Effects of forage source on chewing and rumen fermentation in lactating dairy cows

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SUMMARY

The objective of this experiment was to evaluate the effects of partial replacement of corn silage with long alfalfa hay and/or coarse chopped wheat straw on chewing activity and rumen fermentation in late lactating dairy cows fed diets with 50 % forage on dry matter basis. Twelve late lactating Holstein primiparous cows including four cows equipped with a rumen cannula, averaging 210 ± 20 d in milk and weighing 575 ± 50 kg were randomly assigned in a 4x4 Latin square design. During each of four 21-d periods, cows were fed 4 total mixed diets that varied in the forage sources : 1) CS (50 % corn silage), 2) CSW (35 % corn silage + 15 % wheat straw), 3) CSA (35 % corn silage + 15 % alfalfa hay), 4) CSWA (25 % corn silage + 10 % wheat straw + 15 % alfalfa hay). The physically effective neutral detergent fiber (peNDF) contents of the diets which were determined by using the Penn State Particle Separator were as 33.2, 36.3, 33.6, and 35.3 % of DM for CS, CSW, CSA, and CSWA diets, respectively. The proportion of particles retained on the 19.0 mm screen of the CS was lower (P < 0.001) than in the others group (3, 14, 12, and 24 % of DM, for CS, CSW, CSA, and CSWA diets, respectively). Dry matter intake was not affected by the diets but peNDF intakes were lower (P < 0.001) with CS and CSA than other diets. Cows spent more time eating the CSW, CSA and CSWA diets compared with CS diet (P < 0.001). Cows fed with CSW spent more time ruminating than cows fed CS diet (P < 0.05). Total chewing time was higher (P < 0.01) in CSW (662 min/d) and CSWA (633 min/d) groups than CS (521 min/d). Mean rumen pH values, ammonia content (NH_3-N), acetate/propionate ratio, and total concentration of volatile fatty acids in the rumen fluid were not affected by the treatments. However, the time (h/d) where the pH was under 5.8 value, was increased when cows fed the CS diet (P < 0.001). It is concluded that forage source may have an effect on the chewing activity and rumen pH related to the peNDF and fiber structure. The proportion of particles > 19.0 mm and peNDF may be used as predictor of chewing activity.

Keywords : forage sources, particle size, physically effective NDF, chewing activity, rumen fermentation.

RÉSUMÉ

Effets de la source de fourrage sur la mastication et les fermentations ruminales chez des vaches en lactation.

Le but de ce travail est d'évaluer l'effet d'un remplacement partiel de l'ensilage de maïs contenu dans la ration par de la luzerne et/ou de la paille de blé sur l'activité de mastication et les fermentations ruminales chez des vaches en fin de lactation recevant une ration comportant 50 % de fourrage. Douze vaches de race Holstein dont quatre munies de canules ruminales, qui sont au 210 ± 20^{e} jour de lactation, et pesant 575 ± 50 kg ont été soumises à un protocole en carré latin 4x4. Au cours d'une période de 21 jours, les animaux ont été nourris avec quatre régimes différents du point de vue de la source de fourrage : 1) CS (50 % d'ensilage de maïs), 2) CSW (35 % d'ensilage de maïs et 15 % de paille de blé), 3) CSA (35 % d'ensilage de maïs et 15 % de luzerne) et 4) CSWA (25 % d'ensilage de maïs, 10 % de paille de blé et 15 % de luzerne). L'autre moitié du régime est composé d'un concentré identique pour les quatre régimes. Dans la ration, le pourcentage de peNDF (physically effective neutral detergent fiber) a été obtenu au moyen d'un séparateur de particules de type Penn State Particle Seperator, et était de 33,2, 36,3, 33,6, et 35,3 % (par rapport à la matière sèche) respectivement pour les régimes CS, CSW, CSA, et CSWA. La proportion de particules qui ont une taille inférieure à 19 mm était plus faible (P < 0.001) pour le groupe CS par rapport aux autres groupes (3, 14, 12, et 24 % MS pour les groupes CS, CSW, CSA, et CSWA, respectivement). Le type de régime n'a pas eu d'influence sur la digestibilité de la matière sèche de la ration. Néanmoins, la digestibilité de peNDF a été plus basse dans les groupes CS et CSA que dans les autres groupes (P < 0.001). En comparaison avec le régime CS, les animaux des groupes CSW, CSA et CSWA passaient plus de temps à manger leurs repas (P < 0.001). Le temps de rumination du groupe CSW était plus long par rapport au groupe CS (P < 0.05). La durée totale de mastication était plus longue pour les groupes CSW (662 min/j) et CSWA (633 min/j) par rapport au groupe CS (521 min/j) (P < 0.01). Le pH, la teneur en ammoniac (N-NH_3), le rapport acétate/propionate, la concentration totale en acides gras volatiles dans le rumen et la quantité de matières sèches n'ont pas été affectés par les différents traitements. Cependant, la durée pendant laquelle le pH est inférieur à 5.8 durant la journée, a augmenté quand les animaux ont reçu le régime CS (P < 0.001). Il a été conclu que la source de fourrage peut avoir un effet sur l'activité de mastication et le pH du rumen. Ceci peut être lié à la valeur peNDF et à la structure des fibres de la ration. La proportion de particules supérieures à 19 mm et la valeur peNDF peuvent être utilisées comme valeurs prédictives de l'activité de mastication.

Mots-clés : sources de fourrage, taille de particule, peNDF, mastication, fermentations ruminales.

Introduction

Ruminants require fiber in coarse physical form [18] for a more effective chewing and ruminal activity. Increasing fiber content and forage particle size in diet effectively increases chewing activity resulting in increased saliva flow, rumen pH, acetate-to-propionate ratio, and milk fat levels [6, 17]. Increasing chewing activity and salivary buffer production are believed to be indicators of improving the dietary effect on rumen health and function [25].

Corn silage is one the most popular forages fed to dairy cows because it has good agronomic characteristics, yields high concentrations of nutrients, ensiles well, and incorporates easily into total mixed ration (TMR) [15]. Most commercial dairy rations contain high levels of concentrate and high quality corn silages that are often finely chopped. These types of diets are highly fermentable in the rumen and encourage maximum milk production ; however, they can lead to a variety of metabolic disorders, including subclinical ruminal acidosis, reduced fiber digestion, milk fat depression, displaced abomasum, lameness, and fat cow syndrome [18]. These negative effects may be decreased using the coarse forage sources such as alfalfa hay and wheat straw. Coarse forage sources have an important effect by contributing to the physical form of the ration [16]. The concept of physically effective NDF (peNDF) was introduced [14] to relate the physical characteristics of feeds to rumen pH by assessing the effects of feed particle size on chewing activity.

Alfalfa forage, conserved as silage or hay, is a major component of diets fed to lactating dairy cows due to its palatable taste, high dry matter (DM) yield and crude protein (CP) content. Similarly, wheat straw is available in large amounts in many regions of world and is commonly used for lactating cows as a balancing matter because of high fiber and volume, and low contain of nutrients.

The aim of this experiment was to investigate the effects of partial replacement of corn silage with long alfalfa hay and/or coarse chopped wheat straw on dry matter intake (DMI), intake of peNDF, chewing activity and rumen fermentation in late lactating Holstein primiparous dairy cows.

Materials and Methods

FORAGES

Corn silage was obtained from Uludag University, Faculty of Veterinary Medicine farm. Whole plant corn (hybrid C955, Monsanto Company, St. Louis, MO, USA) was harvested at about 26.5 % DM using a self-propelled forage harvester without kernel processing (Tosun Tarim, Izmir, Turkey) set to obtain a 10.0 mm theoretical cut length (TCL). The chopped forage was placed in a horizontal silo (300 ton capacity), covered with nylon plastic, and ensiled for approximately 3 months. Alfalfa was harvested during second cutting in a middle flowering stage and preserved as alfalfa hay in small rectangular bales of 20 kg. The hay was stored in barns. Wheat straw was chopped using a miller rotary hay mill (Model No : S8002, Tosun Tarim) equipped with a 5-cm screen.

COWS AND DIETS

Twelve lactating Holstein primiparous cows averaging 210 ± 20 d in milk and weighing 575 ± 50 kg were randomly assigned in a 4 ∞ 4 Latin square design. One of the three cows in each group was cannulated ruminally with soft plastic cannulas of 10 cm internal diameter (Ankom, pliable rumen cannula # 29, 4 inches, NY, USA). Cows were housed in individual tie stalls and all diets were formulated for a 600 kg cow producing 20 kg/d of milk with 3.6 % fat and 3.0 % true protein by using the NRC guidelines [18]. Throughout the experiment, cows were fed twice daily (0900 and 2100 h) at 110 % of expected intake with TMR that was mixed daily by hand. Each period consisted of 14 d of adaptation to diets and 7 d of experimental measurements. Cows were fed diets with 50 : 50 forage to concentrate ratio (DM basis). During each period, animals were offered one of the four diets that varied in the forage source, proportion and particle size (Table I) : 1) 50 % corn silage (CS), 2) 35 % corn silage + 15 % wheat straw (CSW), 3) 35 % corn silage + 15 % alfalfa hay (CSA) and 4) 25 % corn silage + 10 % wheat straw + 15 % alfalfa hay (CSWA).

Feeds offered and orts were measured and recorded daily during the last 7 d of each period to calculate feed intake. The TMR samples were collected once weekly for particle distribution analysis. One kg of each TMR samples (CS, CSW, CSA, and CSWA) was obtained weekly. Particle size distributions of TMR samples were determined using the Penn State Particle Separator (PSPS) containing 3 sieves (19, 8 and 1.18 mm) and a pan (Table II) as described by KONONOFF et al. [11]. The TMR samples were dried in a forced-air oven at 55°C for 48 h for chemical analyses. The peNDF is defined as that dietary fiber source which effectively stimulates rumination and salivation. The peNDF is an estimate of physically effective fiber and is calculated by multiplying the proportion of feed greater than 1.18 mm in length by total ration NDF [14].

CHEWING ACTIVITIES

Eating and ruminating behaviors were monitored visually for a 24-h period in twelve cows during the days of ruminal pH monitoring. Eating and ruminating activities were noted every 5 min, and each activity was assumed to persist for the entire 5-min. To estimate the time spent eating, ruminating and total chewing per kilogram of DMI, NDF and peNDF, the actual intake for that day was used. A period of rumination was defined as at least 5 min of rumination occurring after at least 5 min without rumination activity. Total chewing (TC) time was determined as the sum of total eating and ruminating times. The number of chews per day was calculated (Eating chews [number day] = -5854 + 84.75 ∞ eating time [minutes per day] ; ruminating chews [number day] = -81 + 71.29 ∞ ruminating time [minutes per day] ; total chews [number day] = -12390 + 80.59 ∞ total chewing time [minutes per day]) as described by ALLEN [1].

RUMINAL PH AND VFA CONCENTRATIONS

During each period, ruminal pH was measured from 0900 (d 19) to 0800 (d 20) with 1 h intervals in rumen cannulated cows. Ruminal fluid was obtained as grab samples from ventral of the rumen and then was immediately measured pH using the pH meter (Inolab pH, serial no : 00200018, pH-Electrode SenTix 41, D-82362, Weiheim, Germany). The ruminal pH 5.8 was chosen as bench mark to determine incidence of sub-clinical rumen acidosis [7]. If the pH was lower than 5.8 between two consecutive measurement intervals, the one hour period in between was recorded as an hour value.

Item	CS[1]	CSW[2]	CSA[3]	CSWA[4]
Ingredients, % of Dry Matter				
Corn silage[5]	50.00	35.00	35.00	25.00
Wheat straw[6]	0.00	15.00	0.00	10.00
Alfalfa hay[7]	0.00	0.00	15.00	15.00
Barley grain ground	10.81	13.74	14.39	13.19
Wheat grain ground	14.27	16.82	14.27	17.92
Soybean meal ground, 44 % CP	16.47	11.47	12.89	10.72
Sunflower meal ground	7.15	6.74	7.15	6.91
Calcium carbonate	1.07	1.01	1.07	1.04
Vitamin-mineral premix[8]	0.05	0.05	0.05	0.05
Salt (NaCl)	0.18	0.17	0.18	0.17
Total, %	100	100	100	100
Chemical composition,				
Dry matter (DM), %	59.05	67.71	67.21	73.51
Crude protein, % of DM	16.13	15.19	17.38	16.45
Ether extract, % of DM	3.18	3.08	2.98	2.54
Neutral detergent fiber, % of DM	39.57	43.21	38.17	41.53
FNDF[9], % of DM	27.82	31.06	26.96	29.11
Acid detergent fiber, % of DM	23.31	23.36	23.18	25.63
Acid detergent lignin, % of DM	3.85	4.71	4.51	5.10
NFC[10], % of DM	35.82	32.46	34.85	32.60
Starch, % of DM	27.61	24.78	26.00	25.39
Ash, % of DM	5.30	6.06	6.62	6.88
NEL[11], Mcal/kg, of DM	1.63	1.58	1.61	1.57

[1]CS = 50 % corn silage and 50 % concentrate, [2]CSW = 35 % corn silage + 15 % wheat straw and 50 % concentrate, [3]CSA = 35 % corn silage + 15 % alfalfa hay and 50 % concentrate, and [4]CSWA = 25 % corn silage + 10 % wheat straw + 15 % alfalfa hay and 50 % concentrate.[5]Corn silage analysis (DM basis) : NDF, 55.64 %, [6]Wheat straw (DM basis) : NDF, 77.22 %, [7]Alfalfa hay (DM basis) : NDF, 49.89 %. [8]Supplied per kilogram of premix (Kavimix VM, Kartal Kimya A.S., Gebze, Turkey) : Vitamin A 12000000 IU, Vitamin D_3 3000000 IU, Vitamin E 30 g, Mn 50 g, Fe 50 g, Zn 50 g, Cu 10 g, I 0.8 g, Co 0.1 g, Se 0.15 g, Antioxidant 10 g. [9]FNDF : Percentage neutral detergent fiber from forage, calculated from ingredient analysis.[10]NFC : Nonfiber carbohydrate, %; calculated as : 100 - (NDF, % + CP, % + EE, % + ash, %). [11]NEL : Net energy lactation ; Calculated from NRC [18].

TABLE I. Ingredients and chemical composition of the total mixed diets.

Ruminal fluid was collected during each period from 0900 (d 19) to 0700 (d 20) with 2 h intervals from multiple sites in the rumen for volatile fatty acids (VFA) and ammonia content (NH_3-N) determination. Samples were immediately squeezed through 4 layers of cheesecloth with a mesh size of 250 µm. Samples of 10 ml were acidified with 0.5 ml of 50 % H_2SO_4 and used to determine VFA, and 5 ml of filtrate was preserved by adding 1 ml of 1 % H_2SO_4 and used to determine NH_3-N. The samples were subsequently stored frozen at -20 ˚C until analyzed. These samples were prepared for analysis as follows : 1) samples tubes containing 5 ml rumen fluid were thawed and shaken, then the contents were transferred into centrifuge tube and centrifuged at 10 000 rpm, 4 °C for 15 min ; 2) supernatant (1 ml) was transferred into a microfuge tube, 0.2 ml of 25 % metaphosphoric acid was added, and the mixture was vortexed before incubating at room temperature for 30 min ; and 3) supernatant was transferred into a gas chromatography (GC) sample vial for analysis by GC (Hewlett Packard Agilent Technologies 6890N Network GC System, Serial CN10447002, China) with GP 10 % SP-1200/1 % H_3PO_4 on 80/100 Chromosorb and using 6'∞2 mm ID glass column (Supelco, Bellefonte, PA, USA). Ammonia content of ruminal samples was determined using the method described by ANNINO [2].

CHEMICAL ANALYSES

The dietary samples were dried in forced-air oven at 55 ˚C for 48 h for measurement of DM content and then ground through a 1-mm diameter screen using a laboratory 3303 Mill (Hundenge, Sweden). Analytical DM content of the dietary samples was determined by drying at 105˚C for 12 h, and

	CS[1]	CSW[2]	CSA[3]	CSWA[4]	SE	P
% DM retained						
> 19.0 mm	3[c]	14[b]	12[b]	24[a]	1.2	***
19.0 to 8.0 mm	41[a]	27[b]	27[bc]	19[c]	2.1	**
8.0 to 1.18 mm	40[b]	43[a]	49[a]	42[b]	1.3	***
< 1.18 mm	16[a]	16[a]	12[b]	15[a]	0.7	*
X_{gm}[5](mm)	5.08[d]	5.33[c]	5.46[b]	6.15[a]	0.4	***
S_{gm}[6] (mm)	2.70[d]	3.02[b]	2.79[c]	3.26[a]	0.2	***
pef[7]	0.84[b]	0.84[b]	0.88[a]	0.85[b]	0.1	***
peNDF[8], % of DM	33.24[b]	36.30[a]	33.59[b]	35.30[a]	0.2	***
peNDFI[9], kg/d of DM	5.1[b]	5.8[a]	5.2[b]	5.6[a]	0.04	***
NDFI[10], kg/d of DM	6.1[b]	6.9[a]	5.9[b]	6.6[a]	0.08	***
DMI[11], kg/d	15.32	16.00	15.53	15.84	0.1	NS

[1]CS = 50 % corn silage and 50 % concentrate, [2]CSW = 35 % corn silage + 15 % wheat straw and 50 % concentrate, [3]CSA = 35 % corn silage + 15 % alfalfa hay and 50 % concentrate, and [4]CSWA = 25 % corn silage + 10 % wheat straw + 15 % alfalfa hay and 50 % concentrate.[5]X_{gm} = Calculated average particle size.[6]S_{gm} = Calculated standard deviation.[7]Physical effectiveness factors (pef) were determined by the fraction retained on a 1.18 mm sieve using horizontal shaking with PSPS.[8]peNDF was measured as the NDF content of the TMR multiplied by the pef from Mertens [14]. [9]peNDFI = peNDF intake.[10]NDFI = NDF intake[11]DMI = Dry matter intake
NS : P>0.05 * P < 0.05 ** P < 0.01 *** P < 0.001 [a,b,c,d] Means in the same row with different superscripts differ according to P value indicated.

TABLE II. Dietary particle size distribution, peNDF, peNDFI, NDFI and DMI values of total mixed diets (DM basis).

crude protein was determined by the KJELDAHL method [3]. Ash was determined by combustion at 550 °C for 6 h. The NDF, Acid Detergent Fiber (ADF) and Acid Detergent Lignin contents were determined using the methods described by VAN SOEST et al. [23] with heat-stable amylase (Sigma No : A-3306, Sigma Chemical Co., St Louis, MO, USA) and sodium sulfite used in the NDF procedure. Starch was measured on composited samples as described by BAL et al. [4].

STATISTICAL ANALYSES

Feed intake, pH, NH_3-N, VFA and chewing activity data were analyzed as a 4x4 Latin square. Data were analyzed using General Model procedure and Repeated Measures of SPSS. The model included treatments, period on two-way interaction between treatments and period. Treatments and period were considered fixed effect. Rumen NH_3-N, pH, and VFA concentration were analyzed by use of repeated measurements procedure, as well as variance analyze. Feed intake, chewing activity data, duration of pH < 5.8, mean separation for TMR particle size, NDF intake, and peNDF were determined using the one-way ANOVA, followed by the Tukey test procedure. The Pearson correlation coefficients were estimated using correlation procedure of SPSS. All statistical analyses were performed with SPSS software (version 10.0, SPSS Inc, Chicago, USA).

Results

PARTICLE SIZE DISTRIBUTION OF TMR, DRY MATTER INTAKE, AND PHYSICALLY EFFECTIVE FIBER

Particle size distributions of the diets and intakes of DM, NDF, and peNDF are presented in Table II. The proportion of particles retained on the 19.0 mm screen of the CS diet was lower (P < 0.001) than in the other diets. Intake of DM (averaged 15.7 kg/d) did not differ between the diets, although CS diet had less DM content than the others due to the highest ratio of corn silage in among the diets. Physical effectiveness factor (pef) of the CSA was higher (P < 0.001) than those of other groups. The peNDF contents of the diets were 33.24, 36.30, 33.59 and 35.30 % of DM, for CS, CSW, CSA and CSWA diets, respectively. Cows fed diets containing CSW and CSWA had greater peNDF (% of DM), NDFI, and peNDFI than did cows fed CS and CSA (P < 0.001).

CHEWING ACTIVITIES

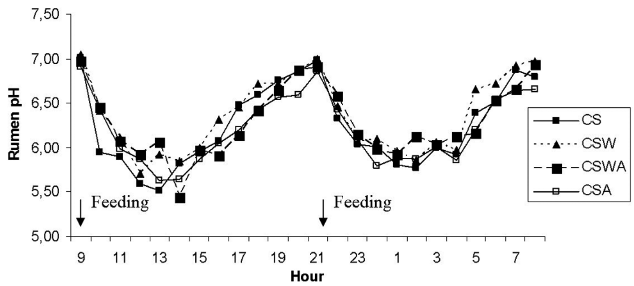
Chewing activities are reported in Table III. Time spent eating ranged from 2.6 to 3.7 h/d. The eating time (min/d), number of eating chews, and eating time per kg of DMI were decreased when cows fed with CS (P < 0.01). Time spent ruminating ranged from 6.1 to 7.4 h/d. The ruminating time (min/d) and number of ruminating chews were lower in cows fed with CS than in cows fed with CSW, but ruminating per

Dependent variable	CS[1]	CSW[2]	CSA[3]	CSWA[4]	SE	P
Eating						
Chews/d	7565[b]	12543[a]	11167[a]	13109[a]	558.3	***
Chews/kg of DM	494[b]	783[a]	719[ab]	828[a]	34.7	**
Chews/kg of NDF	1240[b]	1767[ab]	1893[a]	1986[a]	82.5	**
Min/d	158.3[b]	217.1[a]	200.8[a]	223.8[a]	6.7	***
Min/kg of DM	10.1[b]	13.6[a]	12.9[a]	14.1[a]	0.4	***
Min/kg of NDF	25.5[b]	31.4[ab]	33.9[a]	34.1[a]	1.0	**
Min/kg of peNDF	31.1[b]	37.4[ab]	38.6[ab]	40.0[a]	1.1	*
Ruminating						
Chews/d	21621[b]	27473[a]	24116[ab]	24947[ab]	806.5	*
Chews/kg of DM	1411	1717	1552	1575	50.3	NS
Chews/kg of NDF	3544	3869	4087	3780	120.0	NS
Min/d	363.3[b]	445.5[a]	398.3[ab]	410.0[ab]	11.3	*
Min/kg of DM	23.1	27.8	25.7	25.9	0.7	NS
Min/kg of NDF	58.5	64.4	67.2	62.3	1.7	NS
Min/kg of peNDF	71.2	76.8	76.6	73.2	2.0	NS
Total chewing						
Chews/d	29651[b]	41001[a]	35897[ab]	38684[a]	1257.6	**
Chews/kg of DM	1935[b]	2563[a]	2311[ab]	2442[ab]	77.7	*
Chews/kg of NDF	4861	5774	6084	5861	182.1	NS
Min/d	521.7[b]	662.5[a]	599.2[ab]	633.8[a]	15.9	**
Min/kg of DM	33.2[b]	41.4[a]	38.6[a]	40.1[a]	1.0	**
Min/kg of NDF	84.0[b]	95.8[ab]	101.1[a]	96.4[ab]	2.3	*
Min/kg of peNDF	102.3	114.2	115.2	113.2	2.6	NS

[1]CS = 50 % corn silage and 50 % concentrate, [2]CSW = 35 % corn silage + 15 % wheat straw and 50 % concentrate, [3]CSA = 35 % corn silage + 15 % alfalfa hay and 50 % concentrate, and [4]CSWA = 25 % corn silage + 10 % wheat straw + 15 % alfalfa hay and 50 % concentrate.
NS : P>0.05 * P < 0.05 ** P < 0.01 *** P < 0.001
[a,b,c,d] Means in the same row with different superscripts differ according to P value indicated.

TABLE III. Effects of dietary treatments on chewing behavior.

kg of DMI and intake of NDF (NDFI) were not affected by treatments. Rumination time of animals fed with CSW was 82 min longer (P < 0.05) than of these only fed corn silage as forage source (CS) (445 and 363 min/d for CSW and CS, respectively). The total chewing time (min/d) and number of chews (chews/d) were higher in wheat straw added (CSW and CSWA) groups than in the CS group (P < 0.01). Total time spent chewing per day ranged from 8.7 to 11.0 h/d. Total chewing time for cows fed corn silage as the sole source of forage averaged 521 min/d. Supplementing diets with alfalfa hay and wheat straw increased chewing time (662, 599, and 633 min/d for CSW, CSA, and CSWA, respectively).



CS = 50 % corn silage and 50 % concentrate, CSW = 35 % corn silage + 15 % wheat straw and 50 % concentrate, CSA = 35 % corn silage + 15 % alfalfa hay and 50 % concentrate, and CSWA = 25 % corn silage + 10 % wheat straw + 15 % alfalfa hay and 50 % concentrate.

FIGURE 1. Diurnal variation of rumen pH affected by diets varying in forage source.

RUMINAL PH AND RUMEN FERMENTATION

Effects of forage sources on rumen pH, total VFA, NH_3-N, and molar proportion of individual VFA are presented in Table IV and diurnal rumen pH are presented in Figure 1. The

Dependent Variable	CS[1]	CSW[2]	CSA[3]	CSWA[4]	SE	P
pH						
Mean	6.26	6.42	6.24	6.30	0.1	NS
pH < 5.8, h/d	7.3^{a}	4.5^{b}	5.0^{b}	5.3^{b}	0.3	***
VFA						
Total, mmol/L	103.0	100.6	105.9	106.9	7.2	NS
Mol/100 mol						
Acetate (A)	66.4	70.5	67.2	67.9	2.4	NS
Propionate (P)	16.7	15.8	17.0	18.3	2.0	NS
Butyrate	12.7	10.6	11.6	10.5	0.8	NS
Isobutyrate	0.6	0.6	0.6	0.6	0.04	NS
Isovalerate	1.7	1.7	1.7	1.4	0.2	NS
n-Valerate	1.7	1.5	1.9	1.7	0.3	NS
A/P	4.0	4.5	4.1	4.0	0.6	NS
NH_3- N, mg/dL	20.2	17.9	20.7	17.4	2.5	NS

[1]CS = 50 % corn silage and 50 % concentrate, [2]CSW = 35 % corn silage + 15 % wheat straw and 50 % concentrate, [3]CSA = 35 % corn silage + 15 % alfalfa hay and 50 % concentrate, and [4]CSWA = 25 % corn silage + 10 % wheat straw + 15 % alfalfa hay and 50 % concentrate.
NS : P>0.05 ***P < 0.001
[a,b,c,d] Means in the same row with different superscripts differ according to P value indicated.

TABLE IV. Effects of dietary treatments on ruminal pH, VFA and NH_3-N values.

mean of pH, VFA, acetate/propionate (A/P) and NH_3-N were not affected by treatments. However, total concentrations of VFA and rumen pH were linearly increased by hour of sampling after the a.m. and p.m. feeding in all of treatments (data not shown). The highest pH values were observed just before the 9-am feeding, while the lowest pH values were 1400 h. In addition the time (h/d) where the pH value was under 5.8, was increased when cows fed the CS diet (7.3, 4.5, 5.0 and 5.3 h/d for CS, CSW, CSA and CSWA, respectively) (P < 0.001).

Discussion

Many dietary factors may influence dry matter intake in lactating cattle such as physical characteristics, ingredient and nutrient compositions of diets. In this study, the daily DMI was not influenced by partial replacement of corn silage with alfalfa hay and/or wheat straw. POORE et al. [20] and BROWN et al. [9] reported that partial replacement of alfalfa hay with wheat straw did not affect DMI in lactating cows. On the other hand, WEST et al. [24] stated when corn silage was replaced with bermudagrass or alfalfa hay (15 % or 30 %), DMI decreased from 22.9 to 22.0 kg/d. RODE et al. [21] observed that DMI was affected by long forage but not by chopped or ground forage in diets of dairy cows. In addition, KONONOFF et al. [11] reported that reducing forage particle size may increase DMI when cows consumed average 25.7-28.0 kg/d. In our study, particle size longer than 19 mm was increased when alfalfa hay and/or wheat straw was included in diets (Table II). This increase did not affect DMI and further no correlation was found between intake of particles > 19.0 mm and DMI (Table V). The lack of difference in DMI may be due to the late lactating period (DMI averaged 15.3-16.0 kg/d).

Eating times, time spent eating per kilogram of DMI and chews per day were increased (P < 0.001) when cows fed with wheat straw and/or alfalfa hay, probably due to forage sources**.**

The relationship between intake variables and animal responses associated with forage sources were investigated using Pearson correlation coefficients (Table V). In parallel to our findings, several researchers [7, 8] observed a positive correlation between the ruminating time and the peNDF. BEAUCHEMIN et al. [5] reported that addition of 10 % alfalfa hay (equivalent to 2.3 kg/d) increased rumination time when compared with cows fed coarse silage without alfalfa hay. However, the amount of hay added in their study appears to be an important factor, because adding 20 % alfalfa hay (equivalent to 4.2 kg/d) did not consistently increase rumination time.

	1	2	3	4	5	6	7
1. Intake of DM, kg/d							
2. Intake of NDF, kg/d	0.36*						
3. Intake of peNDF, % of DM	0.10	0.70**					
4. Intake of particles > 19.0 mm, kg/d	0.20	0.41**	0.52**				
5. Eating time, min/d	-0.02	0.15	0.43**	0.48**			
6. Ruminating time, min/d	0.02	0.08	0.29*	0.22	0.53**		
7. Total chewing time, min/d	0.01	0.12	0.39**	0.36*	0.80**	0.93**	
8. pH < 5.8, h	0.21	-0.45**	-0.45**	-0.50**	-0.43**	-0.27	-0.37**

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

TABLE V. Pearson correlation coefficients for parameter measurements among 8 observations.

In that study, for adequate fiber diets, addition of alfalfa hay either had little effect or decreased rumination time [5].

Time spent total chewing per kilogram of DMI was also increased When a part of the corn silage was replaced with alfalfa hay and/or wheat straw (P < 0.01). BEAUCHEMIN and YANG [7] found that in multiparous Holstein the TC was 12-13 h/d when cows fed with corn silage as forage, but in our study it was 8.7 h/d. It is assumed that the reason for this decrease was due to less dry matter intake in our study (21 versus 15 kg/d, DM basis). We observed chewing time per kg of DMI as 34 min ; similar to the 35 min value reported by BEAUCHEMIN and YANG [7].

In this study, it was determined that increasing peNDF of the diets and the intake of amount particles > 19.0 mm may be among the factors affecting total chewing activity of dairy cattle. KONONOFF et al. [11] reported that chewing activity was increased when proportion of TMR particles > 19.0 mm increased. In the present study, intake of amount particles > 19.0 mm was positively correlated eating (r =0.48, P < 0.01) and total chewing time (r =0.36, P < 0.01) (Table V). Although intake of amount particles > 19.0 mm fed with CSWA was higher (P < 0.001) than CSW, TC of CSWA was lower. This result may be related to, the higher peNDF content of CSW.

When replacing corn silage with alfalfa hay and wheat straw, we expected that forage sources were to affect ruminal pH, because of higher fermentability, small particle size of corn silage and higher buffering capacity of alfalfa hay and wheat straw. However, the mean daily rumen pH value in rumen fluid was not significantly affected by the dietary treatments. This situation can be due to the high NDF and peNDF values of diets used in our study. The NRC [18] recommends that the DM of dairy diets contain a minimum of 25 % NDF and 75 % of the NDF should be supplied by forages. Also Mertens [14] suggested that 22 % of ration DM needs to consist of peNDF in order to maintain rumen pH above 6.0. Mean daily rumen pH ranged from 6.24 to 6.42 in our study. These results agree with the results of MERTENS [14], who reported that the relationship between peNDF and rumen pH is quadratic, and that the effect of peNDF on rumen pH is limited at higher peNDF levels. OWENS et al. [19] concluded that the rumen pH is affected by the total VFA concentration in rumen fluid. For our study, it can be said that the lack of the influence of diet on VFA may also mean that rumen pH values are not likely to be affected as well.

The ruminal pH 6.2 and 5.8 were chosen as benchmarks based on in vitro observations that ruminal activity is compromised when ruminal pH drops below 6.2 [22], and the incidence of sub-clinical acidosis increases when ruminal pH falls below 5.8 [7]. In the present study, the time (h/d) where the pH was under 5.8 increased (P < 0.001) when cows fed CS diet. Lower ruminal pH of cows fed diets with corn silage as the only forage source might be partially explained by the high availability of rapidly fermentable starch in corn silage. The higher duration of pH < 5.8 in CS compared with other diets might also be associated with shorter (P < 0.001) eating time (Table III). The increase in saliva output during eating could enable the cow to buffer large quantities of fermentation acid produced soon after the feed is consumed. In the present study the duration of pH < 5.8 was negatively correlated with the peNDF (r =-0.45, P < 0.01) and total chewing time (r =-0.37, P < 0.01) of diets (Table V). A negative correlation was also found between the amount of particle size larger than 19.0 mm and the duration of time pH was lower than 5.8 (r =-0.50, P < 0.01). According to these results cows fed with CS (15.0 kg/d, DMI) might be more susceptible to acidosis than cows in other groups. In the event of increasing DMI, the risk may be higher.

The VFA concentrations were low compared with those observed in other studies [8, 10]. This can be explained by higher NDF and lower starch contents of the diets in our study. KRAUSE et al. [13] stated that the concentration of propionate increased when particle size was decreased. In addition KRAUSE and COMNBS [12] reported that propionate concentration tended to increase when alfalfa silage was partially replaced by corn silage, probably due to increase in the amount of dietary starch. It is likely that the propionate concentrations were not affected due to the similar starch contents of the diets. Using 15 % wheat straw caused a no significant increase in A/P values in our study. BEAUCHEMIN and YANG [7] reported that the A/P of cows which fed with only corn silage as forage source was nearly 2, but in the present study we found it as 4. It is assumed that the reason for this increase was the high NDF and low starch contents of the diets in our study compared with the study of BEAUCHEMIN and YANG (32.6 % versus 39.57 % of DM) [7]. However, BEAUCHEMIN and YANG [7] used kernel processed corn silage which can be a contributing factor to the discrepancy between the results.

Conclusion

The partial replacement of corn silage with alfalfa hay and/ or wheat straw has no effect on DMI. Using alfalfa hay and wheat straw in the diets increases chewing activity and the buffering capacity within the rumen due to increased saliva secretion, and consequently may improve the rumen pH status. It is concluded that forage source may have an effect on the chewing activity and rumen pH related to the intake, peNDF and fiber structure. The proportion of particles > 19.0 mm and peNDF may be used as a predictor of chewing activity and sub-clinical ruminal acidosis.

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