty calves (28 male and 32 female) were randomly assigned to 1 of 4 treatments in a randomized complete block design. Treatments in a 2 × 2 factorial arrangement were (1) lower-CP ground starter feed mixed with alkali-processed WS (LP-PWS), (2) lower-CP ground starter feed mixed with unprocessed WS (LP-WS), (3) higher-CP ground starter feed mixed with alkali-processed WS (HP-PWS), and (4) higher-CP ground starter feed mixed with unprocessed WS (HP-WS). Wheat straw was fed at 4.75% of dry matter (DM), and low-protein (LP) and high-protein (HP) starter feed contained 19.5 and 23.5% CP, respectively. The calves were weaned on d 60 and remained in the study until d 75. During the experiment, the calves received 4.2 kg of whole milk per day and had free access to fresh water and starter feed. The interaction between WS processing and protein content of starter tended to be significant for starter feed intake, average daily gain (ADG), and body weight (BW); calves fed HP-PWS tended to have greater ADG and final BW than other treatments. The results showed that feeding HP ground starter feed increased ADG and feed efficiency compared with LP groups during the preweaning and the overall periods. Moreover, weaning and final BW were higher in HP-fed calves than in LP-fed calves. Apparent digestibilities of acid detergent fiber (ADF), starch, and CP were greater in calves fed HP than in calves fed LP starter feed. The HP ground starter feed increased rumen propionate and ammonia concentrations. Wheat straw processing had no effect on intake and growth of

calves but increased DM, ADF, and neutral detergent fiber digestibilities and decreased ruminal pH. Using processed wheat straw (PWS) mixed with starter feed tended to decrease rumination time and ruminal acetate concentration in calves. Final body barrel and withers height tended to be greater in calves fed PWS. Overall, the results indicated that HP content of ground starter feed (23.5%) could be recommended for Holstein calves. Furthermore, PWS inclusion in the ground starter diet increased fiber digestibility but had no effect on calf performance. Moreover, calves fed HP-PWS had greater ADG and final BW than other treatments.

**Key words:** calf nutrition, forage quality, weaning

### INTRODUCTION

The first months of life have long-lasting consequences on the physiological function of calves, and future milk yield has a positive relationship with ADG during the first 65 d of age (Soberon et al., 2012). Early dry feed consumption fosters early rumen microbial development, resulting in greater rumen metabolic activity (Anderson et al., 1987).

Rumen epithelium development is stimulated by VFA production during microbial fermentation (Baldwin et al., 2004), and rumen muscle development, rumen movements, and rumen capacity are influenced by the physical form of starter feed (Beharka et al., 1998). Several researchers (Castells et al., 2012; Mirzaei et al., 2017) have shown that providing chopped forages improved starter feed intake by calves. However, wheat straw (WS) has lower protein content and apparent digestibility of fiber and protein than alfalfa hay (Castells et al., 2012) and therefore may influence calves' performance.

Wheat straw is very low in N and high in cellulose and hemicellulose, which are only partly available to animals because of poor digestibility due to the presence of lignin and silica (Ahmed et al., 2002). Therefore, it may be important to improve the quality of WS to increase efficient utilization by the gastrointes-

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tinal tract of ruminants. Alkali processing of WS has been demonstrated to improve the digestibility and availability of cellulose and hemicellulose as well as N content (Haddad et al., 1994). Alkali processing of WS improves ruminal and total-tract fiber digestion and might increase the availability of energy for rumen microbial production. Haddad et al. (1998) reported that the inclusion of up to 20% alkali-treated WS in the diets of lactating cows resulted in ruminal function and performance similar to those of cows fed diets that contained alfalfa haylage only.

In addition to ruminal energy availability, adequate N availability is also needed to optimize ruminal fermentation (Casper et al., 1999). Adequate starter CP is essential to maintain ruminal fermentation and growth of calves. Increasing dietary CP content enhanced DMI (Drackley et al., 2002; Stamey et al., 2012), ruminal bacteria growth, nutrient digestibility, microbial protein synthesis, N utilization (Chanthakhoun et al., 2012), and growth performance (Stamey et al., 2012). However, results of feeding different starter protein contents have been inconsistent. Hill et al. (2007) detected no differences in the performance of calves fed 18 or 22% CP starters. However, Drackley et al. (2002) found that calves fed starters containing 22% CP were more efficient than those fed 18% CP. Akayezu et al. (1994) also indicated that BW of calves increased depending on the increase of starter protein content; maximum growth was obtained with 19.6% CP, and no advantage was detected at 22.4% CP. The NRC (2001) has recommended that starter feed should contain 20% CP on a DM basis (18% CP as-fed basis). Although field reports have suggested that starter feeds containing 20% or more CP may have some benefits after weaning, beneficial effects of calf starter feeds with high protein content have not been clearly documented. More recently, Makizadeh et al. (2020) reported that calves fed a diet with higher digestibility had greater withers and hip heights when they were fed starter feed with higher CP (21 vs. 18% of DM). This finding showed that the availability of energy can affect responses of calves to starter CP content.

Thus far, to our knowledge, the combined effects of WS processing (**WSP**) with different ground starter CP contents on the intake and performance of calves have not been examined. We tested whether calves fed alkali-processed WS responded differently depending on N availability in the starter diet. We hypothesized that alkali processing of WS mixed in ground starter feed with high CP content may have potential to enhance performance of dairy calves. Thus, the aim of the current study was to evaluate the effects of WS alkali processing and dietary CP content on intake, digestibility, behavior, and growth performance in Holstein

calves. We also aimed to discover any possible interactions of CP content and WSP.

## MATERIALS AND METHODS

The present experiment was carried out at Feka Agriculture and Animal Husbandry, Isfahan, Iran, from February to May 2017. Animal procedures were performed in accordance with protocol no. 19293, approved by the Iranian Council of Animal Care (1995).

### *Animals, Management, and Diets*

Sixty Holstein calves with an average initial BW of  $39.9 \pm 1.5$  kg were assigned to 4 treatments (7 male and 8 female calves per treatment). The calves were separated from their dams immediately after birth, weighed, and moved to individual pens ( $1.2 \times 2.5$  m). The gate in front of each pen had 2 openings for access to milk and feed buckets mounted on the outside. Pens were located under a 3-sided, roofed barn and were bedded with wood shavings, with bedding replaced every 24 h and fresh bedding added as needed.

The animals were fed a total of 4 L of colostrum, with 1.5 L fed within 2 h of life and 2.5 L fed 8 h after the first feeding. On d 2 of life, transition milk (4 L) was fed in 2 equal meals. The calves were blocked by date of birth and, within block, randomly assigned to 1 of 4 treatments, using initial BW and sex as secondary blocking factors on d 3 of age. Whole milk samples were obtained weekly and analyzed for fat, CP, lactose, and total solids, using an infrared spectrophotometer (Foss MilkoScan, Foss Electric, Hillerød, Denmark). The calves received 4.2 L/d of whole milk (mean composition:  $3.2 \pm 0.13\%$  fat,  $3.1 \pm 0.01\%$  CP,  $4.6 \pm 0.15\%$  lactose, and  $11.5 \pm 0.52\%$  total solids) in steel buckets twice daily at 0800 and 1600 h from d 3 to 60 of age.

The calves were weaned at d 60 of age and fed the experimental diets until d 75 of age. Calves were fed the ground starter feeds in mash form with different CP contents mixed with alkali-processed or unprocessed WS. Treatments were (1) lower-CP starter feed mixed with alkali-processed WS (**LP-PWS**), (2) lower-CP starter feed mixed with unprocessed WS (**LP-WS**), (3) higher-CP starter feed mixed with alkali-processed WS (**HP-PWS**), and (4) higher-CP starter feed mixed with unprocessed WS (**HP-WS**). Wheat straw was fed at 4.75% of DM, and low-protein (**LP**) and high-protein (**HP**) starter contained 19.5 and 23.5% CP, respectively. The calves had free access to water (via nipple drinkers) throughout the study, and ad libitum starter diet intake was achieved by offering an amount that resulted in orts of 5 to 10% of offered feed after 24 h. Feed refusal from each calf was collected before

**Table 1.** Ingredients and chemical composition of experimental diets on DM basis<sup>1</sup>

Item	LP		HP	
	PWS	WS	PWS	WS
Ingredient composition, % of DM				
Wheat straw	—	4.75	—	4.75
Processed wheat straw	4.75	—	4.75	—
Barley grain, ground	9.71	9.71	10.20	10.20
Corn grain, ground	39.63	39.63	28.49	28.49
Wheat grain, ground	4.87	4.87	5.12	5.12
Soybean meal (44% CP)	30.45	30.45	40.93	40.93
Wheat bran	3.55	3.55	3.55	3.55
Calcium carbonate	1.30	1.30	1.30	1.30
Fat powder <sup>2</sup>	1.74	1.74	1.74	1.74
Vitamin and mineral premix <sup>3</sup>	0.60	0.60	0.60	0.60
Salt	0.44	0.44	0.44	0.44
Sodium bicarbonate	1.70	1.70	1.70	1.70
Sodium bentonite	0.44	0.44	0.44	0.44
Dicalcium phosphate	0.44	0.44	0.44	0.44
Monensin	0.41	0.41	0.41	0.41
Chemical composition				
DM	91.5	91.5	91.6	91.6
CP	19.5	19.5	23.5	23.5
MP <sup>4</sup>	10.4	10.4	11.2	11.2
NDF	17.5	18.1	18.3	18.9
peNDF <sup>5</sup>	8.20	8.80	8.50	9.10
Ether extract	4.80	4.80	4.80	4.80
Starch	38	38	30.8	30.8
Ca <sup>4</sup>	0.90	0.90	0.90	0.90
P <sup>4</sup>	0.45	0.45	0.60	0.60
Na <sup>4</sup>	0.84	0.83	0.84	0.83
Ash	8.20	8.20	8.40	8.40
NFC <sup>6</sup>	50.4	51.1	46.1	48.8
Lignin	1.04	1.10	1.08	1.14
ME <sup>4</sup> (Mcal/kg)	2.89	2.89	2.85	2.85
NEG <sup>4</sup> (Mcal/kg)	1.28	1.28	1.26	1.26

<sup>1</sup>LP = lower-protein starter, containing 19.5% CP; HP = higher-protein starter, containing 23.5% CP; PWS = processed wheat straw; WS = unprocessed wheat straw.

<sup>2</sup>Nurifat-99, Global Agri Resources Private Limited, Hyderabad, India. Composition: moisture 0.5%, crude fat 99.5% (C16:0, 85%; C18:0, 10%; C18:1, 2%; C18:2, 3%).

<sup>3</sup>Contained, per kilogram of supplement: 1,500,000 IU of vitamin A, 380,000 IU of vitamin D, 13,000 IU of vitamin E, 12,000 mg of Mn, 2000 mg of Fe, 4,000 mg of Cu, 18,000 mg of Zn, 250 mg of I, and 125 mg of Se (Vetaque, Sirjan, Iran).

<sup>4</sup>Calculated from NRC (2001).

<sup>5</sup>Physically effective NDF.

<sup>6</sup>NFC = OM - (NDF + CP + EE), where EE = ether extract.

0800 h throughout the study. All diets were formulated according to the Cornell Net Carbohydrate and Protein System (version 5.1; Fox et al., 2004). The ingredient and nutrient compositions of the starter diets are given in Table 1.

The particle size distribution of WS was determined using the 3-screen Penn State Particle Separator (Kononoff et al., 2003). Wheat straw was chopped (particle size distribution: 0% >19 mm, 6.5 ± 0.6% >8 mm, 50.5 ± 1.2% >1.18, and 42.9 ± 2.1% <1.18 mm; geometric mean particle size 2.1 ± 0.1 mm) using a harvesting machine with a screen size regulator (Golchin Trasher Hay Co., Isfahan, Iran). Ten kilograms of WS was treated with 650 g of NaOH dissolved in 10 L of tap water (Haddad et al., 1998). The NaOH solution was

sprayed with a hand garden sprayer on the chopped WS and mixed thoroughly to achieve uniform wetting. Processed WS was aerated for 3 to 4 d in an open environment to be dried before feeding to the calves.

### Sampling and Laboratory Analyses

Starter feed intake and total DMI (milk plus starter feed) was determined daily and averaged by 10-d periods, and individual BW were recorded every 10 d. Pre- and postweaning plus overall means of ADG and feed efficiency (FE = kg BW gain/kg total DMI) were calculated. Samples of feed and orts were collected every week throughout the study and stored at -20°C until chemical analysis. Subsamples of feed and refusals were

mixed thoroughly, dried, ground to pass a 1-mm screen in a mill (Ogaw Seiki Co. Ltd., Tokyo, Japan), and analyzed (AOAC International, 2002) for DM (method 925.40), CP (method 2001.11), ether extract (method 920.39), ash (method 942.05), starch (Zhu et al., 2016), and NDF (using heat-resistant  $\alpha$ -amylase and sodium sulfite) and ADF, according to Van Soest et al. (1991) with the Ankom Fiber Analyzer system (Ankom Technology, Macedon, NY). Whole milk composition was measured by MilkoScan (Foss Electric) every week. Fecal scores were recorded daily at 0800 h using the following scale: 1 = normal, 2 = soft to loose, 3 = loose to watery, 4 = watery, mucous, slightly bloody, 5 = watery, mucous, and bloody (Khan et al., 2007).

Body measurements of each calf, including body length (distance between the points of shoulder and rump), withers height (distance from base of the front feet to the withers), body barrel (circumference of the belly before feeding), heart girth (circumference of the chest), hip height (distance from base of the rear feet to hook bones), and hip width (distance between the points of hook bones), were measured with calipers at the start of the experiment (d 3), at weaning (d 60), and at the end of the study (d 75) according to the method described by Khan et al. (2007).

Data on feeding behavior attributes (times spent standing, ruminating, lying, eating, and in non-nutritional behavior) monitored by direct observations were collected once weekly 2 wk before and 2 wk after weaning according to the procedure described in Castells et al. (2012). Before and after weaning, the calves were observed for 2 h after solid feed was offered at 0800 h, because solid feed and morning milk feeding were offered at 0800 h. Therefore, total observation time per animal was 8 h for the entire experiment.

Ruminal fluid samples were collected at 2 h after the morning feeding on d 35 and 70 of age, using a stomach tube fitted to a vacuum pump; the first 10 mL were discarded because of possible saliva contamination, and rumen pH was measured immediately (HI 8318; Hanna Instruments, Cluj-Napoca, Romania). The samples were squeezed through 4 layers of cheesecloth, and a 10-mL aliquot was preserved with 2 mL of 25% meta-phosphoric acid and frozen at  $-20^{\circ}\text{C}$  until analysis. After thawing at room temperature, ruminal fluid samples were analyzed for VFA using GLC (model CP-9002, Chrompack, Delft, the Netherlands) as previously described (Bal et al., 2000). For  $\text{NH}_3$  measurement, the ruminal fluid filtrates were centrifuged at  $3,000 \times g$  at  $4^{\circ}\text{C}$  for 20 min and analyzed by the colorimetric phenol-hypochlorite method (Broderick and Kang, 1980).

Fecal grab samples were collected for 5 d after weaning to evaluate nutrient digestibility. Sampling was performed every 6 h over a 12-h period between 2

feeding times (2 samples) from 5 calves per treatment. The samples were frozen ( $-20^{\circ}\text{C}$ ) before being dried. Samples then were dried in a forced-air oven at  $60^{\circ}\text{C}$  for 72 h, ground to pass through a 1-mm screen, and analyzed for starch, DM, ash, ADF, NDF, and CP as described for feeds. Apparent total-tract digestibility of nutrients was determined using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977). Percentage of acid-insoluble ash was calculated from the equation  $[(W_f - W_e)/W_s] \times 100$ , where  $W_f$  = weight of crucible with ash,  $W_e$  = weight of empty crucible, and  $W_s$  = weight of sample DM. Each duplicate 5-g sample of feed or feces (dried and ground) was weighed into a 50-mL crucible, dried (2 h) in a forced-air oven ( $135^{\circ}\text{C}$ ), cooled in a desiccator to room temperature, re-weighed ( $W_s$ ), and then ashed overnight at  $450^{\circ}\text{C}$ . The ash was transferred to a 600-mL Berzelius beaker (without spout), and 100 mL of 2 *N* HCl was added. The mixture was then boiled for 5 min on a hotplate. The hot hydrolysate was filtered (Whatman No. 41) and washed free of acid with hot distilled water ( $85$  to  $100^{\circ}\text{C}$ ). The ash and filter paper were then transferred back into the crucible and ashed overnight at  $450^{\circ}\text{C}$ . The crucible and contents were cooled in a desiccator to room temperature, weighed while containing ash ( $W_f$ ), and re-weighed immediately after emptying ( $W_e$ ).

### Statistical Analysis

Statistical analyses were conducted for 3 periods: preweaning, postweaning, and the entire experiment, using PROC MIXED (SAS version 9.1; SAS Institute, 2002) with the individual calf as the experimental unit. Starter feed intake, ADG, and FE were analyzed as repeated measures with 10-d periods as the repeated variable, using the following model:  $Y_{ijk} = \mu + PL_i + WSP_j + W_k + (PL \times W)_{ik} + (WSP \times W)_{jk} + (PL \times WSP)_{ij} + (PL \times WSP \times W)_{ijk} + \beta(X_i - \bar{X}) + \varepsilon_{ijk}$ , where  $Y_{ijk}$  is the dependent variable;  $\mu$  is the overall mean;  $PL_i$  is the effect of starter CP content;  $WSP_j$  is the effect of WS processing;  $W_k$  is the effect of time;  $(PL \times W)_{ik}$  is the effect of the interaction between starter CP content and time;  $(WSP \times W)_{jk}$  is the effect of the interaction between WS processing and time;  $(PL \times WSP)_{ij}$  is the interaction between starter CP content and WS processing;  $(PL \times WSP \times W)_{ijk}$  is the tripartite effect of starter CP content, WS processing, and time;  $\beta(X_i - \bar{X})$  is the covariate variable (initial BW and structural data); and  $\varepsilon_{ijk}$  is the overall error term. The autoregressive (order 1) covariance structure was the best fit for these data, as determined by the lowest Akaike information criterion. Digestibility, ruminal fermentation, and fecal score variables were ana-

lyzed using the following model:  $Y_{ijk} = \mu + PL_i + WSP_j + (PL \times WSP)_{ij} + \varepsilon_{ijk}$ , where  $Y_{ijk}$  is the dependent variable;  $\mu$  is the overall mean;  $PL_i$  is the effect of starter CP content;  $WSP_j$  is the effect of WS processing;  $(PL \times WSP)_{ij}$  is the interaction between starter CP content and WS processing; and  $\varepsilon_{ijk}$  is the overall error term. The means were compared using least squares means adjusted by the Tukey procedure, and significant differences and tendencies were stated at  $P \leq 0.05$  and  $0.05 < P \leq 0.10$ , respectively.

## RESULTS

### Starter Feed Intake, ADG, and FE

Least squares means for average daily starter feed intake, ADG, and FE are listed in Table 2. In general, WSP had no effect on starter feed intake, ADG, FE, or final BW of calves. Starter CP content had no effect on mean daily starter diet intake during preweaning (average 0.49 kg/d), postweaning (average 2.20 kg/d), or overall (average 0.91 kg/d) periods. As expected, HP-fed calves had greater MP intake in preweaning (55.3 vs. 48.1 g/d;  $P = 0.006$ ) and overall (102.1 vs. 94.0 g/d;  $P = 0.046$ ) periods than LP-fed calves. Calves fed HP had greater ADG preweaning (0.67 vs. 0.61 kg/d;  $P = 0.0001$ ) and tended to have greater ADG overall (0.77 vs. 0.74 kg/d;  $P = 0.071$ ) than calves fed LP. The HP starter increased FE in preweaning (0.57 vs. 0.53;  $P = 0.002$ ) and overall (0.58 vs. 0.54;  $P = 0.003$ ) periods. In parallel with ADG, feeding HP starter increased weaning (79.9 vs. 76.2 kg;  $P = 0.002$ ) and final (95.9 vs. 92.8 kg;  $P = 0.027$ ) BW compared with the LP groups.

An interaction for MP intake in the overall period ( $P = 0.038$ ) between WSP and CP content was observed (Table 2). Overall starter feed intake ( $P = 0.056$ ), overall ADG ( $P = 0.059$ ), and final BW ( $P = 0.072$ ) tended to be greater in calves fed HP-PWS than calves fed other treatments. The fecal score tended to be greater ( $P = 0.059$ ) in HP-WS calves than in the other calves on d 35 and 70 of age.

### Digestibility

An interaction was observed for DM and OM digestibilities ( $P < 0.001$ ) between WSP and CP content, such that calves fed LP-WS had the lowest digestibility compared with other treatments (Table 3). Regardless of WSP, starch (88.5 vs. 83.7%;  $P < 0.001$ ), CP (76.6 vs. 71.3%;  $P < 0.001$ ), and ADF (43.6 vs. 41.9%;  $P < 0.001$ ) digestibilities were greater in HP-fed calves than in LP-fed calves. The processing of WS increased DM (77.8 vs. 76.5%;  $P = 0.021$ ), NDF (52.4 vs. 47.0%;  $P < 0.001$ ), and ADF (44.7 vs. 40.8%;  $P < 0.001$ ) digest-

ibilities compared with those in WS-fed calves, regardless of CP content.

### Structural Growth

Regardless of WSP, calves that received HP treatments had greater weaning heart girth than those that received LP (90 vs. 88.8 cm;  $P = 0.027$ , Table 4). Weaning body barrel tended to be greater in calves fed HP than in calves fed LP (96.5 vs. 95.5 cm;  $P = 0.090$ ) at weaning time. Regardless of CP content, processed wheat straw (PWS) supplementation tended to increase final body barrel (100.1 vs. 98.9 cm;  $P = 0.092$ ), weaning withers height (85.4 vs. 84.8 cm;  $P = 0.093$ ), and final hip height (90.9 vs. 90.3 cm;  $P = 0.098$ ) compared with calves fed WS. No interaction was detected between WSP and CP content for structural growth.

### Animal Behavior

No interaction was detected between CP content and WSP for the times devoted to standing, ruminating, eating, and non-nutritional behavior (Table 5). An interaction of CP content and WSP influenced lying time ( $P = 0.031$ ) during the postweaning period, with the least lying time for HP-PWS calves and the greatest lying time for HP-WS. Calves fed WS diets, regardless of CP content, tended to spend less time performing non-nutritional behavior (5.54 vs. 8.10 min;  $P = 0.103$ ) and more time performing rumination (26.1 vs. 22.3 min;  $P = 0.103$ ) than calves fed PWS diets.

### Rumen Parameters

The data for rumen parameters and fecal score are presented in Table 6. No interaction was detected between WSP and CP content with respect to rumen parameters. Protein content had no effect on rumen pH (d 35 and 70 of age); however, feeding HP starter increased rumen ammonia at d 35 (14.3 vs. 12.3 mg/dL;  $P = 0.055$ ) and 70 (9.1 vs. 7.5 mg/dL;  $P = 0.037$ ) compared with LP groups. The total VFA concentration (106.1 vs. 103.9 mmol/L;  $P = 0.076$ ) and the acetate-to-propionate ratio (1.36 vs. 1.41 mol/100 mol;  $P = 0.076$ ) in the rumen tended to be greater and lower in calves fed HP than in calves fed LP on d 70 of age, respectively. High-CP starter increased molar proportion of propionate in the rumen on d 35 (35.1 vs. 33.9 mol/100 mol;  $P = 0.025$ ) and 70 (38.7 vs. 37.1 mol/100 mol;  $P = 0.039$ ) of age.

Regardless of CP content, calves fed WS mixed with starter feeds had greater ruminal pH on d 35 (5.8 vs. 5.6;  $P = 0.003$ ) and 70 (5.9 vs. 5.7;  $P = 0.009$ ) of age compared with calves fed PWS. Total VFA concentra-

**Table 2.** Least squares means of DMI, ADG, feed efficiency, BW, and fecal score of dairy calves (n = 15 per treatment) fed starter containing either higher (HP) or lower (LP) content of protein with either processed wheat straw (PWS) or unprocessed wheat straw (WS)

Item	LP			HP			Treatment effect <sup>1</sup>						
	PWS	WS	SEM	PWS	WS	SEM	CP	WSP	CP	WSP	CP	WSP	CP × WSP
Total DMI, kg/d													
Prewaning	1.11	1.10	0.024	1.14	1.10	0.024	0.759	0.142	0.289	0.91	0.06	0.91	0.65
Postweaning	2.13	2.34	0.132	2.25	2.10	0.132	0.631	0.868	0.169	0.80	0.68	0.80	0.62
Overall	1.36	1.40	0.038	1.42	1.34	0.038	0.782	0.576	0.085	0.99	0.73	0.99	0.67
Starter intake, kg/d													
Prewaning	0.46	0.48	0.034	0.53	0.47	0.034	0.185	0.415	0.081	0.39	0.75	0.39	0.84
Postweaning	2.13	2.33	0.132	2.25	2.10	0.132	0.631	0.868	0.169	0.79	0.68	0.79	0.62
Overall	0.87	0.94	0.038	0.96	0.87	0.038	0.838	0.809	0.056	0.96	0.93	0.96	0.78
MP intake, g/d													
Prewaning	46.97	49.27	2.56	58.74	51.94	2.56	0.006	0.382	0.077	0.39	0.75	0.39	0.84
Postweaning	221.2	242.4	14.30	251.5	233.6	14.30	0.456	0.909	0.175	0.79	0.68	0.79	0.62
Overall	90.52 <sup>b</sup>	97.55 <sup>ab</sup>	4.04	106.91 <sup>a</sup>	97.34 <sup>ab</sup>	4.04	0.046	0.756	0.038	0.96	0.93	0.96	0.78
ADG, kg/d													
Prewaning	0.61	0.61	0.016	0.69	0.65	0.016	0.0001	0.243	0.179	0.15	0.04	0.15	0.11
Postweaning	1.11	1.17	0.043	1.10	1.04	0.043	0.110	0.948	0.178	0.14	0.08	0.14	0.15
Overall	0.73	0.75	0.017	0.79	0.75	0.017	0.071	0.479	0.059	0.18	<0.001	0.18	0.12
Feed efficiency													
Prewaning	0.52	0.54	0.017	0.57	0.58	0.017	0.002	0.308	0.777	0.97	0.46	0.97	0.99
Postweaning	0.58	0.59	0.049	0.55	0.60	0.049	0.899	0.550	0.652	0.16	0.15	0.49	0.73
Overall	0.53	0.55	0.018	0.57	0.59	0.018	0.003	0.239	0.902	0.98	0.14	0.98	0.99
BW, kg													
Initial	39.9	40.0	0.01	39.9	39.9	0.01	0.905	0.905	0.905	—	—	—	—
Weaning	76.1	76.3	1.11	81.1	78.7	1.11	0.002	0.339	0.260	0.99	0.73	0.99	0.99
Final	92.0	93.7	1.33	97.5	94.3	1.33	0.027	0.592	0.072	0.91	0.56	0.91	0.94
Fecal score													
Prewaning	1.16	1.25	0.037	1.20	1.15	0.037	0.450	0.597	0.059	0.99	<0.001	0.99	0.17
Postweaning	1.12	1.19	0.028	1.15	1.11	0.028	0.451	0.598	0.059	0.98	0.15	0.98	0.17

<sup>a,b</sup>Means within a row with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup>Contrasts for WSP (wheat straw processing), CP (protein content of starter), and interactions (CP × WSP; WSP × Time; CP × Time; CP × WSP × Time).

**Table 3.** Least squares means of nutrient digestibility of dairy calves (n = 15 per treatment) fed starter containing either higher (HP) or lower (LP) content of protein with either processed wheat straw (PWS) or unprocessed wheat straw (WS) during the postweaning period (d 70–75)

Item	LP		HP		SEM	Treatment effect <sup>1</sup>		
	PWS	WS	PWS	WS		CP	WSP	CP × WSP
DM	76.1 <sup>b</sup>	74.5 <sup>c</sup>	79.5 <sup>a</sup>	78.4 <sup>a</sup>	0.48	0.578	0.021	<0.001
OM	76.7 <sup>bc</sup>	75.3 <sup>c</sup>	80.3 <sup>a</sup>	79.1 <sup>ab</sup>	0.97	0.912	0.211	<0.001
CP	71.0	71.6	76.2	77.1	1.27	<0.001	0.605	0.938
Starch	83.2	84.1	88.1	89.0	0.61	<0.001	0.161	0.987
Ether extract	85.8	86.8	85.1	85.0	0.77	0.119	0.635	0.499
NDF	51.4	46.7	53.3	47.3	1.05	0.253	<0.001	0.550
ADF	44.6	39.2	44.8	42.4	0.43	<0.001	<0.001	0.268

<sup>a-c</sup>Means within a row with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup>Contrasts for WSP (wheat straw processing), CP (protein content of starter), and interaction (CP × WSP).

tions (d 35 and 70 of age) and proportions of acetate (d 35 and 70 of age) and propionate (d 35 and 70 of age) in the rumen did not differ across WS processing. Feeding PWS increased the acetate-to-propionate ratio (1.52 vs. 1.45 mol/100 mol;  $P = 0.045$ ) in the rumen on d 35 of age.

## DISCUSSION

Our study examined the effects of a ground starter feed with different CP contents mixed with alkali-processed or unprocessed WS on the growth performance, digestibility, ruminal fermentation, and behavior of dairy calves. Our hypothesis was that WS alkali processing may improve digestibility in calves fed diets with different protein content, and therefore may improve rumen conditions and growth performance of dairy calves.

Interactions between dietary CP content and WSP on starter feed intake in the overall period tended to be significant, where HP-PWS and LP-WS tended to have greater intake than other treatments. This tendency for greater starter feed intake in those diets was because of the numerically greater starter diet intake in the preweaning period in calves fed HP-PWS and in the postweaning period in calves fed LP-WS. Calves fed LP-WS had the lowest OM and DM digestibilities and probably increased their starter feed intake to gain more energy and compensate for the lack of energy due to lack of digestibility. In dairy cows (Mesfin and Ktaw, 2010) and beef cattle (Ahmed et al., 2002), processed straw has been found to increase DMI. Several studies (Haddad et al., 1994, 1998) have demonstrated the effectiveness of NaOH to improve nutritive value and palatability of WS, which will increase cows' consump-

**Table 4.** Least squares means of structural growth of dairy calves (n = 15 per treatment) fed starter containing either higher (HP) or lower (LP) content of protein with either processed wheat straw (PWS) or unprocessed wheat straw (WS)

Item	LP		HP		SEM	Treatment effect <sup>1</sup>		
	PWS	WS	PWS	WS		CP	WSP	CP × WSP
Heart girth								
Weaning	88.66	89.01	90.64	89.34	0.518	0.027	0.359	0.120
Final	91.39	91.37	91.84	91.47	0.497	0.595	0.700	0.726
Body barrel								
Weaning	95.60	95.40	97.20	95.90	0.601	0.090	0.199	0.399
Final	99.77	98.77	100.33	99.02	0.683	0.551	0.092	0.817
Body length								
Weaning	56.69	56.57	56.22	56.66	0.328	0.559	0.629	0.403
Final	58.12	58.10	58.20	58.37	0.365	0.599	0.875	0.767
Hip width								
Weaning	20.47	20.62	20.57	20.72	0.163	0.539	0.358	1.000
Final	21.10	21.25	21.14	21.44	0.148	0.312	0.074	0.822
Withers height								
Weaning	85.59	84.94	85.22	84.62	0.365	0.357	0.093	0.947
Final	86.65	86.22	86.72	86.12	0.384	0.966	0.180	0.829
Hip height								
Weaning	89.75	89.15	89.10	88.62	0.425	0.157	0.208	0.875
Final	91.37	90.24	90.47	90.35	0.375	0.298	0.098	0.177

<sup>1</sup>Contrasts for WSP (wheat straw processing), CP (protein content of starter), and interaction (CP × WSP).

**Table 5.** Least squares means of total time devoted to performing different behaviors (min) during 8 h of observation of dairy calves (n = 15 per treatment) fed starter containing either higher (HP) or lower (LP) content of protein with either processed wheat straw (PWS) or unprocessed wheat straw (WS)

Item	LP		HP		SEM	Treatment effect <sup>1</sup>		
	PWS	WS	PWS	WS		CP	WSP	CP × WSP
Standing								
Prewaning	61.2	57.6	58.8	56.0	3.36	0.553	0.353	0.908
Postweaning	60.4	64.1	64.5	53.9	4.33	0.489	0.428	0.106
Lying								
Prewaning	113.5	110.9	111.2	111.9	3.95	0.859	0.812	0.682
Postweaning	106.4 <sup>ab</sup>	100.4 <sup>ab</sup>	96.1 <sup>b</sup>	112.5 <sup>a</sup>	5.07	0.869	0.312	0.031
Eating								
Prewaning	35.4	36.5	36.8	37.4	2.28	0.654	0.658	0.869
Postweaning	34.5	35.2	38.8	32.5	2.83	0.771	0.336	0.223
Rumination								
Prewaning	20.7	25.6	23.9	26.6	2.28	0.353	0.103	0.623
Postweaning	30.9	34.9	32.5	35.5	3.37	0.738	0.298	0.879
Non-nutritive oral behaviors								
Prewaning	9.2	9.3	8.9	8.7	1.00	0.638	0.920	0.890
Postweaning	7.9	5.5	8.2	5.6	1.51	0.889	0.103	0.946

<sup>a,b</sup>Means within a row with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup>Contrasts for WSP (wheat straw processing), CP (protein content of starter), and interaction (CP × WSP).

tion of straw. In contrast, in the current experiment, WSP had no effect on calf performance and intake, which is probably due to the low proportion of WSP in the diets and hence low consumption by calves.

In the current experiment, CP content had no effect on starter diet intake. The effects of starter CP on starter feed intake have been inconsistent. Trials by Akayezu et al. (1994), Hill et al. (2007), Ozkaya and Tokar (2012), and Daneshvar et al. (2017) showed that

18 to 20% CP starter is adequate for calves; however, Drackley et al. (2002) found that calves fed starters containing 22% CP were more efficient than calves fed starter feeds with 18% CP. Moreover, Stamey et al. (2012) reported that, during the weaning period, starter with 25% CP (DM basis) promoted greater starter feed intake than the conventional starter (19.5% CP). Senevirathne et al. (2017) reported that calves fed HP (23.5%) had less or similar DMI compared with those

**Table 6.** Least squares means of rumen fermentation parameters of dairy calves (15 per treatment) fed starter containing either higher (HP) or lower (LP) content of protein with either processed (PWS) or unprocessed (WS) wheat straw

Item	LP		HP		SEM	Treatment effect <sup>1</sup>		
	PWS	WS	PWS	WS		CP	WSP	CP × WSP
pH								
d 35	5.53	5.80	5.65	5.80	0.058	0.297	0.003	0.297
d 70	5.75	6.00	5.73	5.88	0.066	0.274	0.009	0.459
Total VFA (mmol/L)								
d 35	97.2	96.5	100.1	96.7	1.31	0.288	0.160	0.358
d 70	103.8	103.9	108.0	104.3	1.11	0.076	0.139	0.129
Acetate (mol/100 mol)								
d 35	51.8	50.2	52.3	50.1	1.05	0.883	0.104	0.758
d 70	52.57	51.73	53.28	51.39	0.773	0.817	0.116	0.518
Propionate (mol/100 mol)								
d 35	33.25	34.56	35.41	34.84	0.439	0.025	0.424	0.066
d 70	36.75	37.46	39.35	37.95	0.442	0.039	0.599	0.130
Butyrate (mol/100 mol)								
d 35	8.54	8.40	8.84	8.14	0.259	0.926	0.145	0.307
d 70	9.26	9.35	9.94	9.43	0.339	0.293	0.556	0.409
Acetate:propionate								
d 35	1.56	1.46	1.48	1.44	0.030	0.148	0.045	0.281
d 70	1.43	1.39	1.36	1.36	0.030	0.076	0.369	0.369
N-NH <sub>3</sub> (mg/dL)								
d 35	12.52	12.01	14.49	14.01	0.939	0.055	0.627	0.974
d 70	7.73	7.29	9.12	8.98	0.653	0.037	0.668	0.826

<sup>1</sup>Contrasts for WSP (wheat straw processing), CP (protein content of starter), and interaction (CP × WSP).



fed LP (20%) treatment. The greater CP intake may have reduced feed intakes.

Because of a tendency toward greater starter diet intake, calves fed HP-PWS tended to have greater ADG than other calves. Probably the calves consuming the HP-PWS treatment gained more energy and protein due to the higher starter feed consumption and therefore grew more. This suggests that the combination of WS processing and higher protein levels in the starter can lead to improved daily growth. Wheat straw processing had no effect on ADG and BW, which is probably due to similar starter feed intakes; however, calves fed HP had greater ADG and BW in preweaning and overall periods. Although starter feed intake was similar between calves fed HP and LP diets, HP-fed calves received a greater amount of MP than LP-fed calves, which contributed to the growth of calves. Senevirathne et al. (2017) noted that calves fed HP had lower starter diet intake and similar ADG and concluded that providing a greater amount of CP from starter may maintain BW, even though calves had less starter feed intake or total DMI because of the similar CP intake. In agreement with our results, Stamey et al. (2012) reported that calves fed 25.5% CP had greater BW than those fed 19.5% CP and noted that provision of higher CP from starter during the weaning period supported lean tissue growth. We conclude that greater MP intake from the starter may potentially increase rumen development (Stamey et al., 2012) and BW gain of calves without affecting the starter feed intake.

Due to greater ADG and similar starter feed intake, calves fed HP had greater FE than calves fed LP diets. In agreement with our results, efficiency increased linearly with an increase of starter CP content from 15 to 22.3% (Akayezu et al., 1994) or 20 to 23% (Senevirathne et al., 2017).

In the current study, calves fed LP-WS had the lowest OM and DM digestibility, which is likely due to lower fiber digestibility in WS diets and lower CP and starch digestibilities in LP diets. Decreased digestibility in calves fed LP-WS led to these calves being unable to grow more than other calves, even with increasing starter diet intake. This indicates that the use of starter feeds with lower levels of protein and unprocessed WS can have a negative effect on calf performance by reducing digestibility. The observed increase in the digestibilities of CP, ADF, and starch in calves fed HP may have resulted from stimulated ruminal microbial growth due to higher dietary CP. The greater digestibility resulted in higher propionate proportion in calves fed HP than in calves fed LP. Increased concentrations of AA, peptides, ammonia N, and branched-chain VFA in the rumen with increased dietary CP content have been observed to stimulate ruminal bacteria growth

and to promote microbial enzyme secretion (Liu et al., 2014). In agreement with our results, Senevirathne et al. (2017) reported greater digestibility in calves fed HP (23.5%) than in calves fed LP (20%).

Processing of WS increased DM, ADF, and NDF digestibilities. Similar to our findings, in dairy cows, Haddad et al. (1998) reported that alkali processing of WS increased the extent of NDF digestion by 24.6%. Ghasemi et al. (2014) reported that ADF and NDF digestibilities were higher in alkali-processed WS than in unprocessed WS. Alkali processing of WS reduced the crystalline structure of cellulose and the esterified crosslinks between lignin and hemicellulose. These changes increase the swelling capacity of cell walls and facilitate the penetration of microbial enzymes, which leads to greater digestion of structural carbohydrates (Hendriks and Zeeman, 2009).

Small differences were found in frame sizes among treatments; however, starter CP content was significant for heart girth and tended to be significant for body barrel. Senevirathne et al. (2017), Daneshvar et al. (2017), and Stamey et al. (2012) reported that starter CP content did not affect body length and withers height; however, Ozkaya and Toker (2012) reported that calves fed HP diets (22% CP) had greater body length and withers height than calves fed LP (18% CP). We expected that due to higher ADG and MP intake, calves that consumed HP diets would have larger body sizes than calves fed LP. Our results, however, showed that increased ADG or BW does not necessarily lead to a detectable increase in body frame. Although HP-fed calves had more ADG and BW, we detected no difference in body size between HP- and LP-fed calves. This indicates that 19.5% CP in calf starter is sufficient to promote near-maximum structural growth of calves from birth to 10 wk of age as long as the starter feed consumption is adequate and starter diet formulation meets energy requirements.

The PWS-fed calves tended to have higher hip and withers heights. The effect of WSP on the performance of calves has not been studied so far, and, for this reason, the results of this experiment cannot be compared with other experiments. Probably a slight increase in the body frame of PWS-fed calves is due to the increased digestibility of fiber that would increase energy availability.

The decrease in time devoted to lying observed with HP-PWS treatment might be related to the greater starter feed intake compared with other treatments. In fact, these calves spent more time eating compared with other treatments (38.8 vs. 34.0 min). Unprocessed WS had lower fiber digestibility than PWS and probably required more rumination for passage through the rumen. Thus, when forage fiber digestibility was

lower, calves spent more time ruminating, but when forage fiber digestibility was higher, as with PWS, they spent less time ruminating. In confirmation of our results, Welch and Smith (1970) stated that increasing the quality of forages decreased the time devoted to rumination. In that experiment, the animals that were fed straw ruminated 578 min, and the animals fed excellent-quality hay ruminated a shorter time of 369 min.

In the current study, higher time spent in rumination improved rumen function and resulted in a more stable pH of WS diets compared with PWS diets. Non-nutritive oral behaviors are often considered an index for poor welfare because they are believed to be related to frustrated feeding activity (Castells et al., 2012; Mirzaei et al., 2017). The present results suggest that providing calves with PWS may have some adverse effects on welfare and rumen environment. To our knowledge, no study has investigated the effect of CP content on calf behavior. In the current experiment, CP content had no effect on calves' behavior, which is primarily due to similar starter diet intake between HP and LP treatments.

Ruminal pH was lower in calves fed PWS at 5 and 10 wk of age. Ruminal pH decreased when PWS diet was fed, which may be due to the enhanced fermentability of PWS, as evidenced by significant improvement in fiber digestibility. Because of greater fiber digestibility, calves fed PWS tended to have greater acetate proportion and acetate-to-propionate ratio than calves fed WS. On the other hand, WS-supplemented calves had higher rumination activity than PWS-supplemented calves, and this caused higher rumen pH due to increasing saliva production (Krause and Oetzel, 2006), which can help to buffer the ruminal fluid. In agreement with our results, Ghasemi et al. (2014) reported that sheep fed NaOH-processed straw had lower rumen pH than those fed unprocessed straw.

Calves fed HP had greater digestibility and were expected to have lower ruminal pH than calves fed LP. Increased ADF, CP, and starch digestibilities did not cause any change in the rumen pH, although they caused a slight increase in propionate concentration. In line with our results and in high-producing dairy cows, Rafiee-Yarandi et al. (2019) reported that feeding different dietary CP contents changed nutrient digestibility but did not affect rumen pH. As expected, calves fed HP had higher rumen ammonia than calves fed LP. Daneshvar et al. (2017) in calves and Rafiee-Yarandi et al. (2019) in dairy cows noted that rumen ammonia values increased in a linear manner as dietary CP increased. The propionate and total VFA concentrations were higher and acetate-to-propionate ratio tended to be lower in HP-fed calves than in LP-fed calves. The

increase in starch digestibility in HP-fed calves probably caused an increase in propionate and total VFA production. Daneshvar et al. (2017) noted that calves fed LP (20%) had greater butyrate (preweaning) and lower propionate (postweaning) than calves fed HP (24%).

## CONCLUSIONS

Ground starter feed with higher CP increased digestibility, ADG, FE, and BW compared with lower CP. Moreover, feeding a ground starter diet with CP content higher than NRC recommendations can improve the ADG, BW, and FE of calves without influencing structural growth or starter feed intake. Alkali-processed WS increased fiber digestibility and tended to increase hip and withers heights, but it decreased rumen pH compared with unprocessed WS. The ADG and starter feed intake of calves were not influenced by WS processing. Moreover, the inclusion of PWS and 23.5% CP in the starter tended to improve starter feed intake, ADG, and BW of dairy calves. We conclude that increasing fiber digestibility through alkali processing of WS may improve calf performance in starter feed with higher protein content.

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## REFERENCES

- Ahmed, S., M. J. Khan, M. Shahjalal, and K. M. S. Islam. 2002. Effects of feeding urea and soybean meal-treated rice straw on digestibility of feed nutrients and growth performance of bull calves. *Asian-Aust. J. Anim. Sci.* 15:522–527. <https://doi.org/10.5713/ajas.2002.522>.
- Akayezu, J. M., J. G. Linn, D. E. Otterby, W. P. Hansen, and D. G. Johnson. 1994. Evaluation of calf starters containing different amounts of crude protein for growth of Holstein calves. *J. Dairy Sci.* 77:1882–1889. [https://doi.org/10.3168/jds.S0022-0302\(94\)77130-7](https://doi.org/10.3168/jds.S0022-0302(94)77130-7).
- Anderson, K. L., T. G. Nagaraja, and J. L. Morrill. 1987. Rumen metabolic development in calves weaned conventionally or early. *J. Dairy Sci.* 70:1000–1005. [https://doi.org/10.3168/jds.S0022-0302\(87\)80105-4](https://doi.org/10.3168/jds.S0022-0302(87)80105-4).
- AOAC International. 2002. Official Methods of Analysis. 17th ed. AOAC International, Arlington, VA.
- Bal, M. A., R. D. Shaver, A. G. Jirovec, K. J. Shinnors, and J. G. Coors. 2000. Crop processing and chop length of corn silage: Effects on intake, digestion, and milk production by dairy cows. *J. Dairy Sci.* 83:1264–1273. [https://doi.org/10.3168/jds.S0022-0302\(00\)74993-9](https://doi.org/10.3168/jds.S0022-0302(00)74993-9).
- Baldwin, R. L., K. R. McLeod, and J. L. Klitz. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and post-weaning ruminant. *J. Dairy Sci.* 87:55–65.

- Beharka, A. A., T. G. Nagaraja, J. L. Morrill, G. A. Kennedy, and R. D. Klemm. 1998. Effects of form of the diet on anatomical, microbial and fermentative development of the rumen of neonatal calves. *J. Dairy Sci.* 81:1946–1955. [https://doi.org/10.3168/jds.S0022-0302\(98\)75768-6](https://doi.org/10.3168/jds.S0022-0302(98)75768-6).
- Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64–75. [https://doi.org/10.3168/jds.S0022-0302\(80\)82888-8](https://doi.org/10.3168/jds.S0022-0302(80)82888-8).
- Casper, D. P., H. A. Maiga, M. J. Brouk, and D. J. Schingoethe. 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. *J. Dairy Sci.* 82:1779–1790. [https://doi.org/10.3168/jds.S0022-0302\(99\)75408-1](https://doi.org/10.3168/jds.S0022-0302(99)75408-1).
- Castells, L., A. Bach, G. Araujo, C. Montoro, and M. Terré. 2012. Effect of different forage sources on performance and feeding behavior of Holstein calves. *J. Dairy Sci.* 95:286–293. <https://doi.org/10.3168/jds.2011-4405>.
- Chanthakhoun, V., M. Wanapat, and J. Berg. 2012. Level of crude protein in concentrate supplements influenced rumen characteristics, microbial protein synthesis and digestibility in swamp buffaloes (*Bubalus bubalis*). *Livest. Sci.* 144:197–204. <https://doi.org/10.1016/j.livsci.2011.11.011>.
- Daneshvar, D., M. Khorvash, E. Ghasemi, and A. H. Mahdavi. 2017. Combination effects of milk feeding methods and starter crude protein concentration: Evaluation on performance and health of Holstein male calves. *Anim. Feed Sci. Technol.* 223:1–12. <https://doi.org/10.1016/j.anifeeds.2016.10.025>.
- Drackley, J. K., K. S. Bartlett, and R. M. Blome. 2002. Protein content of milk replacers and calf starters for replacement calves. [www.livestocktrail.uiuc.edu/dairy/paperDisplay.cfm?ContentID=339](http://www.livestocktrail.uiuc.edu/dairy/paperDisplay.cfm?ContentID=339).
- Fox, D. G., L. O. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. Technol.* 112:29–78. <https://doi.org/10.1016/j.anifeeds.2003.10.006>.
- Ghasemi, E., M. Khorvash, G. R. Ghorbani, and F. Elmamouz. 2014. Effects of straw treatment and nitrogen supplementation on digestibility, intake and physiological responses of water intake as well as urine and faecal characteristics. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 98:100–106. <https://doi.org/10.1111/jpn.12052>.
- Haddad, S. G., R. J. Grant, and S. D. Kachman. 1998. Effect of wheat straw treated with alkali on ruminal function and lactational performance of dairy cows. *J. Dairy Sci.* 81:1956–1965. [https://doi.org/10.3168/jds.S0022-0302\(98\)75769-8](https://doi.org/10.3168/jds.S0022-0302(98)75769-8).
- Haddad, S. G., R. J. Grant, and T. J. Klopfenstein. 1994. Digestibility of alkali-treated wheat straw measured in vitro or in vivo using Holstein heifers. *J. Anim. Sci.* 72:3258–3265.
- Hendriks, A. T. W. M., and G. Zeeman. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* 100:10–18. <https://doi.org/10.1016/j.biortech.2008.05.027>.
- Hill, T. M., J. M. Aldrich, R. L. Schlotterbeck, and H. G. Bateman II. 2007. Protein concentrations for starters fed to transported neonatal calves. *Prof. Anim. Sci.* 23:123–134. [https://doi.org/10.15232/S1080-7446\(15\)30952-9](https://doi.org/10.15232/S1080-7446(15)30952-9).
- Iranian Council of Animal Care. 1995. Guide to the Care and Use of Experimental Animals. Vol. 1. Isfahan University of Technology, Isfahan, Iran.
- Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, K. S. Ki, T. Y. Hur, G. H. Suh, S. J. Kang, and Y. J. Choi. 2007. Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. *J. Dairy Sci.* 90:3376–3387. <https://doi.org/10.3168/jds.2007-0104>.
- Kononoff, P. J., A. J. Heinrichs, and D. R. Buckmaster. 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. *J. Dairy Sci.* 86:1858–1863. [https://doi.org/10.3168/jds.S0022-0302\(03\)73773-4](https://doi.org/10.3168/jds.S0022-0302(03)73773-4).
- Krause, K. M., and G. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim. Feed Sci. Technol.* 126:215–236. <https://doi.org/10.1016/j.anifeeds.2005.08.004>.
- Liu, Q., C. Wang, C. X. Pei, H. Y. Li, Y. X. Wang, S. L. Zhang, Y. L. Zhang, J. P. He, H. Wang, W. Z. Yang, Y. S. Bai, Z. G. Shi, and X. N. Liu. 2014. Effects of isovalerate supplementation on microbial status and rumen enzyme profile in steers fed on corn stover based diet. *Livest. Sci.* 161:60–68. <https://doi.org/10.1016/j.livsci.2013.12.034>.
- Makizadeh, H., M. Kazemi-Bonchenari, H. Mansoori-Yarahmadi, J. Fakhræi, H. Khanaki, J. K. Drackley, and M. H. Ghaffari. 2020. Corn processing and crude protein content in calf starter: Effects on growth performance, ruminal fermentation, and blood metabolites. *J. Dairy Sci.* 103:9037–9053. <https://doi.org/10.3168/jds.2020-18578>.
- Mesfin, R., and G. Ktaw. 2010. Effect of feeding urea treated wheat straw based diet on biological performances and economic benefits of lactating Boran-Friesian crossbred dairy cows. *Livest. Res. Rural Dev.* 22:12–18.
- Mirzaei, M., M. Khorvash, G. R. Ghorbani, M. Kazemi-Bonchenari, and M. H. Ghaffari. 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>.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Ozkaya, S., and M. T. Toker. 2012. Effect of amount of milk fed, weaning age and starter protein level on growth performance in Holstein calves. *Arch. Tierzucht* 55:234–244. <https://doi.org/10.5194/aab-55-234-2012>.
- Rafiee-Yarandi, H., M. Alikhani, G. R. Ghorbani, M. Heydari, and P. Rezamand. 2019. Dietary protein level and corn processing method: Lactation performance, milk fatty acid composition, rumen and blood parameters of lactation dairy cows. *Livest. Sci.* 221:95–104. <https://doi.org/10.1016/j.livsci.2019.01.019>.
- SAS Institute. 2002. SAS User's Guide: Statistics. Version 9.1. SAS Institute Inc., Cary, NC.
- Senevirathne, N. D., J. L. Anderson, W. R. Gibbons, and J. A. Clapper. 2017. Growth performance of calves fed microbially enhanced soy protein in pelleted starters. *J. Dairy Sci.* 100:199–212. <https://doi.org/10.3168/jds.2016-11221>.
- Soberon, F., E. Raffrenato, R. W. Everett, and M. E. Van Amburgh. 2012. Prewaning milk replacer intake and effects on long-term productivity of dairy calves. *J. Dairy Sci.* 95:783–793. <https://doi.org/10.3168/jds.2011-4391>.
- Stamey, J. A., N. A. Janovick, A. F. Kertz, and J. K. Drackley. 2012. Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program. *J. Dairy Sci.* 95:3327–3336. <https://doi.org/10.3168/jds.2011-5107>.
- Van Keulen, V., and B. H. Young. 1977. Evaluation of acid-insoluble ash as natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44:282–287. <https://doi.org/10.2527/jas1977.442282x>.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 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](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Welch, J. G., and A. M. Smith. 1970. Forage quality and rumination time in cattle. *J. Dairy Sci.* 53:797–800. [https://doi.org/10.3168/jds.S0022-0302\(70\)86293-2](https://doi.org/10.3168/jds.S0022-0302(70)86293-2).
- Zhu, L., C. Jones, Q. Guo, L. Lewis, C. R. Stark, and S. Alavi. 2016. An evaluation of total starch and starch gelatinization methodologies in pelleted animal feed. *J. Anim. Sci.* 94:1501–1507. <https://doi.org/10.2527/jas.2015-9822>.