Iranian Journal of Applied Animal Science

Forage Essentiality for Young Holstein Calves: Superiority of Wheat Straw over Alfalfa Hay?

Research Article

A. Nikkhah[1*], M. Alimirzaei[2] and H. Kazemi[3]

[1] Department of Animal Science, University of Zanjan, Zanjan, Iran
[2] Aras-Dam Arsham Co., Poldasht, West Azarbayjan, Iran
[3] Department of Animal Science, Agriculture and Natural Resources Campus, University of Tehran, Karaj, Iran

Received on: 11 Jan 2024
Revised on: 6 Jun 2024
Accepted on: 8 Jul 2024
Online Published on: Sep 2024

*Correspondence E-mail: anikkha@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

The objective of this study was to determine the effects of feeding starter diets containing either chopped wheat straw (WS), chopped alfalfa hay (ALF) or their mixture (WA), and forage free starter diet (CO) on feed and water intakes, growth rate, blood metabolites, feeding behavior, ruminal pH, and structural body characteristics of Holstein calves. Sixty newborn Holstein calves (30 males and 30 females) were blocked based on sex and randomly assigned to one of four dietary treatments. The treatments included feeding dry starter diets with 1) no forage (CO), 2) chopped wheat straw (WS), 3) chopped alfalfa hay (ALF), or 4) a 50:50 mixture of wheat straw and alfalfa hay (WA). The dietary inclusion rate of forage was 5% from d 3 to 21 and 10% from d 22 until weaning and 2 weeks post-weaning. The starter diets were offered as totally mixed rations. Feed intake of individual calves was measured daily and body weight (BW) was monitored biweekly. Jugular blood samples were taken to measure serum metabolites on d 21 and 63. The calves in the WS group had greater (P<0.05) starter DM (1327 *vs.* 902 (g/d]), and neutral detergent fiber (NDF) (233 *vs.* 98 (g/d]) intakes than calves in the CO group. Calves fed forage had greater acid-detergent fiber (ADF) intake than calves fed CO diet (P<0.01). The total starter intake preweaning (100 *vs.* 76 and 73 (kg]) and dry matter intake (DMI) post-weaning (63 *vs.* 43 and 42 (kg]) were greater (P<0.05) for WS compared with ALF and CO calves, respectively. The average daily gain (average daily gain (ADG), 727 *vs.* 633 (g/d]) and average BW (65 *vs.* 60 (kg]) tended to be greater for WS relative to others (P<0.10). The average final body weight (BW) (97.7 *vs.* 85.3 (kg]) and total post-weaning weight gain (13.4 *vs.* 9.5 (kg]) were greater in WS compared with ALF (P<0.05), but was similar among WS, CO and WA groups. Ruminal pH tended to be greater (P=0.09) for WS (6.28) and WA (6.36) than for CO (5.61) and ALF (5.88). There was a tendency to decrease in non-nutritive oral behaviors for WS (21 min/d) compared with CO (59 (min/d]). Overall, findings suggest that forage in calf starter diets (mashed) could improve DMI and growth, with chopped WS being more effective than ALF under the conditions of this study. To imply, the indigestible and physically effective fiber of WS was able to stabilize rumen conditions and successfully stimulate greater starter intake.

KEY WORDS alfalfa hay, calf growth, dry matter intake, starter diet, wheat straw.

INTRODUCTION

Enhancing nutritional management, health, and growth of pre-weaning calves is a major investment to improve future herd productivity and sustainability (NASEM, 2021; Nikkhah and Alimirzaei, 2022a). The role of early life nutrition in determining later performance and metabolic status of mature dairy cows has been well addressed

(Cantor *et al*. 2019; Keogh *et al*. 2021; Meale *et al*. 2021). As such, the modern calf nutrition has increasingly placed an emphasis on optimizing weight gain and wellbeing of young calves. For instance, concurrent increases in milk, starter, and water intakes have been proposed for maximizing pre-weaning calf immunity and performance (Nikkhah and Alimirzaei, 2022b). Moreover, elevated starter intake during weaning has been reported to be positively related to future milk yield (Heinrichs and Heinrichs, 2011). Feeding forage to pre-weaned calves is one of the most controversial aspects of dairy calf raising (Xiao *et al*. 2020, Keshavarz *et al*. 2023).

Decreased feed intake, nutrient digestibility, and weight gain have been demonstrated in calves fed higher levels of forage (Hill *et al*. 2009; Hosseini *et al*. 2019). However, forage inclusion could prevent reductions in ruminal pH due to higher intake of grains in the calf starter diet (Bagheri *et al*. 2021). Differences in forage type, level, physical form, timing of forage feeding, grain type, and milk feeding method may have influenced the different outcomes. Providing adequate nutrients from both milk and starter concentrate to maintain average daily gain (ADG) of calves above 0.5 kg/d would be likely associated with increased milk yield in their first lactation (Galsinger *et al*. 2020).

Alfalfa hay, corn silage, and wheat straw are the most common forage sources for ruminants worldwide, differing in their physicochemical characteristics (Kahyani *et al*. 2019). Wheat straw may contain low proportion of potentially digestible NDF (pdNDF) than AH causing slow digestion rate in the rumen. Although not much investigated in pre-weaning calves, wheat straw may be useful in providing proper amounts of physically effective NDF to promote rumination and prevent ruminal pH reduction in dairy cows (Kahyani *et al*. 2019). Pre-weaning calf dry matter intake (DMI), growth rate, nutritional behavior, and welfare may be influenced by forage inclusion in starter diets (Terré *et al*. 2015; Mirzaei *et al*. 2017; Bagheri *et al*. 2021; Nikkhah and Alimirzaei, 2022c). In a recent meta-analysis, inclusion of hay in the starter diet led to positive calf performance when fed with finely processed or texturized starters (Ghaffari and Kertz, 2021). However, the paucity of data exists on how forage type (e.g., wheat straw vs. alfalfa hay) can affect rumen physiology, growth, immunity, and nutritional behavior of young daily calves.

Ruminal development and feed intake of dairy calves are two main factors that affect the successful performance of weaned and post-weaned calves (Nikkhah and Alimirzaei, 2021). It has been known that ruminal fermentation products such as volatile fatty acids (VFA) play important roles in the timely development of the rumen papillae and metabolic function of the rumen epithelium (Khan *et al*. 2016).

In an early study (Warner *et al*. 1956), it was concluded that grains, and not necessarily forages, promote rumen development. In accordance with that study, Wu *et al*. (2018) found no effects of forage supplementation on feed intake, growth, and rumen fermentation of 3 to 15 d-old calves. The primary mechanism describing such responses would be that forages are less digestible than concentrates and may have filling effect on young calves' rumen, which would reflect in decreased voluntary feed intake and delayed rumen development (Nikkhah and Alimirzaei, 2022c). In other words, fiber is slowly digested in the rumen in favor of acetate production which is less metabolized in the rumen epithelial cells, when compared with butyrate and propionate (Khan *et al*. 2016). The shift towards increased acetate proportion was also reported for hay fed calves (Poier *et al*. 2022). Nevertheless, rumen muscular development may be enhanced by including fibrous feeds in calf starters (Heinrichs and Lesmeister, 2005). As such, forage inclusion in calf starters may modulate rumen pH. Laarman and Oba (2011) reported that hay intake is negatively associated with ruminal acidosis during the weaning transition. Rumination and subsequently salivation can increase as a result of forage provision to young calves (Pazoki *et al*. 2017). Calves fed 100% high quality hay consumed more physically effective neutral-detergent fiber (peNDF) which is related to longer rumination time (Poier *et al*. 2022). In fact, pre-weaning calf responses to dietary forage have been different among studies, and the results have been inconsistent. In some studies (Hill *et al*. 2008; Hill *et al*. 2009), feeding forage has decreased feed intake and ADG of pre-weaned calves. While, no differences (Mirzaei *et al*. 2015; Wu *et al*. 2018) or improved feed intake and ADG (Castells *et al*. 2015; Daneshvar *et al*. 2015) have been reported by others. In a recent study (Terler *et al*. 2023), Holstein calves fed 70% of concentrate had greater ADG and final BW but ticker keratin layer and wider papillae in rumen, indicating the risk of ruminal acidosis in those calves. In a similar study, calves fed 100% high quality hay had similar growth performance and nutrient intake when compared with calves receiving 70% concentrate (Terler *et al*. 2022).

Given the importance of physically effective fiber for mature dairy cows in preventing sub-acute ruminal acidosis (SARA) and its negative effects on performance and welfare, attention to fiber requirements of young calves may be necessary. This is to avoid adverse effects of low ruminal pH on feed intake and calf performance and immunity. We hypothesized that feeding less digestible forage such as wheat straw alone or in combination with alfalfa hay can optimize rumen conditions and improve starter feed intake and growth of young calves. Therefore, the objective of the current study was to determine effects of including either wheat straw, alfalfa hay, or their combination in the calf starter on nutrient intake, blood metabolites, rumen pH, fecal score and pH, feeding behavior, growth, body weight, and structural body growth indices of young Holstein calves.

MATERIALS AND METHODS

Experimental design, treatments, and animal feeding and care

This study was conducted in a large commercial dairy farm (2600 dairy cattle, Behroozi Dairy Co., Tehran, Iran) from December 2021 to March 2022. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Tehran University of Medical Sciences (TUMS) (IACUC protocol number: (93-02-45-26666]). Sixty newborn Holstein calves (30 males and 30 females; 41 ± 1.4 (kg] BW) were blocked by sex and randomly assigned to one of four dietary treatments. The treatments included starter diets containing either 1) no forage (CO), 2) chopped wheat straw (WS), 3) chopped alfalfa hay (ALF), or 4) a 50:50 combination of chopped wheat straw and alfalfa hay (WA). The dietary forage inclusion rate was 5% from d 3 to 21, and 10% from d 22 until weaning (d 63) and 2 weeks post-weaning. The starter diets were formulated using AminoCow software program (AminoCow®, 3.5.2 Mepron-Evonik, USA). The expected calf growth rate was 800 g/d and calf body weight was 42 kg. The dietary ingredients and chemical composition of the dietary treatments are presented in Table 1. The starter diet's ingredients were ground using a 3-mL sieved hammer mill before mixing. Thus, a relatively course starter feed was prepared. The starter and forage were mixed using a 17 (M^3] Feeder-Mixer Wagon (Seko-5 Samurai, Japan) for about 15 min. Both wheat straw and alfalfa hay were chopped using the above Feeder-Mixer Wagon. Five samples from each forage were taken and particle size distribution was determined using a Penn State Particle Separator as described by Kononoff *et al.* (2003). The particle size distributions of the forages are given in Table 2. Wheat straw and alfalfa hay had respectively 5.0% and 18.0% crude protein (CP), 80.0% and 42.0% NDF, 53.9% and 31.1% acid-detergent fiber (ADF), and 6.5 and 27.5% non-fiber carbohydrate (NFC) (DM-based). All calves had free access to starter feed and clean water throughout the study. In the post-weaning period (64-83 d of calf age), the CO diet was switched to a totally mixed ration with 90:10 concentrate to forage ratio similar to treatment 4 or WA as a farm routine. The other three treatments continued to be fed as they were in the pre-weaning period.

All calves were born in individual maternity pens (8 pens for 80 close-up cows) bedded with dry wheat straw.

The bedding materials of the maternity pens were removed after each calving and then were washed, disinfected, and renewed with fresh dry wheat straw. After parturition, umbilical cord was submerged in a 7% iodine solution for about 15 s. Calves were separated from their dams within 1 h of calving and weighed before transferring to individual pens. The individual calf pens (1.2×2.4 m) were made of concrete within a roofed modern facility including 70 individual pens. The individual pens were equipped with water and starter feed containing buckets. Calves were bedded with sawdust, which was removed and renewed daily throughout the study. All calves received high quality maternal colostrum (Brix Index>22) using a pacifier head bottle 3 times daily with the first meal offered approximately at 10% of birth body weight (BBW) within the first 2 h of life. Notably, if the maternal colostrum was inadequate in IgG concentrations (i.e., Brix Index<22), high quality pooled colostrum (stored in the refrigerator) was fed to ensure adequate passive immunity transfer. The quality of colostrum was determined by a refractometer (LH-T32, EMIN, Hanoi, Vietnam) in accordance with the farm protocol. The next colostrum meal (2 L) was fed 12 h after the first meal. The last meal was fed for about 2 L at 8 h after the second meal. Transition milk was fed to the calves for the next two days or until d 3 of age. Afterwards, milk feeding started according to a step-up and step-down feeding method. The experimental calves were fed pasteurized whole milk (13% DM, 3.1% crude protein, and 3.5% fat) twice daily at 06:00 am and 17:00 pm in equal amounts through plastic buckets. The pasteurized whole milk was offered at 10% of BBW for the first week, then increased gradually to reach 20% of BBW at week 5, and remained constant until week 8. After that, milk consumption was reduced to approximately 1-1.2 (l/d] to enable weaning at 63 d of age. Clean water was provided to the calves from d 3 of age until weaning for *ad-libitum* intake. In the post-weaning period, the calves had free access to their own water nipples connected to a central water supply system. The solid starters or dietary treatments were offered to the experimental calves from d 3 of age until the end of study or d 83. Water and solid starter diets were renewed every day, and refusals were collected and measured daily to calculate water and DM intakes of individual calves. The ambient temperature and relative humidity were recorded daily by a digital thermometer (HTC-4) located at the middle of the calf-raising facility right above the individual calf pens.

BW, feed sampling procedures, and skeletal growth indices

Immediately after birth, before moving the calves to individual pens, initial or BBW was recorded for each calf using a digital scale.

Table 1 Ingredients and chemical composition of the dietary treatments

Item	CO	WS		ALF		WA	
	3-63 d	5% (3-21 d)	10% (22-63 d)	5% (3-21 d)	10% (22-63 d)	5% (3-21 d)	10% (22-63 d)
Ingredient (% DM-based)							
Alfalfa hay (chopped)	-	-	-	5.00	10.0	2.5	5.0
Wheat Straw (chopped)	-	5.00	10.00	-	-	2.5	5.0
Corn grain (ground)	46.70	44.33	42.00	44.33	42.00	44.3	42.00
Barley grain (ground)	20.00	19.00	18.00	19.00	18.00	19.0	18.00
Soybean Meal	27.70	26.30	25.00	26.30	25.00	26.3	25.00
Limestone	1.33	1.26	1.20	1.26	1.20	1.3	1.20
Salt	0.20	0.19	0.01	0.19	0.01	0.2	0.01
Sodium bicarbonate	1.00	0.95	1.00	0.95	1.00	0.95	1.00
Vitamin and Mineral Supplement[2]	1.33	1.26	1.20	1.26	1.20	1.3	1.20
Toxin binder[3]	1.80	1.71	1.62	1.71	1.62	1.7	1.62
Chemical composition							
Metabolizable energy (Mcal/kg]	2.65	2.62	2.60	2.65	2.65	2.63	2.62
Crude protein (%)	19.4	18.60	17.83	19.15	18.9	19.15	18.38
Fat (%)	2.82	2.77	2.72	2.78	2.74	2.77	2.73
Acid-detergent fiber (ADF %)	4.57	7.03	9.50	6.21	7.85	6.21	8.67
Neutral detergent fiber (NDF %)	10.9	14.25	17.61	12.88	14.85	12.88	16.23
Starch (%)	45.5	40.95	43.23	43.30	41.09	43.26	41.02
Ca (%)	0.87	0.84	0.81	0.90	0.93	0.87	0.87
P (%)	0.54	0.51	0.49	0.52	0.51	0.52	0.50
Mg (%)	0.22	0.21	0.21	0.22	0.23	0.22	0.22
K (%)	0.90	0.93	0.96	0.94	0.99	0.94	0.97
Na (%)	0.42	0.40	0.39	0.40	0.39	0.40	0.39
Cl (%)	0.21	0.21	0.22	0.22	0.32	0.22	0.22
S (%)	0.43	0.41	0.40	0.42	0.41	0.42	0.41

[1] CO: starter diet without forage; WS: starter diet with chopped wheat straw; ALF: starter diet with chopped alfalfa hay and WA: starter diet with a 50:50 mixture of chopped wheat straw and chopped alfalfa hay.
[2] Each kg of VMS contained: vitamin A: 700000 IU; vitamin D3: 100000 IU; vitamin E: 4000 IU; Ca: 90 g; Mg (magnesium oxide): 200 g; S: 20 g; Fe: 500 mg; Cu: 3000 mg; Mn: 6000 mg; Z: 8000 mg; Co: 80 mg; I: 120 mg; Se: 90 mg; Antioxidant agent: 1000 mg and Carrier: 1000 g.
[3] Magnotox (commercial name), manufactured by Vivan company, Mashhad, Iran.

Table 2 Particle size distribution of chopped wheat straw, chopped alfalfa hay, and their 50:50 mixture A (% of DM retained on each sieve; mean±SD)

Sieve [1]	Forage treatment		
	Wheat straw	Alfalfa hay	Wheat straw + alfalfa hay
Upper	13.72±2.18	10.95±2.34	4.71±2.24
Middle	40.98±2.18	29.45±2.34	30.00±2.24
Lower	31.58±2.18	35.22±2.34	33.75±2.24
Bottom Pan	13.7±2.18	24.38±2.34	31.54±2.24
Average particle size (mm)	8.48	6.63	5.56

[1] The Penn State Particle Separator was used to separate forage particle size into long (>19 mm], medium (>8 mm], lower (>1.8 mm], and fine (<1.8 mm] fractions (Kononoff *et al.* 2003).

Afterwards, all valves were weighed biweekly (i.e., 14-d intervals) throughout the study to obtain total weight gain and average daily gain (ADG) in both pre- and post-weaning periods for individual calves. In addition, weaning and final BWs were recorded on d 63 and 83, respectively. The starter feed was renewed every day at 1000 h with the daily allocation increasing by 50% when refusals were less than 20% of the amount offered. This amount was increased by 100% in the post-weaning period because calves were grown and feed intake had increased considerably. Feed efficiency for individual calves was calculated by dividing BW gain values by DMI separately for each 14-d interval according to Khan *et al.* (2007).

The skeletal growth indices of the experimental calves were measured on d 21, 50, and 63 of age using the method described by Khan *et al.* (2011). The withers height, heart girth, body depth, and hip width were recorded for all calves.

Blood sampling, processing, and analysis

The jugular blood was sampled at 4 h after the morning milk feeding on d 21 and d 63. The blood samples were collected using 6-mL EDTA-free tubes and were placed inside an ice container to allow clotting. The blood tubes were centrifuged (Shimifan, Tehran-Iran) at 3000 × *g* for 15 minutes to separate serum. The serum was harvested using a sterile plastic sampler and spilled into micro tubes (3 mL) for storage at -20 ˚C for later metabolite analysis. The serum samples were sent to a veterinary laboratory (Dampar-var, Shahriyar, Tehran, Iran) for measuring glucose, urea (BUN), beta-hydroxybutyrate (BHBA), total protein, and albumen concentrations. Serum total proteins, albumin, urea nitrogen, and glucose were measured using Pars Azmoon kits and related procedures (Pars Azmoon Co., Tehran, Iran). Globulin concentrations were obtained by deducting albumin from total protein. The concentrations of BHBA were measured using commercial kits with a Technicon-RA 1000 Autoanalyzer (DRG Co., Marburg, Germany).

Fecal and rumen pH measurements

The fecal score was determined and recorded daily by an experienced technician at 0600 h before the morning milk feeding. The fecal scoring was based on fecal fluidity (1=normal, 2=soft, 3=runny, and 4=watery) according to Larson *et al.* (1977). A fecal score greater than 3 was considered as diarrhea. The 0.5-score intervals were considered to better differentiate among different fecal appearances. Fecal pH was measured using a pH meter (Crison Instruments, Barcelona, Spain) on d 50 and 63 of calf age in two consecutive days for each session and the average was calculated. Fecal samples were taken either directly by rectal sampling or using newly dropped excretions. Ten g of the sampled feces was added to 10 mL of distilled water to make a homogenized mixture. The prepared mixture was then used for pH measurements. The rumen pH was measured at weaning at 4 h after starter feeding (i.e., 1400 h) by using a flexible plastic oral stomach tube with a bulbous-shaped terminal to prevent possible injuries during sampling.

A suction pump attached to the other end of the tube enabled the rumen fluid to be extracted immediately (Klopp *et al.* 2018). To avoid saliva contamination, samples were taken by a professional experienced technician and the initial secretions were removed. The collected rumen fluids were used to determine pH. The highly contaminated samples were thrown away and sampling was repeated. The rectal temperature of the individual calves was recorded weekly using a digital thermometer (Rossmax TG380, Berneck, Switzerland) at 1200 h during the pre-weaning period.

Feeding behavior

The calf feeding behavior was monitored according to Castells *et al.* (2012). Ten calves per each treatment were randomly selected to record their feeding behavior. All behaviors were monitored by direct visual observations. Eating, drinking, lying, standing, ruminating, and non-nutritive oral behavior (tongue rolling, licking of pen and feed and water buckets, and playing with bedding materials) were recorded. All behaviors were monitored 4 times including at 2 weeks before weaning, 1 week before weaning, 1 week after weaning, and 2 weeks after weaning. In the pre-weaning period, feeding behaviors were recorded for 1 h immediately after milk feeding and 1 h immediately after offering the starter feed. In the post-weaning period, behaviors were recorded twice daily after each starter feeding. As a result, each calf was monitored for 8 hours. During the 1 h monitoring period, each behavior was recorded at the beginning of each and every minute and was considered for the entire 1 minute if observed.

Statistical analysis

Data for initial, weaning, and final BW; pre-weaning, post-weaning, and total BW gains and starter intakes; rumen and fecal pH; structural body growth indices; and blood metabolites were analyzed using a mixed-effects model with fixed effects of treatment, sex, and treatment × sex, plus the random effect of calf within sex × treatment (SAS, 2003). For repeated measures of BW, fecal score, rectal temperature, and starter nutrient intake, mixed models with fixed effects treatment, sex, time, treatment × sex, treatment × time, sex × time, and treatment × sex × time as well as the random effect of calf within treatment × sex × time were used. The interactions were illustrated in graphs as necessary. To take into account the dissimilar correlations of the repeated measures on the same subject, mixed models with different covariance structures (e.g., compound symmetry, compound symmetry heterogeneous, autoregressive (1), autoregressive heterogeneous (1), unstructured] were tested to adopt the structure producing the least fit criteria (Wang and Goonewardene, 2004). Initial BW values were modeled as covariate. The PDIFF option of SAS with Tukey multiple-range test (SAS, 2003) was used to separate means. The data were presented as least square means and standard errors of differences. The significant differences were declared at $P \leq 0.05$ and tendencies for significance were declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

Nutrient and water intakes, BW, and BW gains

The daily starter and total (milk+starter) DMI as well as starter metabolizable energy (ME) intake were greater for WS than for CO (Table 3, P<0.05). The daily starter CP intake tended to be greater for WS than for CO (P=0.08). The daily starter and total DMI and starter ME and CP intakes were not significantly different among CO, ALF and WA (P>0.10, Table 3). The daily starter starch intake was greater for WS than for CO and ALF (P<0.05).

Table 3 Effects of feeding different forages (3-83 d) in starter diet as total mixed ration (TMR) on nutrient intake, body weight (BW), average daily gain (ADG), fecal score, and rectal temperature of Holstein calves

Item	Treatment (Trt)[1]				Sex		P-value						
	CO (n=15)	WS (n=15)	ALF (n=15)	WA (n=15)	Male	Female	SE	Trt	Time	Sex	Trt × Time	Trt × Sex	Trt × Time × Sex
Milk DMI (g/d) (d 3-62)	329	329	329	330	330	329	0.80	0.69	<0.001	0.13	0.57	0.05	0.01
Starter DMI (g/d)	902.2^{b}	1326.5^{a}	997.5^{ab}	1272.0^{ab}	1288	961	155.7	0.02	<0.001	<0.01	<0.001	0.92	0.04
Total DMI (g/d)	1121.5^{b}	1546^{a}	1217.0^{ab}	1492.0^{ab}	1508	1180	155.7	0.02	<0.001	<0.01	<0.001	0.93	0.04
Starter CP intake (g/d)	175.0^{b}	236.6^{a}	188.5^{ab}	233.9^{ab}	239	178	27.10	0.08	<0.01	<0.001	<0.001	0.80	0.03
Starter ME intake (Mcal/d)	2.39^{b}	3.47^{a}	2.64^{ab}	3.33^{ab}	3.39	2.52	0.41	0.02	<0.001	<0.001	<0.001	0.92	0.04
Starter starch intake (g/d)	410.5^{b}	573.0^{a}	410.1^{b}	522.0^{ab}	548	409	65.97	0.03	<0.001	<0.01	<0.001	0.94	0.04
Starter NDF intake (g/d)	98.3^{c}	232.9^{a}	147.3^{bc}	206.0^{ab}	196	147	22.37	<0.001	<0.001	<0.01	<0.001	0.88	0.05
Starter ADF intake (g/d)	41.2^{c}	125.5^{a}	78.0^{b}	110.0^{ab}	101	76	12.57	<0.001	<0.001	<0.01	<0.001	0.82	0.05
Average daily gain (g/d)	612	727	623	663	738	573	30	0.08	<0.001	<0.001	0.33	0.22	<0.01
Body weight (kg)	60.7	65	58.7	62	65.4	57.6	2.62	0.09	<0.001	<0.001	0.04	0.23	<0.001
Water intake (mL/d)	1719	2049	1941	2199	2191	1763	208	0.15	<0.001	<0.01	<0.01	0.68	0.34
Feed:gain	0.6	0.55	0.56	0.57	0.58	0.56	0.05	0.79	<0.001	0.58	0.82	0.49	0.45
Fecal score	2.1	2.1	2.2	2.1	2.1	2.1	0.04	0.65	<0.001	0.73	0.10	0.78	0.99
Rectal temperature (°C)	38.40	38.32	38.32	38.32	38.38	38.30	0.05	0.42	<0.01	0.03	0.03	0.5	0.19

[1] CO: starter diet without forage; WS: starter diet with chopped wheat straw; ALF: starter diet with chopped alfalfa hay and WA: starter diet with a 50:50 mixture of chopped wheat straw and chopped alfalfa hay.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

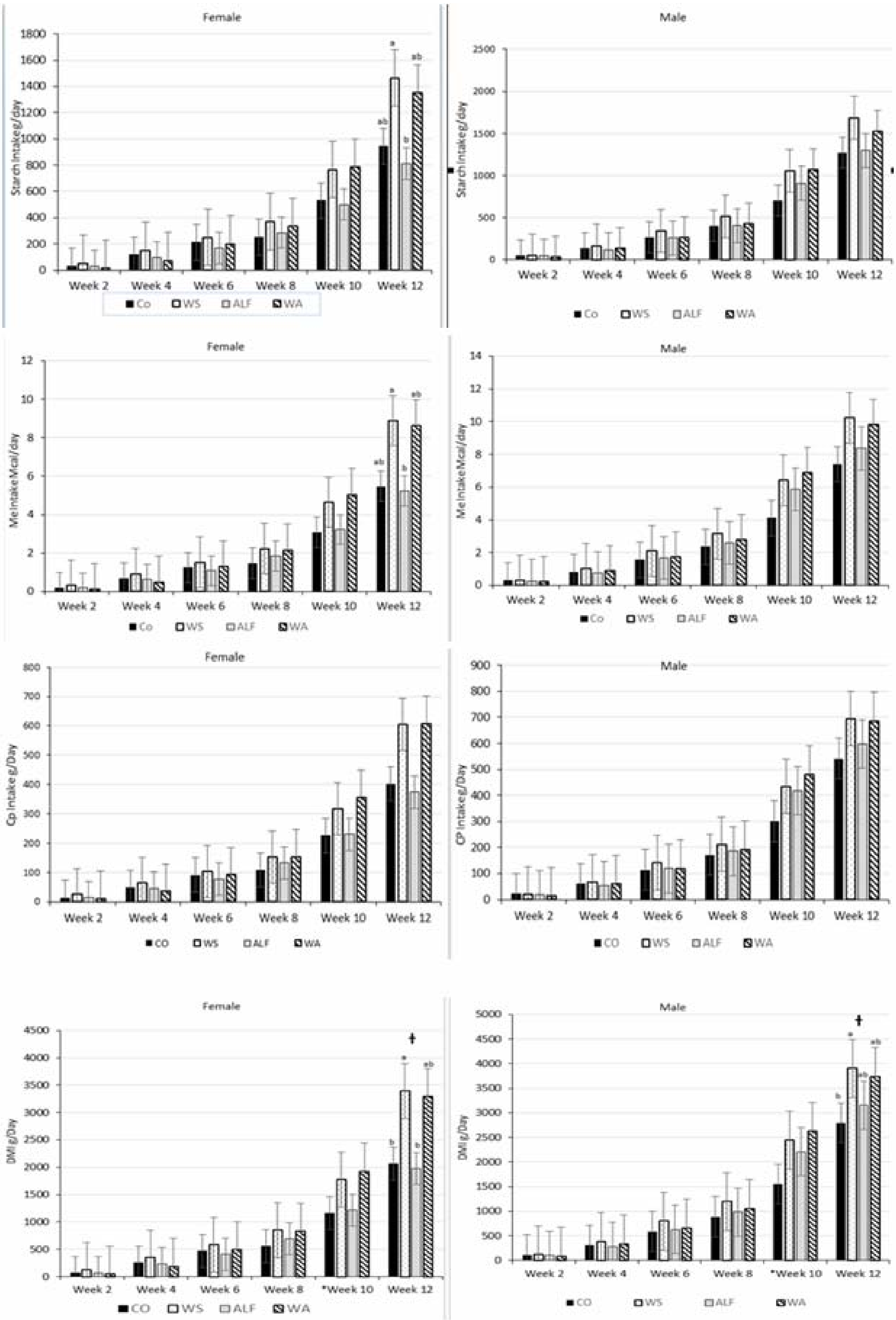
SE: standard error.

A significant interaction of treatment × time × sex was found for total daily DMI (starter+milk) and starter intakes of DM, starch, ME, NDF and ADF (P<0.05, Table 3). At week 12, in female calves; starch, ME and DM intakes were greater (P<0.05) for WS than for ALF (Figure 1), but no significant differences were found among CO, ALF and WA. This finding reiterates that WS was more effective than ALF in stimulating starter nutrient intake. In male calves, total DMI was greater (P<0.05) for WS than for CO, but did not differ among CO, ALF and WA (Figure 1). In male calves at weeks 10 and 12, starter NDF intake was greater (*P* < 0.05) for WS and WA than for CO (Figure 2).

In male calves at week 12, starter NDF intake was greater (P<0.05) for WS *vs*. ALF, as well. Although time- and sex-dependent, WS show improved fiber intake over ALF and CO. In female calves at weeks 10 and 12, daily starter ADF intake was greater (P<0.05) for WS than for CO; while at week 12, starter ADF intake was greater (P<0.05) for WS and WA than for CO and ALF (Figure 2). In male calves, at week 10, daily starter ADF intake was greater (P<0.05) for WS, ALF and WA than for CO; while at week 12, daily starter ADF intake was greater (P<0.05) for WS than for other treatments. These data suggest that the increasing effect of forage on fiber intake is more durable and consistent for WS than for ALF.

The average water intake during the entire experiment was not significantly different among treatments (P>0.10). However, a significant interaction was found between treatment and time, such that at week 8, daily water intake was greater (P<0.05) for forage fed calves than for CO fed calves (Figure 3). At week 8, no significant differences (P>0.10) were found among WS, ALF and WA in daily water intake (Figure 3). These findings support the notion that forage inclusion in calf starter diets can stimulate water intake. The greater water intake may be a physiological response to increased nutrient intake in forage fed calves, especially for WS. The positive influence of increased water intake on rumen development and successful weaning has been reported (Wickramasinghe *et al*. 2019).

Body weight and ADG exhibited a significant interaction among treatment, time and sex (P<0.05, Table 3). In female calves at week 12, BW was greater (P<0.05) for WS than for ALF, while no significant differences existed among WS, CO and WA (Figure 3). In male calves at week 10, ADG was greater (P<0.05) for WS and ALF than for CO and not WA (Figure 3). Feed efficiency did not differ among treatments (P>0.10, Table 3). These data suggest that with the advancing calf age, the superior role of WS over ALF in increasing the BW gain became more evident. Final BW and total post-weaning BW gain were greater (P<0.05) for WS than for ALF (P<0.05, Table 4), demonstrating the superior effect of WS *vs*. ALF on calf growth. These measures were not significantly different among WS, WA, and CO.



Continue on next page

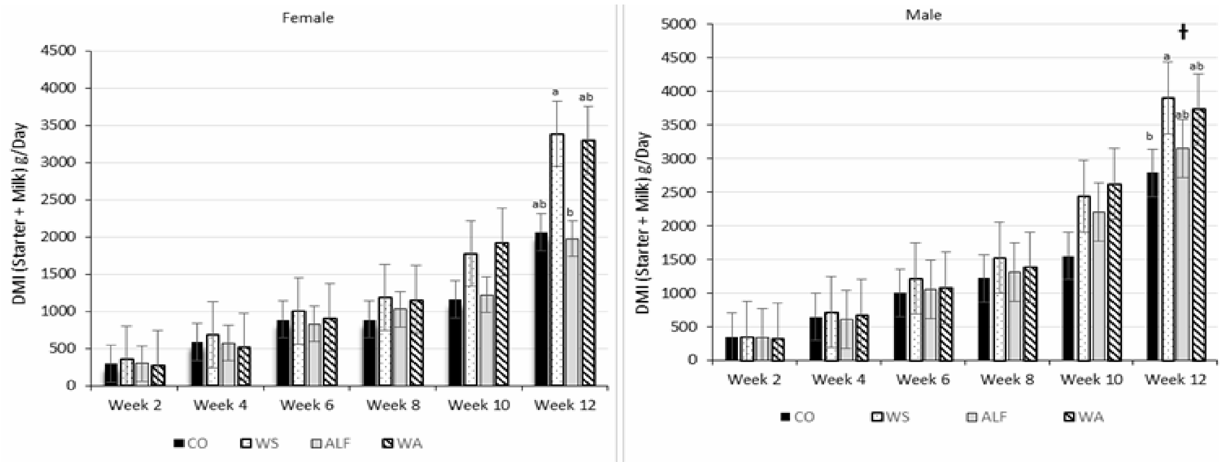


Figure 1 Nutrient intake in male and female calves on starter diets containing either no forage (CO), chopped wheat straw (WS), chopped alfalfa hay (ALF), or a 50:50 mixture of WS and ALF (WA)

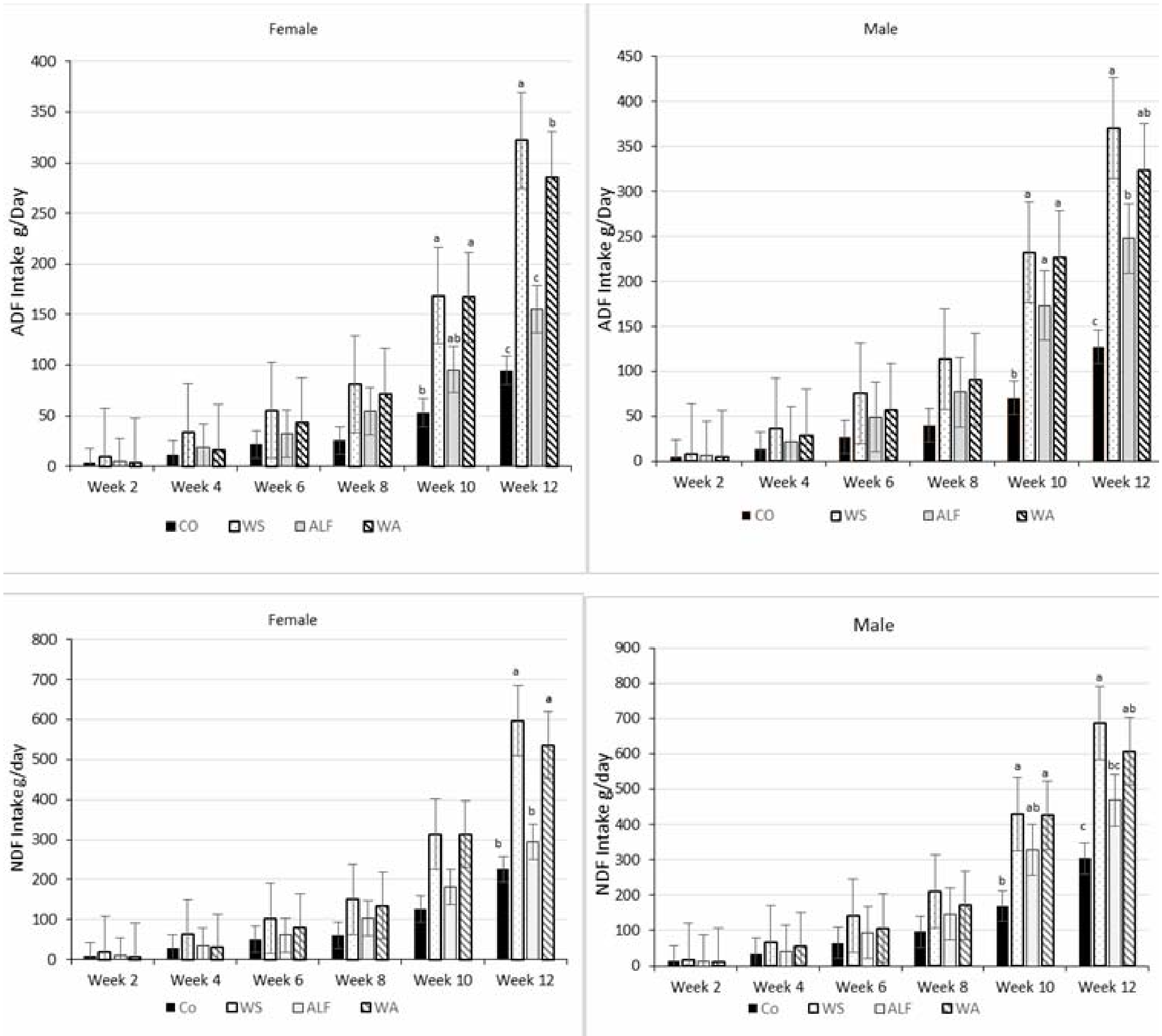


Figure 2 ADF and NDF intake in male and female calves on starter diets containing either no forage (CO), chopped wheat straw (WS), chopped alfalfa hay (ALF), or a 50:50 mixture of WS and ALF (WA)

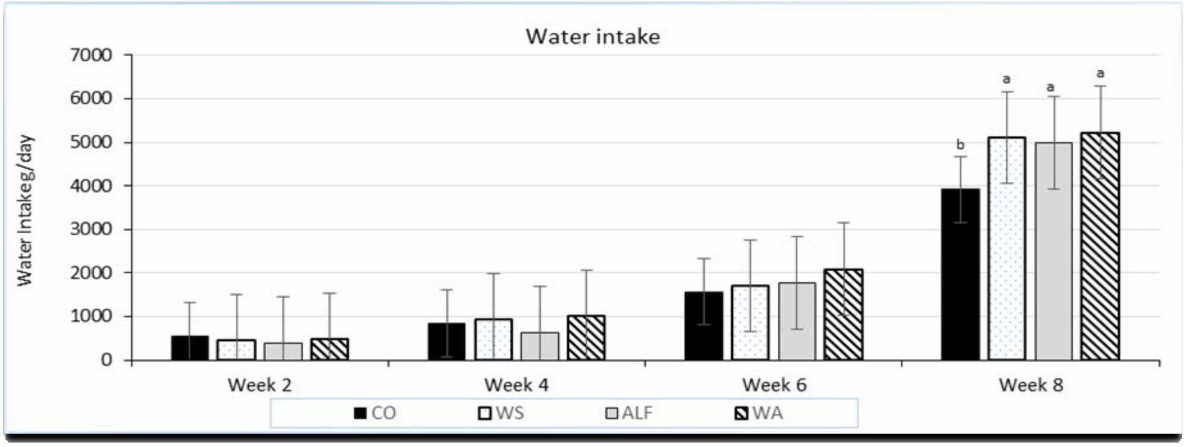


Figure 3 Water intake in the experimental calves on starter diets containing either no forage (CO), chopped wheat straw (WS), chopped alfalfa hay (ALF), or a 50:50 mixture of WS and ALF (WA)

Table 4 Effects of feeding different forages in starter diet as total mixed ration (TMR) on total weight gain, total starter intake and rumen and fecal pH of Holstein calves

Item	Treatment (Trt)[1]				Sex		P-value			
	CO (n=15)	WS (n=15)	ALF (n=15)	WA (n=15)	Male	Female	SE	Trt	Sex	Trt × Sex
Initial body weight (kg)	40.5	42.5	38.5	41.2	42.5	39.0	2.0	0.25	0.02	0.07
Weaning body weight (kg) (d 63)	76.0	84.0	76.0	79.1	84.0	73.0	3.9	0.13	<0.01	0.43
Final body weight (kg) (d 83)	88.3[ab]	97.7[a]	85.3[b]	92.0[ab]	97.6	84.0	3.1	0.03	<0.01	0.21
Pre-weaning weight gain (kg) (d 3-63)	35.4	41.6	37.1	37.8	42.3	33.6	3.3	0.30	<0.01	0.46
Post-weaning weight gain (kg) (d 64-83)	12.2[ab]	13.4[a]	9.5[b]	12.8[ab]	12.9	11.0	1.4	0.04	0.06	0.26
Total weight gain (kg) (d 3-83)	47.8	54.5	47.2	50.6	55.5	44.6	3.3	0.15	<0.001	0.31
Pre-weaning starter DMI (kg) (d 3-63)	33.0	46.3	36.7	43.6	46.6	33.0	6.9	0.21	<0.01	1.00
Post-weaning starter DMI (kg) (d 64-83)	41.5[b]	63.0[a]	43.0[b]	54.2[ab]	56.4	44.4	6.5	<0.01	0.02	0.92
Total starter DMI (kg) (d 3-83)	73.2[b]	100.3[a]	76.1[b]	96.6[ab]	100.4	78.7	12.9	0.02	0.02	0.87
Rumen pH (d 63)	5.61	6.28	5.88	6.36	6.0	6.1	0.3	0.09	0.66	0.21
Fecal pH										
50 d	7.2	6.9	7.2	7.0	6.8	7.3	0.3	0.82	0.04	<0.01
63 d	6.8	6.3	7.2	6.8	6.8	6.8	0.3	0.06	0.92	0.01

[1] CO: starter diet without forage; WS: starter diet with chopped wheat straw; ALF: starter diet with chopped alfalfa hay and WA: starter diet with a 50:50 mixture of chopped wheat straw and chopped alfalfa hay.
DMI: dry matter intake.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

Total (pre- plus post-weaning) and post-weaning starter DMI were greater (P<0.05) for WS than for ALF and CO (Table 4).

These results are in line with, and support, the increased BW gain and growth in the WS fed calves. No significant differences (P>0.10) in total starter DMI were found for WS vs. WA or for CO vs. ALF (Table 4).

It appears that the mixture of WS and ALF was also effective in stimulating total starter DMI. Although numerically higher for WS, total pre-weaning BW gain and starter DMI were not significantly different (P>0.10) among treatments (Table 4).

Rumen and fecal pH

Rumen pH tended to be higher (P=0.09) for WS and WA than for CO and ALF (Table 4). On d 50, fecal pH in male calves was higher (P<0.05) for ALF than for WA, but was similar between “WS *vs*. CO” or “WS *vs*. ALF’ (Figure 4). Although sex-dependent, more undigested or partially digested starch might have escaped to the hindgut for WA than for ALF, thereby increasing the hindgut fermentation and reducing fecal pH. On d 63, in male calves, fecal pH was higher (P<0.05) for ALF and CO than for WS and WA (Figure 5), suggesting an increased hindgut fermentation in calves on WS and WA.

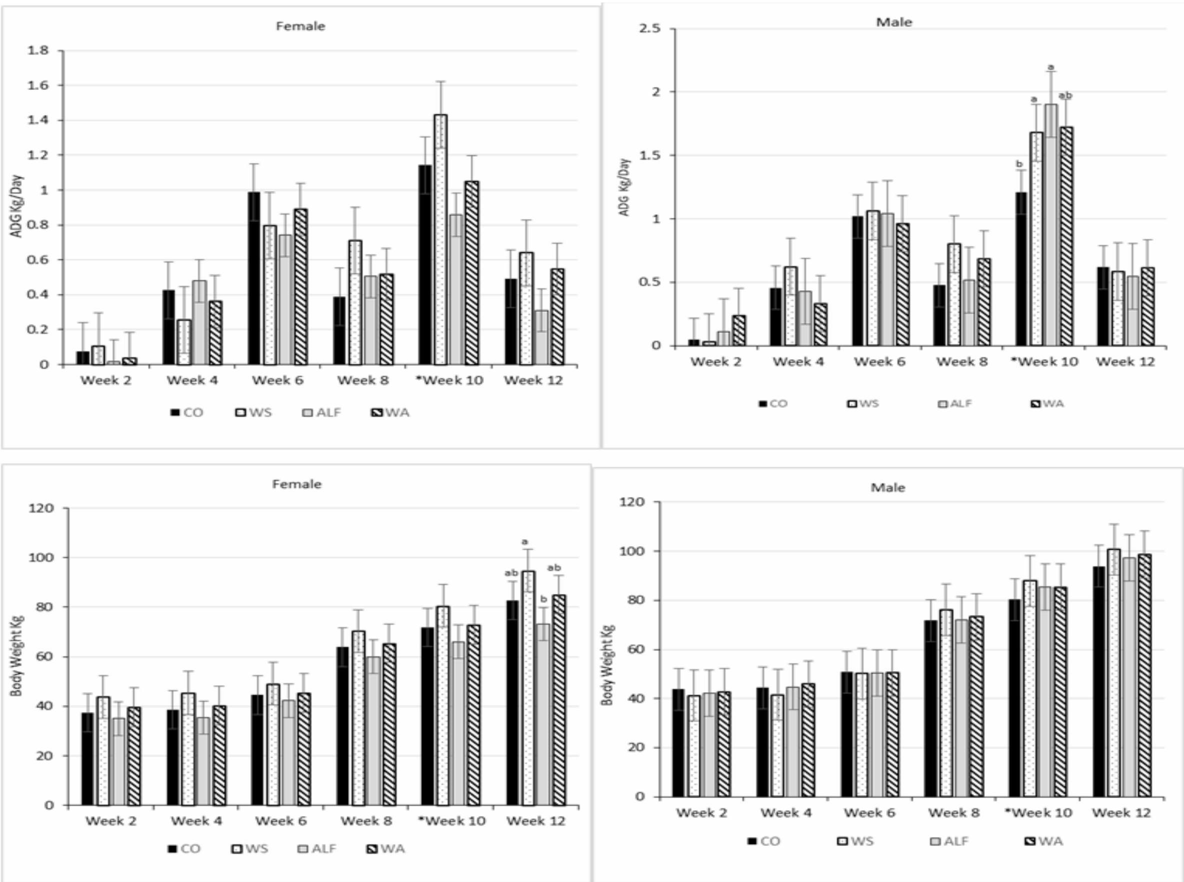


Figure 4 Body weight and average daily gain (ADG) in male and female calves on starter diets containing either no forage (CO), chopped wheat straw (WS), chopped alfalfa hay (ALF), or a 50:50 mixture of WS and ALF (WA)

This effect would not be unexpected given the increased starter DM and starch intake for the WS fed calves. On d 63, in female calves, fecal pH was lower (P<0.05) for WS *vs*. CO; while no significant differences (P>0.10) were observed for “WS *vs*. ALF and WA” and for “CO *vs*. ALF and WA’ (Figure 5). On d 50, fecal pH was greater in female than in male calves (P<0.05, Table 4), suggesting greater hindgut fermentation in male than in female calves. This would be expected since male calves had greater DMI and starch intake than did female calves.

Structural body growth, blood metabolites, and feeding bBehavior

Withers height, heart girth, and hip width were not significantly different among treatments on d 28, 50, and 63 (P>0.10, Table 5). However, body depth on d 63 or at weaning was greater (P<0.05) for WS than for ALF and CO (Table 5). This data was in accordance with the fact that growth rate and BW gain were greater for WS. The structural body growth indices were all greater for male than for female calves (Table 5), which was consistent with the greater BW and BW gain of male *vs*. female calves.

Blood urea nitrogen concentrations at weaning (d 63) tended to be greater (P=0.08) for ALF than for WS. This result would suggest that calves on ALF *vs*. WS may have received greater rumen degradable protein, leading to increased rumen ammonia output and increased hepatic urea synthesis. The higher average dietary CP for alfalfa hay (ALF, 19%) *vs*. wheat straw (WS, 18.2%) containing starters diets would be consistent with this reasoning. However, other blood metabolites including glucose, BHBA, total protein, albumin, globulin on d 21 and 63 were not significantly different (P>0.10) among treatments (Table 6). These similarities would indicate that factors affecting the circulating concentrations of these metabolites were likely unaffected by treatments.

Eating, drinking, laying, standing, and ruminating times were not significantly different among treatments (Table 7). However, total chewing time (eating plus ruminating times) were numerically greater for WS than for CO. Non-nutritive oral behavior and its ratio to chewing time tended to be lower (P<0.10) for WS *vs*. CO (Table 7). These effects suggest improved calf welfare by feeding WS and not necessarily ALF.

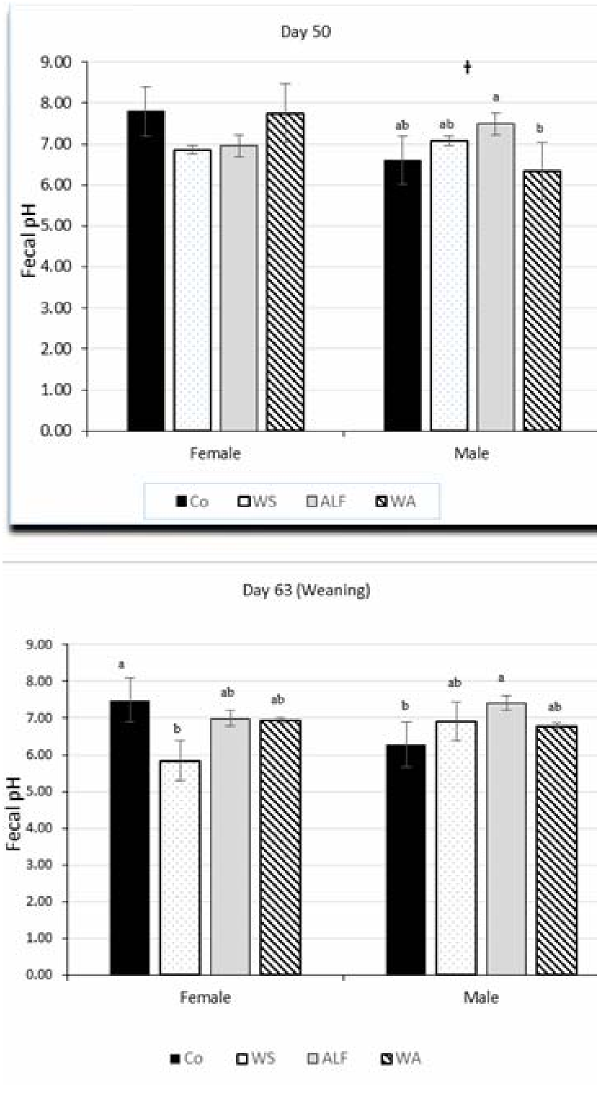


Figure 5 Fecal pH on d 50 and 63 in male and female calves on starter diets containing either no forage (CO), chopped wheat straw (WS), chopped alfalfa hay (ALF), or a 50:50 mixture of WS and ALF (WA)

The results of the present study suggest that WS was a more palatable diet than were ALF and CO in stimulating dry feed intake. In agreement with the current study, Bagheri *et al.* (2021) found that DMI and NDF intakes were greater in calves fed free-choice wheat straw than those fed no forage; although ADG, weaning and final BW, and feed efficiency were not different. In addition, comparing different forage sources in dairy calf diets showed increased starter intake in wheat straw-fed calves relative to either alfalfa- or sugar beet pulp-fed calves (Movahedi *et al.* 2016). More recently, Gasiorek *et al.* (2022) indicated that calves fed low (10% of diet DM) and moderate wheat straw (15% of diet DM) diets had greater DMI post-weaning in the entire experimental period which concur with the findings of the current study. In contrast, DMI in the pre-weaning period was greater for alfalfa hay-fed calves than in wheat straw-fed calves with no difference in the post-weaning period (Omidi-Mirzaei *et al.* 2018). In a meta-analysis conducted to evaluate the effects of forage provision on dairy calves' performance, forage inclusion in calf diets was concluded to potentially improve starter intake with alfalfa hay possessing greater effects on intake than other forage sources (Imani *et al.* 2017). The present study, however, supports wheat straw as an optimum forage for pre and post-weaned calves. Hence, it seems that optimum forage sources and levels in dairy calf diets depend on several other dietary properties such as physical form of forage and starter-starch sources. It has been demonstrated that pelleted starter has more benefits when it is offered with chopped *vs.* pelleted alfalfa hay. Different dietary conditions and environments may produce different results. Moreover, as the increased DMI for the WS diet was more pronounced during the weaning transition and post-weaning periods, rumen conditions were likely more stable for WS than for other treatments. The WS-fed calves had greater rumen pH than did the CO-fed calves, possibly contributing to the greater starter DMI for WS *vs.* CO. Rumen pH below 5.8 for at least 3 h per day is considered as sub-acute ruminal acidosis (SARA; Gozho *et al.* 2005). Reduced feed intake is known to be a physiological consequence of SARA (Krause and Oetzel, 2006). In pre-weaning calves, the occurrence of SARA and subsequent metabolic issues are much less described. For instance, decreased ruminal pH, but not feed intake and weight gain, was reported by Beharka *et al.* (1998) who compared finely ground and unground starter diets.

In another study (Quigley *et al.* 1992), no effects of alfalfa hay supplementation on the rumen pH of calves were found. However, more recent findings (Kim *et al.* 2016; Galsinger *et al.* 2020) suggest that SARA may have similar effects on pre-weaned calves as it does on dairy cows. Ruminal acidosis may occur during the weaning transition when the solid feed intake of calves increases (e.g., > 800 (g/d]) (Laarman and Oba, 2011). Our results support the notion that optimal forage provision during the pre-weaning period may moderate the rumen pH and enhance starter intake, especially for WS *vs.* ALF. The adverse effects of low rumen pH on dairy calf performance have been described (Khan *et al.* 2008).

The daily starter starch intake was not different for WA *vs.* other treatments (Table 3). As such, even the 50:50 mixture of WS and ALF was unable to increase starter starch intake over CO. The daily starter NDF and ADF intakes were greater for WS and WA than for CO ($P<0.05$). The starter NDF and ADF intakes were also greater for WS than for ALF ($P<0.05$, Table 3). Hence, it is evident that chopped wheat straw alone or in combination with chopped alfalfa hay was capable to increase cell wall fiber intake by young calves.

Table 5 Effects of feeding different forages in starter diet as total mixed ration (TMR) on structural growth of Holstein calves

Item	Treatment (Trt)[1]				Sex		P-value			
	CO	WS	ALF	WA	Male	Female	SE	Trt	Sex	Trt × Sex
Withers height (cm)										
28 d	87.5	86.4	87.2	87.2	88.3	86	1.15	0.82	<0.01	0.95
50 d	93.3	93.9	93.0	92.8	94.4	92.2	1.15	0.78	0.01	0.10
63 d	96.2	99.7	98.9	98.7	100.5	97.2	1.05	0.80	<0.01	0.58
Heart girth (cm)										
28 d	85.7	85.8	84.3	84.7	86.3	84	1.3	0.53	0.01	0.23
50 d	93.0	93.4	92.1	93.5	94.5	91.5	1.2	0.68	<0.01	0.20
63 d	99.9	103.2	100.1	101.0	103.0	99.1	1.2	0.23	<0.01	0.17
Body depth (cm)										
28 d	88.4	90.0	85.6	88.1	89.0	87.0	1.9	0.18	0.11	0.42
50 d	100.4	103.8	98.6	102.0	103.4	99.1	2.3	0.14	0.01	0.17
63 d	111.4[b]	119.5[a]	112.0[b]	116.0[ab]	118.0	111.3	2.7	0.01	<0.01	0.30
Hip width (cm)										
28 d	24.9	24.8	24.7	25.0	25.1	24.5	0.4	0.95	0.05	0.05
50 d	27.1	27.7	27.1	27.4	28.0	26.8	0.5	0.60	0.01	<0.01
63 d	29.4	30.6	29.3	30.0	30.6	29.1	0.6	0.16	0.01	0.39

[1] CO: starter diet without forage; WS: starter diet with chopped wheat straw; ALF: starter diet with chopped alfalfa hay and WA: starter diet with a 50:50 mixture of chopped wheat straw and chopped alfalfa hay.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

Table 6 Effects of feeding different forages in starter diet as total mixed ration (TMR) on blood metabolites of Holstein calves

Item	Treatment (Trt)[1]				P-value			
	CO	WS	ALF	WA	SE	Trt	Sex	Trt × Sex
Glucose 21 d (mg/dL)	82.5	82.4	86.7	85.6	4.35	0.70	0.14	0.32
Glucose 63 d (mg/dL)	74.0	75.8	78	72.7	6.58	0.89	0.60	0.54
BUN 21 d (mg/dL)	11.6	11.4	12	11.2	0.53	0.53	0.51	0.59
BUN 63 d (mg/dL)	11.4	10.8	13.2	11.6	0.78	0.08	1.0	0.2
BHBA 21 d (mmol/L)	0.22	0.18	0.23	0.22	0.05	0.76	0.29	0.96
BHBA 63 d (mmol/L)	0.28	0.32	0.4	0.34	0.08	0.60	0.25	0.75
Total protein 21 d (g/dL)	7.82	7.10	7.17	6.77	0.46	0.20	0.82	0.54
Total protein 63 d (g/dL)	7.26	7.18	7.27	7.22	0.28	0.99	0.08	0.23
Albumin 21 d (g/dL)	3.23	3.11	3.27	3.08	0.10	0.57	0.20	0.66
Albumin 63 d (g/dL)	3.55	3.42	3.46	3.35	0.13	0.50	0.08	0.79
Globulin 21 d (g/dL)	4.58	3.98	3.88	3.66	0.33	0.30	0.56	0.69
Globulin 63 d (g/dL)	3.70	3.78	3.81	3.87	0.18	0.91	0.28	0.27
Albumin:globulin 21 d	0.71	0.81	0.89	0.87	0.08	0.51	0.60	0.75
Albumin:globulin 63 d	0.96	0.90	0.92	0.88	0.05	0.63	0.88	0.40

[1] CO: starter diet without forage; WS: starter diet with chopped wheat straw; ALF: starter diet with chopped alfalfa hay and WA: starter diet with a 50:50 mixture of chopped wheat straw and chopped alfalfa hay.
BUN: blood urea nitrogen and BHBA: beta-hydroxybutyrate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

The daily starter NDF and ADF intakes did not differ between ALF and WA; however, daily starter ADF intake was greater for ALF than for CO (Table 3). The NDF and ADF intakes were not different for ALF vs. WA (P>0.10). Increased physically effective fiber intake can stimulate chewing and promote rumen development in pre-weaning calves (Diao *et al.* 2019). Accordingly, increased nutrient and particularly NDF and ADF intakes by WS may have positively affected the rumen development process in the current experiment. The rumen muscular development is likely stimulated by feeding forage to the pre-weaning calves (Beiranvand *et al.* 2014). Wheat straw provides a suitable physical effective NDF source for young calves that may stimulate rumination and contribute to the rumen development (NRC, 2001). In the present study, although ruminating time was not significantly different between the WS and CO fed calves, the WS calves ruminated twice as much as the CO calves (44.8 *vs.* 24.6 (min], respectively). This might have contributed to stimulating chewing activity, saliva production, rumen motility and greater passage rate that could have in turn led to stabilized rumen pH and increased DMI (Khan *et al.* 2016; Wang *et al.* 2022).

Table 7 Effects of feeding different forages in starter diet as total mixed ration (TMR) on feeding behavior of Holstein calves

Item	Treatment (Trt)[1]				P-value			
	CO	WS	ALF	WA	SE	Trt	Sex	Trt × Sex
Eating (min)	150.5	163.0	138.9	145.3	14.8	0.46	0.53	0.40
Drinking (min)	15.3	16.7	15.4	12.9	5.42	0.92	0.69	0.88
Laying (min)	151.6	165.1	161.5	185.4	17.3	0.28	0.96	0.43
Standing without rumination (min)	80.1	65.9	76.9	61.6	12.7	0.44	0.66	0.92
Ruminating (resting+standing) (min)	24.6	44.8	49.1	44.6	11.7	0.17	0.12	0.96
Non-nutritive oral behavior[2] (min)	59.0	20.8	33.8	26.3	10.7	0.09	0.48	0.62
NNOB to chewing activity ratio (%)	38.3	10.0	17.0	14.0	7.0	0.07	0.58	0.92

[1] CO: starter diet without forage; WS: starter diet with chopped wheat straw; ALF: starter diet with chopped alfalfa hay and WA: starter diet with a 50:50 mixture of chopped wheat straw and chopped alfalfa hay.
[2] Licking the pen and tongue rolling were considered as non-nutritive oral behavior (NNOB).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

As such, replacing highly digestible NDF from beet pulp by alfalfa hay with more effective NDF increased rumen pH and chewing activity in dairy calves (Maktabi *et al.* 2016). It has been demonstrated that calves fed a starter diet with straw had elevated rumen pH post-weaning and greater starter intake and BW than did those fed either texturized or pelleted starter without forage (Terré *et al.* 2015), which was in agreement with the present results. More recently, it was found that offering free-choice wheat straw regardless of its particle size (1 or 7 mm) has potential to improve rumen health and calf well-being (Bagheri *et al.* 2021). In agreement with the present study, Hosseini *et al.* (2019) reported that low-quality straw could improve DMI and BW gain of Holstein calves in the post-weaning period. In contrast, high quality alfalfa-fed calves were more efficient that WS-fed calves in the pre-weaning period (Hosseini *et al.* 2019). In another study (Omidi-Mirzaei *et al.* 2018), investigating forage type effects on intake, rumen environment, and growth of dairy calves; alfalfa fed calves had greater DMI, ME intake, weaning BW, and forage intake than WS fed calves in the pre-weaning but not in the post-weaning period. The inconsistent literature results may be attributed to the different milk feeding method, starter diet form, starter and forage chemical composition, forage particle size, and feeding strategy (mixed *vs.* free-choice) among studies. The current results further demonstrated that inclusion of forage in relatively coarse starters could improve calf performance which was in line with Ghaffari and Kertz (2021) reporting that favorable results are obtained when forage is included in finely ground or texturized starters. Positive effects of feeding free-choice chopped barley straw on feed intake and performance of pelleted-starter fed calves has been reported (Castells *et al.* 2012; Gasiorek *et al.* 2022). Including non-forage fiber sources such as beet pulp (Porter *et al.* 2007) and cottonseed hulls (Hill *et al.* 2008) in pelleted and texturized starters did not influence ADG of calves, but slightly improved feed intake in one study (Hill *et al.* 2008).

Such results may provide an insight that young calves require physically effective fiber as do dairy cows. In the current study, the performance data suggest that wheat straw may be a suitable forage choice when ground starters are fed to pre-weaning calves. This suitability may be attributed to the more indigestible and physically effective NDF of wheat straw vs. alfalfa hay.

Including wheat straw in the calf starter has been reported to be more effective when it is supplemented after 21 d of age (Ghahremani *et al.* 2021). In addition, wheat straw-fed calves had greater solid feed intake in either pre- or post-weaning periods with the greatest body weight at weaning for calves receiving wheat straw after 21 d of age. In contrast, post-weaning performance was not affected (Ghahremani *et al.* 2021). It seems that the effects of forage inclusion on calf performance becomes more profound as calves age and starter intake increases. In another recent study investigating calf performance under different levels of wheat straw (0, 10, 15, and 20% of diet DM), greater post-weaning ADG was found for 10% and 15% wheat straw inclusion rates (Gasiorek *et al.* 2022). Also reported that ADG was similar between calves fed wheat straw and alfalfa hay containing diets, which was likely due to similar starter intake.

However, calves fed wheat straw accompanied by higher dietary rumen undegradable protein (RUP): rumen degradable protein (RDP) ratio had the greatest final body weight. The greater weight gain observed in the current study and other reports may be explained by the role of forages in stabilizing the rumen conditions and probably greater feed intake. The present results are in contrast with the findings of Bagheri *et al.* (2021) who found no differences among wheat straw fed calves *vs.* control calves with no wheat straw supplementation. The different experimental conditions might justify the different conclusions.

Male calves had greater nutrient intake, water intake, ADG, BW, total BW gain (pre- + post-weaning), and total starter intake than did female calves.

These data emphasize the greater physiological capacity of male than of female calves in nutrient intake and assimilation as well as growth indices.

This finding suggests that chopped wheat straw alone or in combination with alfalfa hay was able to effectively neutralize the rumen conditions. The positive impact of forage feeding on calf rumen pH has been shown (Nemati *et al.* 2016). Higher average daily ruminal pH was reported for hay-fed calves in comparison with control calves without forage supplementation (Takemura *et al.* 2019). Additionally, control calves had longer duration of rumen pH below 5.6 threshold (Takemura *et al.* 2019). The rumen pH was not different between male and female calves (P>0.10; Table 4). This suggests that factors influencing the rumen pH were not of different magnitude in male *vs.* female calves. As noted above, the WS calves had greater NDF intake than the CO calves. Given the greater content of indigestible NDF in WS relative to CO and ALF diets, it can be postulated that pre-weaned calves especially those in the weaning transition period need more effective NDF to maintain normal rumen pH when the starter contains greater starch. In a study comparing similar proportion of undigested NDF (uNDF) from different forage sources, substituting alfalfa hay with wheat straw in high producing dairy cows resulted in greater ruminal pH (Kahyani *et al.* 2019). More recently, increasing dietary uNDF has been reported to increase rumen pH in beef heifers fed high grain diet (Ran *et al.* 2021). In light of these results and the findings of the current study, it seems that the indigestible NDF content of calf starter diets should be considered, especially when feeding high proportion of grains.

These data indicate that calf sex and age can mediate the possible effects of dietary forage and its type on rumen dynamics, hindgut fermentation, and fecal pH (Neubauer *et al.* 2020). High starch diets could induce ruminal acidosis and hindgut disbiosis particularly in the 3rd lactation dairy cows because of their high DMI (Neubauer *et al.* 2020). As such, in the current study, the higher DMI and starch intake of the WS fed calves may have increased the hindgut starch fermentation and lowered the fecal pH. Infusion of fructans into the abomasum of steers caused watery feces with reduced fecal pH, but did not result in inflammatory responses (Gresley *et al.* 2011). It seems that hindgut acidosis with negative effects on inflammatory status may occur in high producing dairy cows that consume high levels of fermentable carbohydrates (Gresley *et al.* 2011). However, more recently, Abeyta *et al.* (2022) demonstrated that despite decreased fecal pH, the hindgut acidosis did not affect inflammatory biomarkers in feed-restricted dairy cows. The lack of stacked stressors was recommended to describe why the cows did not show inflammatory responses. Since the ruminal acidosis' outcomes in dairy calves may be similar to those in adult cows (Galsinger *et al.* 2020), according to the results of Abeyta *et al.* (2022), it may be assumed that the lack of adverse effects in the WS-fed calves in the present study could be attributed to the minimal stress.

In addition, the increased chewing time by feeding WS *vs.* ALF would support the hypothesis that chopped wheat straw is more effective than chopped alfalfa hay in stimulating fiber intake and thus eating and ruminating activities. This data is consistent with the intake and rumen pH data. Several previous studies (Castells *et al.* 2012; Terré *et al.* 2013; EbnAli *et al.* 2016; Mirzaei *et al.* 2017) have reported a positive relationship between forage intake and chewing activity in dairy calves. The decreased non-nutritive oral behavior observed for WS *vs.* CO calves in the present study was in agreement with other studies (Thomas and Hinks, 1982; Horvath and Miller-Cushon, 2019), showing improved welfare in the WS fed calves.

CONCLUSION

Findings lend support to the essentiality of forage for young Holstein calves. Under the circumstances of the present study, chopped wheat straw was superior over chopped alfalfa hay towards improved calf performance. Increased starter intake or increased intakes of starch, cell wall fibers, protein, and energy, enhanced rumen pH and development, and reduced non-nutritive oral behavior in the WS fed calves should have contributed to their increased body depth and the remarkably improved BW gain. Although all forage containing diets were effective in stimulating more water intake, calf growth and BW gain were greater for WS than for ALF and CO, mainly because of the significantly increased starter intake for WS. The mixture of WS and ALF (i.e., WA) did not offer any metabolic or performance advantages over WS alone. Therefore, based on the results of the present research and under such particular circumstances, chopped wheat straw may be an optimal forage choice for young Holstein calves, especially when compared to chopped alfalfa hay.

ACKNOWLEDGEMENT

Profound thanks to the National Elites Foundation of Iran.

REFERENCES

Abeyta M.A., Horst E.A., Goetz B.M., Mayorga E.J., Rodriguez-Jimenez S., Caratzu M. and Baumgard L.H. (2022). Effects of hindgut acidosis on production, metabolism, and inflammatory biomarkers in feed-restricted lactating dairy cows. *J. Dairy Science.* **106,** 2890-2903.

Bagheri N., Alamouti A.A., Norouzian M.A., Mirzaei M. and Ghaffari M.H. (2021). Effects of wheat straw particle size as a

free-choice provision on growth performance and feeding behaviors of dairy calves. *Animal.* **15,** 1-8.
Beharka A.A., Nagaraja T.G., Morrill J.L., Kennedy G.A. and Klemm R.D. (1998). Effects of form of the diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. *J. Dairy Sci* **88,** 1946-1955.
Beiranvand H., Ghorbani G.R., Khorvash M., Nabipour A., Dehghan-Banadaky M., Homayouni A. and Kargar S. (2014). Interaction of alfalfa hay and sodium propionate on dairy calf performance and rumen development. *J. Dairy Sci*. **97,** 2270-2280.
Cantor M.C., Neave H.W. and Costa J.H. (2019). Current perspectives on the short-and long-term effects of conventional dairy calf raising systems: a comparison with the natural environment. *Trans. Anim. Sci.* **3,** 549-563.
Castells L., Bach A. and Terré M. (2015). Short-and long-term effects of forage supplementation of calves during the preweaning period on performance, reproduction, and milk yield at first lactation. *J. Dairy Sci*. **98,** 4748-4753.
Castells L., Bach A., Araujo G., Montoro C. and Terré M. (2012). Effect of different forage sources on performance and feeding behavior of Holstein calves. *J. Dairy Sci*. **95(1),** 286-293.
Daneshvar D., Khorvash M., Ghasemi E., Mahdavi A.H., Moshiri B., Mirzaei M., Pezeshki A. and Ghaffari M.H. (2015). The effect of restricted milk feeding through conventional or step-down methods with or without forage provision in starter feed on performance of Holstein bull calves. *J. Anim. Sci.* **93,** 3979-3989.
Diao Q., Zhang R. and Fu T. (2019). Review of strategies to promote rumen development in calves. *Animals*. **9,** 490-497.
EbnAli A., Khorvas M., Ghorbani G.R., Mahdavi A.H., Malekkhahi M., Mirzaei M., Pezeshki A. and Ghaffari M.H. (2016). Effects of forage offering method on performance, rumen fermentation, nutrient digestibility and nutritional behaviour in Holstein dairy calves. *J Anim. Physiol. Anim. Nutr.* **100,** 820-827.
Galsinger S.L., Coblentz W.K., Zanton G.I., Ogden R.K. and Akins M.S. (2020). Physiological effects of starter-induced ruminal acidosis in calves before, during, and after weaning. *J. Dairy Sci.* **103,** 2762-2772.
Gasiorek M., Stefanska B., Pruszynska-Oszmatek E., Komisarek J. and Nowak W. (2022). Effects of the straw inclusion in the diet of dairy calves on growth performance, rumen fermentation, and blood metabolites during pre-and post-weaning periods. *J. Anim. Physiol. Anim. Nutr.* **106,** 33-44.
Ghaffari M.H. and Kertz A.F. (2021). Effects of different forms of calf starters on feed intake and growth rate: A systematic review and Bayesian meta-analysis of studies from 1938 to 2021. *Appl. Anim. Sci*. **37,** 273-293.
Ghahremani A., Mahjoubi E., Hossein-Yazdi M., Chamani M., Bahrami M. and Patton R.A. (2021). Forage inclusion in calf starter has the best outcome when it is supplemented since 21 days after birth in Holstein calves. *Trop. Anim. Health Prod.* **53,** 203-210.
Gozho G.N., Plaizier J.C., Krause D.O., Kennedy A.D. and Wittenberg K.M. (2005). Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J. Dairy Sci.* **88,** 1399-1403.
Gresley T.F., Hall M.B. and Armentano L.E. (2011). Ruminant nutrition symposium: Productivity, digestion, and health responses to hindgut acidosis in ruminants. *J. Anim. Sci.* **89,** 1120-1130.
Heinrichs A.J. and Heinrichs B.S. (2011). A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* **94,** 336-341.
Heinrichs A.J. and Lesmeister K.E. (2005). Rumen development in the dairy calf. Calf and heifer rearing: Principles of rearing the modern dairy heifer from calf to calving. University of Nottingham Easter School in Agricultural Science, Nottingham, United Kingdom.
Hill T.M., Bateman II H.G., Aldrich J.M. and Schlotterbeck R.L. (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.
Hill T.M., Bateman II H.G., Aldrich J.M. and Schlotterbeck R.L. (2009). Roughage for diets fed to weaned dairy calves. *Prof. Anim. Sci.* **25,** 283-288.
Horvath K.C. and Miller-Cushon E.K. (2019). Evaluating effects of providing hay on behavioral development and performance of group-housed dairy calves. *J. Dairy Sci*. **102,** 10411-10422.
Hosseini S.H., Mirzaei-Alamouti H., Vazirigohar Mahjoubi E. and Rezamand P. (2019). Effects of whole milk feeding rate and straw level of starter feed on performance, rumen fermentation, blood metabolites, structural growth, and feeding behavior of Holstein calves. *Anim. Feed Sci. Technol.* **255,** 1-11.
Imani M., Mirzaei M., Baghbanzadeh-Nobari B. and Ghaffari M.H. (2017). Effects of forage provision to dairy calves on growth performance and rumen fermentation: A meta-analysis and meta-regression. *J. Dairy Sci.* **100,** 1136-1150.
Kahyani A., Ghorbani G.R., Alikhani M., Ghasemi E., Adeghi-Sefidmazdi A., Beauchemin K.A. and Nasrollahi S.M. (2019). Performance of dairy cows fed diets with similar proportion of undigested neutral detergent fiber with wheat straw substituted fo alfalfa hay, corn silage, or both. *J. Dairy Sci*. **102,** 10903-10915.
Keogh K., Kelly A.K. and Kenny D.A. (2021). Effect of plane of nutrition in early life on the transcriptome of visceral adipose tissue in Angus heifer calves. *Sci. Rep.* **11,** 1-12.
Keshavarz V., Dehghan-banadaky M., Ganjkhanlou M. and Kazemi-Bonchenari M. (2023). Effects of feeding wheat straw or beet pulp in starters supplemented with either soybean oil or palm fatty acids on growth performance and urinary purine derivatives in dairy calves. *Anim. Feed Sci. Technol.* **297,** 11569-11575.
Khan M.A., Weary D.M. and Von Keyserlingk M.A.G. (2011). Hay intake improves performance and rumen development of calves fed higher quantities of milk. *J. Dairy Sci.* **94,** 3547-3553.
Khan M.A., Bach A., Weary D.M. and Von Keyserlingk M.A.G. (2016). Invited review: Transitioning from milk to solid feed in dairy heifers. *J. Dairy Sci.* **99,** 885-902.
Khan M.A., Lee H.J., Lee W.S., Kim H.S., Ki K.S., Hur T.Y., Suh G.H., Kang S.J. and Choi Y.J. (2007). Structural growth, ru-

men development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. *J. Dairy Sci.* **90,** 3376-3387.

Khan M.A., Lee H.J., Lee W.S., Kim H.S., Kim S.B., Park S.B., Baek K.S., Ha J.K. and Choi Y.J. (2008). Starch source evaluation in calf starter: II. Ruminal parameters, rumen development, nutrient digestibilities, and nitrogen utilization in Holstein calves. *J. Dairy Sci.* **91,** 3547-3553.

Kim Y.H., Nagata R., Ohtani N., Ichijo T., Ikuta K. and Sato S. (2016). Effects of dietary forage and calf starter diet on ruminal pH and bacteria in Holstein calves during weaning transition. *Front. Microbiol.* **7,** 1575-1582.

Klopp R.N., Oconitrillo M.J., Sackett A., Hill T.M., Schlotterbeck R.L. and Lascano G.J. (2018). Technical note: A simple rumen collection device for calves: An adaptation of a manual rumen drenching system. *J. Dairy Sci.* **101,** 6155-6158.

Kononoff P.J., Heinrichs A.J. and Buckmaster D.R. (2003). Modification of Penn State forage and total mixed ration particle separator and the effects on moisture content on its measurements. *J. Dairy Sci.* **86,** 1858-1863.

Krause K.M. and Oetzel G.R. (2006). Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim Feed Sci. Technol*. **126(3),** 215-236.

Laarman A.H. and Oba M. (2011). Effect of calf starter on rumen pH of Holstein dairy calves at weaning. *J. Dairy Sci.* **94(11),** 5661-5664.

Larson L.L., Owen F.G., Albright J.L., Appleman R.D., Lamb R.C. and Muller L.D. (1977). Guidelines toward more uniformity in measuring and reporting calf experimental data. *J. Dairy Sci.* **60(6),** 989-991.

Maktabi H., Ghasemi E. and Khorvash M. (2016). Effects of substituting grain with forage or nonforage fiber source on growth performance, rumen fermentation, and chewing activity of dairy calves. *Anim. Feed Sci. Technol.* **221,** 70-78.

Meale S.J., Popova M., Saro C., Martin C., Bernard A., Lagree M., Yáñez-Ruiz D.R., Boudra H., Duval S. and Morgavi D.P. (2021). Early life dietary intervention in dairy calves results in a long-term reduction in methane emissions. *Scient. Rep.* **11,** 1-13.

Mirzaei M., Khorvash M., Ghorbani G.R., Kazemi-Bonchenari M. and Ghaffari M.H. (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.

Mirzaei M., Khorvash M., Ghorbani G.R., Kazemi Bonchenari M., Riasi A., Nabipour A., Van Den V. and Borne J.J.G.C. (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.

Movahedi B., Foroozandeh A.D. and Shakeri P. (2016). Effect 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.

National Academies of Sciences, Engineering, and Medicine (NASEM). (2021). Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. The National Academies Press, Washington, DC.

Nemati M., Amanlou H., Khorvash M., Mirzaei M., Moshiri B. and Ghaffari M.H. (2016). Effect of different alfalfa hay levels on growth performance, rumen fermentation, and structural growth of Holstein dairy calves. *J. Anim. Sci.* **94,** 1141-1148.

Neubauer V., Petri R.M., Humer E., Kröger I., Reisinger N., Baumgartner W., Wagner M. and Zebeli Q. (2020). Starch-rich diet induced rumen acidosis and hindgut dysbiosis in dairy cows of different lactations. *Animals.* **10,** 1727-1736.

Nikkhah A. and Alimirzaei M. (2021). Timing of transfer from individual boxes to group pens in newly weaned Holstein calves: Matters? *Arch. Anim. Sci.* **1,** 101-104.

Nikkhah A. and Alimirzaei M. (2022a). On pre-weaning calf weight gain differences: Opportunities to improve herd productivity, health and longevity. *Biomed. J. Sci. Tech. Res.* **42,** 33420-33425.

Nikkhah A. and Alimirzaei M. (2022b). Boosting concurrent intakes of milk, solid starter and water: The ultimate preweaning calf management success triangle. *J. Clin. Res. Rep.* **11(3),** 1-3.

Nikkhah A. and Alimirzaei M. (2022c). Forage for pre-weaning calves: An update. *World's Vet. J.* **12,** 123-127.

NRC (2001). Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academies Press, Washington, DC.

Omidi-Mirzaei H., Azarfar A., Mirzaei M., Kiani A. and Ghaffari M.H. (2018). Effects of forage source and forage particle size as a free-choice provision on growth performance, rumen fermentation, and behavior of dairy calves fed texturized starters. *J. Dairy Sci.* **101,** 4143-4157.

Pazoki A., Ghorbani G.R., Kargar S., Sadeghi-Sefidmazgi A., Drackley J.K. and Ghaffari M.H. (2017). Growth performance, nutrient digestibility, ruminal fermentation, and rumen development of calves during transition from liquid to solid feed: Effects of physical form of starter feed and forage provision. *Anim. Feed Sci. Technol.* **234,** 173-185.

Poier G., Terler G., Klevenhusen F., Sharma S. and Zebeli Q. (2022). Replacing concentrates with a high quality hay in the starter feed of dairy calves: II. Effects on the development of chewing and gut fermentation, and selected systemic health variables. *J. Dairy Sci.* **105,** 3113-3128.

Porter J.C., Warner R.G. and Pas A.F.K. (2007). Effects of fiber level and physical form of starter on growth and development of dairy calves fed no forage. *Prof. Anim. Sci*. **23,** 395-400.

Quigley III J.D., Steen T.M. and Boehms S.I. (1992). Postprandial changes of selected blood and ruminal metabolites in ruminating calves fed diets with or without hay. *J. Dairy Sci.* **75,** 228-235.

Ran T., Saleem A.M., Beauchemin K.A., Penner G.B. and Yang W. (2021). Processing index of barley grain and dietary undigested neutral detergent fiber concentration affected chewing behavior, ruminal pH, and total tract nutrient digestibility of heifers fed high grain diet. *J. Anim. Sci.* **99,** 1-8.

SAS Institute. (2003). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Takemura K., Shingu H., Mizuguchi H., Kim Y.H., Sato S. and Kushibiki S. (2019). Effects of forage feeding on rumen fermentation, plasma metabolites, and hormones in Holstein calves during pre-and post-weaning periods. *J. Anim Sci*. **97,** 2220-2229.

Terler G., Poier G., Klevenhusen F. and Zebeli Q. (2022). Replacing concentrates with a high-quality hay in the starter feed in dairy calves: I. Effects on nutrient intake, growth performance, and blood metabolic profile. *J. Dairy Sci.* **105,** 2326-2342.

Terler G., Velik M., Poire G., Sener-Aydemir A., Klevenhusen F. and Zebeli Q. (2023). Feeding concentrate with hay of different qualities modulates rumen histology, development of digestive tract as well as slaghter performance and meat quality of young dairy calves. *Arch. Anim. Nutr.* **77,** 171-186.

Terré M., Castells L., Khan M.A. and Bach A. (2015). Interaction between the physical form of the starter feed and straw provision on growth performance of Holstein calves. *J. Dairy Sci.* **98,** 1101-1109.

Terré M., Pedrals E., Dalmau A. and Bach A. (2013). What do preweaned and weaned calves need in the diet: A high fiber content or a forage source? *J. Dairy Sci*. **96,** 5217-5225.

Thomas D.B. and Hinks C.E. (1982). The effect of changing the physical form of roughage on the performance of the early-weaned calf. *J. Anim. Sci.* **35,** 375-384.

Wang Z. and Goonewardene L.A. (2004). The use of MIXED models in the analysis of animal experiments with repeated measures data. *Canadian J. Anim. Sci.* **84,** 1-11.

Wang S., Diao Q.Y., Hu F.M., Bi Y.L., Piao M.Y., Jiang L.S., Sun F., Li H. and Tu Y. (2022). Development of ruminating behavior in Holstein calves between birth and 30 days of age. *J. Dairy Sci.* **105,** 572-584.

Warner R.G., Flatt W.P. and Loosli J.K. (1956). Ruminant nutrition, dietary factors influencing development of ruminant stomach. *J. Agric. Food Chem*. **4,** 788-792.

Wickramasinghe H.K.J.P., Kramer A.J. and Appuhamy J.A.D.R.N. (2019). Drinking water intake of newborn dairy calves and its effects on feed intake, growth performance, health status, and nutrient digestibility. *J. Dairy Sci.* **102,** 377-387.

Wu Z.H., Azarfar A., Simayi A., Li S.L., Jonker A. and Cao Z.J. (2018). Effects of forage type and age at which forage provision is started on growth performance, rumen fermentation, blood metabolites and intestinal enzymes in Holstein calves. *Anim. Prod. Sci.* **58,** 2288-2299.

Xiao J., Alugongo G.M., Li J., Wang Y., Li S. and Cao Z. (2020). How forage feeding early in life influences the growth rate, ruminal environment, and the establishment of feeding behavior in pre-weaned calves. *Animals.* **10,** 188-197.