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Effect of Different Levels of Indigestible Fiber and Particle Size on Intake, Rumen Parameters, Digestibility, Chewing Activity, Milk Yield and Compositions of Holstein Dairy Cows

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Abstract

The experiment conducted to investigate the effects of indigstible NDF (iNDF) and forage particle size (PS) on animal response using eight Holstein lactating dairy cows that assigned to four dietary treatments in a 4 × 4 Latin square design with four 21-day periods (14 days for adaptation and 7 days for sampling). Treatments including diet contained: 1) long alfalfa hay-low iNDF, 2) fine alfalfa hay-low iNDF, 3) long alfalfa hay-high iNDF, and 4) fine alfalfa hay-high iNDF. By increasing iNDF, DMI, digestibility of fat and NDF increased, but the consumption of non-fiber carbohydrate (NFC) and starch decreased. Reduction of PS increased intake of DM and NDF. Rumen pH and NH3-N decreased with the reduction of PS. Total VFA, propionate, and butyrate concentration increased by reduction of PS and decreasing iNDF. Chewing activity increased with greater iNDF. Using eating iNDF as a fibrous index is more sensitive than physically effective NDF (peNDF) especially in the high NFC ration. Cows that consume lower iNDF produced more milk yield, milk protein, lactose and solid non-fat (SNF). Decreasing the iNDF increased DMI, rumen propionate and butyrate, milk yield and protein, but decreased digestibility of DM and rumen pH. In addition, reduction of PS increased DMI, effected nutrient digestibility and rumen fermentation, but the effects were not large enough to affect rumen pH and milk production. The results showed that the iNDF as an index to predict chewing behaviour is more sensitive than DM, NDF, and peNDF>1.18, especially in low iNDF ration to prevent ruminal acidosis.

Keywords: Alfalfa hay; Dairy cow; Indegradable fiber; Particle size; Physically effective NDF

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Introduction

Current milk production levels require dairy cows to consume a suitable amount of total carbohydrate, NDF, peNDF, iNDF, NFC, starch, and soluble sugars. Carbohydrates partitioned into three fractions of potential degradable NDF (pdNDF), NFC, iNDF in diets. The NDF is a nonuniform feed fraction and the extent and nature of its lignification controls its digestibility, in contrary, iNDF is a uniform feed fraction with no digestibility [1]. The peNDF is the fraction of NDF that stimulates chewing, contributes to form ruminal mat, and has a high resistance to escape from the rumen [2,3]. In the peNDF system, forage PS explains all variations in chewing response, but this assumption is not always true because forages with similar PS may elicit substantially different chewing times per kg of NDF. Some characteristics such as digestibility, forage fragility, functional specific gravity [4], and iNDF content may explain the variation in chewing response not explained by peNDF (PS or NDF) [3]. Therefore, focusing on peNDF leads to erroneous thinking about the requirement for fiber because the recommendations for peNDF or PS distributions will not eliminate subacute acidosis, and does not explain all variation in chewing response, especially in the low F:C diet and some studies found the degree of acidosis with similar peNDF levels, but with different F:C ratios, carbohydrate sources, and NFC levels [4,5]. Traditionally, nutritionists have focused on measures of fiber digestibility but recently the focus has included iNDF. As iNDF had no digestibility, is full resistance to passage and associated to form ruminal mat, focusing on iNDF instead of NDF or peNDF in balancing dairy diets lead to take in account the requirement for fiber as effective iNDF (eiNDF) to predict animal response. Therefore, this experiment was conducted to determine the effect of iNDF levels and PS of forage on intake, digestibility, rumen kinetic, chewing activity, milk yield and composition in dairy cows.

Materials and Methods

Determination of indigestible neutral detergent fiber

Using two ruminally fistulated Zel ewes (BW=30.5 ± 1.8 kg); 5 g sample in 4 replications was weighed in sealed nylon bags (7 cm × 8 cm, polyamide, with 15 ± 2 μ pore size) and incubated in the rumen for 240 h [6]. Sheep housed in front shed, fed a total mixed ration (TMR) containing 50% chopped alfalfa hay, 25% wheat straw, 25% barely grain, and mineral/vitamin supplement according to their requirements [7]. On removal, bags were washed using cold water, dried at 55°C for 48 h, residues for the periods were homogenized and analyzed for Kjeldahl N, NDF (with α-amylase being added and without sodium sulfite), and acid detergent lignin [8] (**Table 1**), and multiplied by a fixed factor of 2.4 calculated as ADL × 2.4 [9]. The pdNDF calculated using the following equation: pdNDF=NDF–iNDF [10].

Particle distribution and effectiveness fiber

Feed PS and distribution was determined by dry sieving in four replicates, using the Penn State particle separator. The physical effective factor (pef) of TMRs was determined as the sum of

Table 1. The chemical compositions and particle distribution of experimental rations.

Items	Experimental Treatments[1]				Standard Error	P-Value		
	LL	FL	LH	FH		PS	iNDF	iNDF*PS
Chemical composition %								
Dry matter	67.81	67.96	68.16	68.24	1.072	NS	NS	NS
Organic matter	91.55	91.85	91.45	91.45	1.026	NS	NS	NS
Ether extract	3.15	3.25	3.8	3.7	1.278	NS	NS	NS
Crud protein	15.75	15.65	15.87	15.83	0.085	NS	NS	NS
NDF	33.96^{b}	33.96^{b}	36.35^{a}	36.35^{a}	1.206	*	***	NS
$iNDF_{240}$ [2]	14.76^{b}	14.65^{b}	18.58^{a}	18.34^{a}	1.323	NS	***	***
$iNDF_{2.4}$ [2]	6.77^{a}	6.70^{b}	6.62^{c}	6.14^{d}	0.2481	***	***	***
PdNDF[3]	19.2^{a}	19.31^{a}	17.77^{b}	18.01^{b}	0.183	***	***	***
NFC	41.02^{a}	41.04^{a}	37.43^{b}	37.47^{b}	0.79	NS	***	NS
Lignin	2.82^{a}	2.79^{b}	2.76^{c}	2.56^{d}	0.1041	NS	***	***
ADF	20.88^{a}	20.85^{a}	19.88^{b}	19.88^{b}	0.0891	***	***	***
Starch	27.25^{a}	28.50^{a}	24.25^{b}	25.10^{b}	1.705	NS	***	***
NE_L, Mcal/Kg DM	1.69	1.69	1.68	1.68	0.345	NS	NS	NS
Particle size distribution and physical characteristics % of DM								
19 mm	13.89^{a}	1.66^{c}	13.91^{a}	2.96^{b}	0.008	***	***	***
8 mm	13.63^{c}	16.76^{b}	19.38^{ab}	22.97^{a}	0.892	*	***	NS
1.18 mm	53.17^{c}	63.20^{a}	51.57^{d}	57.93^{b}	0.295	***	***	***
Pan	19.31^{a}	18.40^{b}	15.04^{d}	16.17^{c}	0.283	NS	***	***
Geometric mean, mm[4]	4.31^{b}	3.53^{d}	4.97^{a}	4.05^{c}	0.831	NS	NS	NS
SD of geometric mean, mm[4]	2	1.33	1.91	1.47	0.538	NS	NS	NS
$pef_{>8 mm}$ [5]	27.52^{b}	18.42^{d}	33.29^{a}	25.93^{c}	0.886	***	***	NS
$pef_{>1.18 mm}$ [5]	80.69^{b}	81.62^{b}	84.86^{a}	83.87^{a}	0.913	NS	NS	*
$peNDF_{>8}$ [6], % of DM	9.35^{b}	6.26^{d}	12.10^{a}	9.43^{c}	0.318	***	***	NS
$peNDF_{>1.18}$ [6], % of DM	27.40^{b}	27.71^{b}	30.84^{a}	30.48^{a}	0.328	NS	***	*

^{a,b,c,d} Means within a row with different superscripts differ ($P < 0.05$)

[1]Four treatments including diet contained: 1) long alfalfa hay and low iNDF (LL), 2) fine alfalfa hay and low iNDF (FL), 3) long alfalfa hay and high iNDF (LH), and 4) fine alfalfa hay and high iNDF (FH)

[2]The indigestible NDF that determined after 240h ruminal incubation of feed samples and the iNDF2.4 was calculated multiplied with a fixed factor of 2.4×ADL [9]

[3]The estimation of potentially digestible NDF (pdNDF= NDF – iNDF; [10])

[4]The geometric mean and its standard deviation for chopped hay and rations determined by dry sieving (ASAE, 2007, method S424.1)

[5]The physically effective factor determined as the proportion of DM retained on two (pef>8; [11]) and three sieves of the Penn State particle separator (pef>1.18; [12])

[6]The peNDF>8 and peNDF>1.18 were calculated by multiplying NDF content of each ration on each sieve on pef>8 and pef>1.18, respectively

retained particle on two 19 and 8 mm sieves (pef>8) [11], and three 19, 8, and 1.18 mm sieves (pef>1.18) [12]. The NDF of all materials retained on each sieve were measured [8]. The peNDF>8 and peNDF>1.18 were calculated by multiplying NDF content of each portion on each sieve on pef>8 and pef>1.18, respectively (**Table 2**). The geometric mean and its standard deviation were calculated.

Feeding trail

All procedures used in this study were approved based on proposing a National Ethical Framework for Animal Research in Iran. Eight first lactating Holstein dairy cows (BW=730.5 ± 25.5 kg; DIM=74 ± 11 d) were allotted to a 4 × 4 Latin square design in four 21- day periods (adaptation, 14 -days; sample collection and measurement of chewing activity, 7 days). Four treatments including diet contained: 1) long alfalfa hay-low iNDF (LL), 2) fine alfalfa hay-low iNDF (FL), 3) long alfalfa hay-high iNDF (LH), and 4) fine alfalfa hay-high iNDF (FH; **Table 1**). Unchopped alfalfa was used as long hay. Alfalfa hay was chopped with electric shredder (Sharif Industrial Group, model 1570) with a theoretical cut length of 20 mm. Cows were housed in individual free stalls and fed ad libitum, twice daily at 0900 and 2100 h with TMR, allowing for at least 10% residuals. Cows were weighed twice weekly and DMI was measured daily. Total feces were collected from all cows for 5 days of each period at days 14 - 20, weighted, and daily sampled composited, subsampled, dried at 55°C, and ground through a Wiley mill (1-mm screen). Feed, feces and orts were analyzed for DM, Kjeldahl N, ether extract (EE), organic matter (OM) and ash at 605°C for 3 h [13], NDF and ADF [8]; with α-amylase being added and without sodium sulfite]. The NDF was expressed without residual ash. The NFC was calculated from 100-(%CP+%NDF+%Ash+%EE) [7]. The TMR composites also were analyzed for starch [14]. Using the chemical components of TMRs and feces, intake, and digestibility of nutrients were calculated (**Table 3**).

On day 21 of each period, ruminal fluid (50 mL) from the ventral sac were taken 3 h after morning feeding using the rumenocentesis technique. Ruminal pH was measured immediately after ruminal fluid collection using a pH meter (model 632, Metrohm, Herisau, Switzerland), and samples were frozen at -20°C. The concentration of ammonia nitrogen (NH_3-N) was measured with Kjeltec Auto Analyzer (Model 1030, Tecator Co. Sweden). Ruminal fluid was acidified by mixing with 2.5 mL of HCl 6 mol/L and frozen for future analysis of VFA. Using Cr-mordanted NDF alfalfa [15], the passage rate, ruminal and total mean retention time and transit time of marker were measured [16].

Eating and ruminating activities were monitored visually at 5 min intervals for all cows in each treatment over a 24 h period at day 20 to 21 of each period. Total time spent on chewing was calculated as the total time spent eating and ruminating.

Milk yield was daily recorded, sampled at each morning and evening milking on days 17 to 21 of each period. About 100 mL of each morning and evening daily milk samples were composited and analyzed using a milcoanalyzer (ViA StElle, 11-25020 PoN ARA4ecBS). The energy corrected milk (ECM) yield was calculated using the following equation: ECM= [0.327 × milk yield (kg)+12.95 × fat yield (kg)+7.2 × protein yield].

Statistical analysis

The data of PS were analyzed as a completely randomized design with model effects of diets and two methods of PS measurement

Table 2. Dry matter intake and apparent digestibility of nutrients of experimental rations.

Items	Experimental Treatments[1]				Mean Standard Error	P-Value		
	LL	FL	LH	FH		PS	INDF[2]	iNDF*PS
BW, kg	670	668	660	670	10.432	NS	NS	NS
Intake, kg/day								
Dry matter	19.50^d	20.50^c	20.85^b	21.32^a	0.0327	***	***	***
Organic matter	19.24^c	19.38^b	19.42^b	19.49^a	0.026	***	***	NS
Ether extract	0.66^d	0.69^c	0.81^a	0.79^b	0.002	*	***	*
Crud protein	3.31^b	3.30^b	3.37^a	3.37^a	0.005	NS	***	NS
Neutral detergent fiber	7.14^c	7.16^c	7.29^b	7.32^a	0.009	**	***	NS
$peNDF_{>8}$	1.90^c	2.54^a	1.42^d	2.13^b	0.034	**	NS	NS
$peNDF_{>1.18}$	6.16	6.23	6.29	6.25	0.222	NS	NS	NS
Non fiber carbohydrate	8.91^a	8.95^a	8.05^b	8.10^b	0.056	***	***	NS
Starch	5.72^b	6.01^a	5.14^d	5.35^c	0.069	***	***	***
Apparent digestibility of dry and organic matter and nutrients, g/kg								
Dry matter	592.5	600.2	554.5	582.6	31.3	NS	NS	NS
Organic matter	699.9	713.6	728.6	686.9	11.57	NS	NS	*
Ether extract	724.1^b	778.0^a	800.2^a	770.4^{ab}	18.15	NS	NS	*
Crud protein	722.4	730.4	739.5	702.2	11.99	NS	NS	NS
Neutral detergent fiber	557.8^{ab}	560.0^{ab}	628.9^a	527.6^b	24.34	NS	*	*
Non fiber carbohydrate	819.9	838.7	812.3	822.1	10.51	NS	NS	NS

[a ,b ,c, d] Means within a row with different superscripts differ (P <0.05).
[1]Four treatments including diet contained: 1) long alfalfa hay and low iNDF (LL), 2) fine alfalfa hay and low iNDF (FL), 3) long alfalfa hay and high iNDF (LH), and 4) fine alfalfa hay and high iNDF (FH).
[2] The NDF concentration was measured using α-amylase and without sodium sulphite [8].

Table 3. Rumen pH, N-NH3, and digestion kinetic of cows fed the experimental rations with particle size and two level of indegradable NDF.

Items	Experimental Treatments[1]				Mean Standard Error	P-Value		
	LL	FL	LH	FH		PS	iNDF	iNDF*PS
Rumen pH	6.36[b]	6.25[b]	6.65[a]	6.30[b]	0.092	*	*	NS
NH_3-N, mg/dL	13.53[a]	9.71[b]	12.04[a]	9.41[b]	0.413	***	NS	NS
Total volatile fatty acid, mM/L	141.93[b]	147.04[a]	136.93[bc]	139.53[b]	2.221	***	**	NS
Acetate, mM/L	84.21	88.45	86.32	87.85	1.752	NS	NS	NS
Propionate, mM/L	34.65[a]	35.21[a]	29.87[b]	31.23[b]	1.121	NS	***	NS
Isobutyrate, mM/L	1.43	1.55	1.38	1.33	0.132	NS	NS	NS
Butyrate, mM/L	16.32[a]	16.17[a]	14.43[b]	14.23[b]	0.631	*	**	NS
Isovalerate, mM/L	2.41	2.54	2.11	1.98	0.142	NS	NS	NS
Valerate, mM/L	2.91	3.12	2.82	2.91	0.153	NS	NS	NS
Acetate: Propionate	2.42[b]	2.54[b]	2.89[a]	2.80[a]	0.113	NS	***	NS
Rumen digestion kinetic								
Ruminal passage rate, %/h	2.96[b]	3.31[a]	2.85[b]	3.14[a]	0.101	**	NS	NS
Ruminal mean retention time, h	33.78[a]	30.21[b]	35.09[a]	31.85[b]	0.107	***	***	NS
Lower compartment passage rate, %/h	4.21[b]	4.03[c]	4.71[a]	3.96[c]	0.001	***	***	***
Lower compartment mean retention, h	23.75[b]	24.81[b]	21.23[c]	25.25[a]	0.697	NS	*	NS
Time delay, h	9.14	13.39	13.38	7.37	3.412	NS	NS	NS
Total mean retention time, h	66.68[b]	68.45[b]	69.70[a]	64.47[c]	0.775	***	NS	NS

[a,b,c]Means within a row with different superscripts differ (P <0.05).
[1]Four treatments including diet contained: 1) long alfalfa hay and low iNDF (LL), 2) fine alfalfa hay and low iNDF (FL), 3) long alfalfa hay and high iNDF (LH), and 4) fine alfalfa hay and high iNDF (FH).

using the REML variance component and PROC MIXED of SAS® (2002). Mean separation was determined using the PDIFF procedure, and significance was declared at p<0.05. The experimental data were analyzed in a 4 × 4 Latin square design by following model:

$$Y_{ijkl}=\mu+C_i+P_j+PS_{(k)}+iNDF_{(l)}+iNDF*PS_{(hi)}+eij_{(kl)}$$

Where, Yijkl was depended variable; µ is overall mean; Ci, effect of cow (1, 2, ..., 8); Pj, the effect of the experimental period j (1,2,3, and 4); PS(h) (coarse and fine), iNDF (i)(low and high), iNDF*PS(hi) the interaction of iNDF and PS, and eij(kl), was experimental error. Means were separated using Duncan's multiple range tests with an alpha level of 0.05.

Results

Characteristics of diets

Dry and organic matter, EE, CP, and NEl content of rations were similar but their NDF, iNDF240, iNDF2.4, NFC, lignin, ADF, and starch content were different (**Table 1**). Reduction of PS decreased particles remaining on the sieve 19 mm but increased particles on 8 and 1.18 mm sieves and was led to a significant reduction in the geometric mean of particles while their standard deviations were similar. The value of pef>8 and peNDF>8 of the treatments affected by PS reduction, but pef>1.18 was affected by iNDF*PS.

Feed intake and digestibility

With decreasing dietary iNDF, intake of DM, OM, ash, EE, and NDF decreased but the consumption of starch increased. Reduction of PS increased intake of DM, OM, ash, EE, NDF (**Table 2**). However, intake of DM and OM were not affected by iNDF*PS but EE and ash were affected at higher levels of iNDF and small PS lead to more consumption of ash and EE. Protein intake was not affected by PS but was affected by levels of iNDF. The digestibility of DM, OM, CP, and NFC were similar between treatments, but the digestibility of EE and NDF affected by both iNDF and by iNDF*PS, respectively (**Table 2**).

Rumen fermentation and kinetics

Reduction of PS and decreasing of iNDF decreased rumen pH. Reduction of PS decreased NH3-N in rumen environment. The total concentration of VFA affected by iNDF (p=0.0054) and PS (p<0.0001) except for concentration of propionate, butyrate, and the acetate: propionate ratio; the concentration of acetate, isobutyrate, isovalerate and valerate were similar. The ruminal particulate passage rate was higher in treatment that had fine PS. In contrary, the ruminal mean retention times were higher in treatments that had large PS. Lower compartment passage rate affected forage PS (p<0.0001) and iNDF (p<0.0001). The NLH treatment had a greater passage rate in lower compartment than other treatments. Mean retention in lower compartment affected by iNDF of ration (**Table 3**).

Chewing behavior

Reduction of PS and increasing of iNDF decreased and increased eating time. Eating time as min/kg of DM, and peNDF>1.18 had similar trend and decreased when PS reduced. Eating time as min/kg of NDF and pdNDF decreased when PS reduced, but as min/kg of peNDF>8 decreased when PS and iNDF content increased; and as min/kg of iNDF decreased when iNDF increased in rations. Rumination as min/kg of DM, NDF, and peNDF>1.18 decreased when PS reduced and iNDF increased, but as min/kg of pdNDF decreased when PS reduced. Rumination as min/kg of peNDF>8 was greater in FL and FH in comparison to LL and LH. The PS had no effect on rumination, as min/kg of iNDF. Total chewing time as min/kg of DM, CP, NDF, and pdNDF had similar trend and decreased when PS of rations reduced. Total chewing time as min/kg of peNDF>8 was greater in LH in comparison to other treatments and decreased by PS and iNDF increasing. Total chewing time as min/kg of peNDF>1.18 and min/kg of pdNDF decreased when PS reduced. The PS had no effect on total chewing time as min/kg of iNDF but the iNDF of the ration and iNDF*PS had.

Milk yield and composition

Milk yield, FCM, and ECM decreased by increasing of iNDF content of rations, but PS and iNDF*PS had no effect. Milk fat percentage and yield increased and decreased by increasing of iNDF. In contrary, milk protein percentage and yield decreased and increased by increasing of iNDF. The percentage of lactose and SNF and milk density were similar among treatments. The percentage of milk protein reduced in low iNDF diets in comparison to high iNDF diets. Cows received large PS and lower iNDF had a greater yield of milk, fat, protein, lactose, and SNF. Reducing of forage PS in low iNDF diets only decreased the SNF content, but in high iNDF diets reduced yield of protein, lactose, and SNF. Energy and protein efficiency deceased when PS and NFC content of ration reduced.

Discussion

Particle size distribution and physically effective fiber

The PS distribution varied between the rations for the upper, middle, lower sieves, and bottom pan. Treatment LL and LH had the highest amount of DM on 19 mm sieves. One way to describe a target for how a forage or diet should look was to set a minimum for the percentage on the top sieves and the guideline is 15% of as fed forage mass retained on the top sieves to ensure the minimum requirement of dairy cattle [12]. Heinrichs et al. [17] reported that commercial dairy rations contain 7% (1 to 43%) of the particles greater than 19.0 mm. Kononoff [12] found that DMI depends on length size and diets containing alfalfa haylage as a forage source, increasing the portion of particles>19 mm increased chewing and ruminating activity and negatively correlated with the amount of time the rumen pH was below 5.8. However, in all treatment the percentage of particle on the top sieves were lower that 15%. Using three sieves results in higher pef values than when two sieves are used. When three sieves are used the pef>1.18 values for forages with differing chop lengths are not very different, as shown for LL *vs.* FL and LH *vs.* FH (**Table 1**). Yang and Beauchemin [18] and Kononoff [12] compared peNDF>8 and peNDF>1.18, and found that peNDF>1.18 contains a large pool of particles, was always lower than peNDF>1.18 but the peNDF>1.18 values were closer to 21% suggested by Mertens [2]. Therefore, the peNDF>8 is not accurate. However, using pef>1.18 when diets has forages with varying chop lengths, show only small differences that confirmed by Yang and Beauchemin [18]. With two sieves, the pef ranged from 0.184 to 0.333, but with three sieves the pef ranged from 0.811 to 0.849, with no difference in low and high iNDF rations that contained long and fine chopped Alfalfa. When two sieves were used the PS and iNDF affected on peNDF>8. However, when three sieves were used the PS had no effect on peNDF>1.18 but the iNDF and iNDF*PS affected on peNDF>1.18 (**Table 1**). Consequently, there is variation between the measured pef obtained using several methods. The peNDF>8 was lower than the peNDF>1.18. The geometric means of particle in treatments were greater than 3.5-mm. The ration had different geometric means, but their SD was low and similar that leaded to the reduction of feed selection, sorting and increased uniformity.

Feed intake and digestibility

The intake response to reduced forage PS and fiber level is not always consistent, but depending on the reduction extent, forage and concentrate type, F:C ratio, forage digestibility, rumen degradation rate, fragility, and functional specific gravity [3,16]. In experimental rations, the NDF content was higher than the minimum recommendation (from 33.96 to 36.35%). Generally, at high NDF diets rumen fill limits DMI, reducing of PS increases DMI because decreases the filling effects and increases passage rate (**Table 3**). The DM, OM, and NDF intake increased by reducing of PS similar to previous studies Teimouri Yansari [4], Yang and Beauchemin [18], Teimouri and Kononoff [12]. However Tafaj et al. [19] and Zebeli et al. [20] reported that reducing the PS had no effect on DMI. Zebeli et al. [21] found that in lactating dairy cattle, daily DMI averaged 23.0 ± 0.17 kg or 3.5 ± 0.03 kg/100 kg of BW, intakes of peNDF>1.18 and peNDF>8 averaged 4.9 ± 0.16 kg and 3.3 ± 0.19 kg, respectively. However, in this experiment, DMI (as kg/day or kg/100 kg of BW; **Table 2**) and peNDF>8 were lower but the peNDF>1.18 intake was higher than the above average. When peNDF>1.18 is higher than 21% DM, the relationship among DMI and peNDF>1.18 is negative [21]. Although in diets based on forages like alfalfa hay, grass hay, and grains like barley or wheat with relatively higher contents of peNDF>1.18 lower DMI was observed [21]. Reduction of forage PS and rumen filling effect limits-logging seems to increase passage rate and increased DMI.

Dietary PS influences the rumen passage rate and modifies the digestibility especially for low digestible nutrients. Therefore, reduction NDF digestibility may be resulted of reduced rumen digestion because of lower mean retention time in FH. The extent of ruminal NDF digestion depends on the size of the indigestible fraction and the competition among the rates of degradation and passage out of the rumen. Short PS although increases the surface available for microbial but leads to more rapid breakdown of the cell wall and increases the passage rate, reduces rumen retention time, and reduction of digestibility (**Table 3**). However, Yansari et al. [16] and Zebeli et al. [20] found the digestibility of the NDF fraction was not affected by reducing the PS of alfalfa hay. The NFC digestibility was similar between treatments. Reduction of PS increased intake of starch that may decrease ruminal NDF digestion, but may have little to no effect on total tract NDF digestion because of increased NDF digestion in the hind gut. Kononoff [12] found that reduction in PS increased digestibility of all nutrients except ADF and NFC because the increased surface area available for microbial attack, ultimately resulting in a more rapid rate of rumen fermentation and increased intake. These results show that increasing intake of peNDF can increase ruminal fiber digestion by improving rumen function or intestinal fiber digestion.

Volatile fatty acids production

Similar to Poorkasegaran [22] and Teimouri Yansari [4], reduction of PS and iNDF decreased rumen pH and increased the rate of acid production in the rumen. The pH was greater than 6.25 in all treatments. Diets rich in NFC (LL and FL) allow an increase of ruminal fermentation, higher VFA production, and decline ruminal pH therefore it is essential to increase NDF of TMR or peNDF to maintain an optimal ruminal pH (**Table 3**). Reducing PS may reduce the proportion of peNDF in rations and this may negatively affect rumen pH. A content of peNDF>1.18 of about 30-32% is sufficient to maintain ruminal pH of 6.2, lowering the risk of subclinical ruminal acidosis, and preventing milk fat depression without exerting any negative effects on DMI and milk production, which may be problematic when peNDF>1.18 is below 19.5% [20,21]. They recommend that a ratio among peNDF>1.18 and ruminally degradable starch should be lower than 1.45. The low and high iNDF rations had 33.96 and 36.65% NDF therefore it seems that decline ruminal pH is due to reduction of peNDF>8 and peNDF>1.18. The ratio among intake of peNDF>1.18, NFC, and digestible starch were 1.45, 1.44, 1.28, and 1.30; 1.76, 1.71, 1.56, and 1.58 in LL, FL, LH, and FH, respectively. When iNDF decreased in LL and FL treatments, NFC content and intake increased that resulted in increased of butyrate in the rumen which in turn increased ruminal epithelia growth, absorption capacity for VFA, and uptake acetate and butyrate in ruminal epithelia that are positively related to rumen pH. Feeding long PS can shift the site of starch digestion from the rumen to intestine, which reduces rumen pH and the potential for ruminal acidosis. Therefore, nutritionists should be mindful of PS, NDF, iNDF, and NFC independently of peNDF recommendations because they may have even greater effects on variation in rumen pH than ration each alone.

Changes in physical characteristics of the ration resulted in effects on rumen VFA patterns. Kononoff [12] found that total VFA, acetate, propionate, and butyrate and also acetate: propionate ratio increased linearly with a reducing of PS. In animals consuming short alfalfa hay rations containing 3% of the particles>19-mm, digestibility and VFA concentration were highest but mean rumen pH was lowest showing that substrate availability to rumen microbes increased with reduction of PS. In addition, reduction in PS increased digestibility of all nutrients except ADF and NFC.

Reduction of PS decreased NH_3-N but iNDF and iNDF*PS was not influenced on rumen NH_3-N. Kononoff [12] found that ruminal NH_3-N unaffected by diet (averaging 11.24 mg/dL). Oba and Allen [23] found dairy cattle with high DMI and 18% CP averaged about 7.2 mg/dL for NH_3-N. Maulfair [24] and Heinrichs [17] showed that NH_3-N was not affected by PS of forage, rumen fermentable carbohydrates, and their interaction. When forage NDF was decreased from 25.4 to 16.5%, ruminal NH_3-N concentration decreased. Carbohydrate supply profoundly influences the amount of ruminal NH_3-N assimilated into microbial protein.

The PS is important in relative to the rumen fractional rate of passage because feed PS should be reduced to particles small enough to pass through the reticulo-omasal orifice. Most of particles already have an appropriate size to leave rumen but there is a ruminal selective retention mechanism for increasing their retention. The ruminal particulate passage rate was higher in treatment that had fine PS. Rations with smaller PS leave the rumen more rapidly, stay in the rumen for a shorter time, are less available for microbial digestion, and increased DMI. The lower compartment passage rate increased in LH treatment by PS and iNDF. Tafaj et al. [25] found that giving a high NDF diet increased chewing activity, decreased passage rate, and increased nutrients digestibility but giving a diet of low NDF reduced the chewing activity, affected negatively the rumen conditions, and consequently, the ruminal digestion of fiber. Reduction of PS in the high iNDF diet improved the rumen conditions, decrease of the ruminal passage rate, increased retention time, and improved fiber digestibility. Similarly, Tafaj et al. [25] found that increasing the NFC caused an increased passage rate and decrease rumen retention time. Long PS or more NDF or iNDF creates a floating rumen mat that stimulates reticuloruminal contractions. Without these mixing motions the rumen becomes a less dynamic pool, and removal of VFA *via* absorption and fluid passage from the rumen declines, thereby increasing the risk of acidosis.

Chewing activity and eating behavior

Reduction of PS and increasing of iNDF decreased and increased eating time. Rumination as min/kg of DM and NDF decreased when PS reduced and iNDF increased in rations. Total chewing time decreased when PS reduced (**Table 4**). The amount of TMR retained on the 19-mm is positively correlated with ruminating activity [12]. In all treatment the retained particles on the top sieves were lower that 15%, but peNDF>1.18 was greater than 27.4% DM. The dietary peNDF>1.18 and NFC of rations due to their effected on chewing activity and rumen metabolism affect milk fat content therefore dairy cows must consume a sufficient amount of peNDF to achieve a chewing time of 687 min/d or 30 min/kg DM ingested to produce milk having 3.4% of fat [21]. All experimental rations induced chewing time greater than 726.25 min per day that confirmed that increasing the intake of long forage PS and more peNDF increases chewing time.

The time spent on chewing per 1 kg of DM or NDF considered as fibrousness index of forages that attributed but varies depending on the animal breed, body size, level of intake, fiber nature and content, PS, level of DM and peNDF intake, and physiological state [2]. Because not all sources of NDF are equal, a method to discount fiber relative to some standard such as PS, functional specific gravity, fragility, and digestibility or indigestibility; is a necessary part of any fiber requirement system [16,3]. However, the PS and NDF content are more reliable indicators of chewing activity than the NDF content of the forage alone [5]. The pef measurement is used as a discount factor of NDF that is applied to the peNDF system (adjusted NDF based on PS). Also, the mastication efficiency could be measured as time spent chewing per unit of intake. The chewing and ruminating efficiency was markedly increased when PS and iNDF content increased. Rumination is positively related to feeding time and DMI. Cows ruminate 25-80 minutes per kg of roughage consumed and 150 minutes per kg of NDF for long grass hay [2]. This relationship among NDF and chewing response forms the basis of peNDF system. The peNDF is believed to be highly resistant to the passage, stimulates chewing and rumen mat formation. The peNDF is based on the two fundamental properties of feeds that influence eating and ruminative chewing: fiber content and PS. A major assumption of the peNDF system was that forage PS explains all the variation in chewing response, but this assumption is not always true because forages with similar PS or equal NDF content may elicit substantially different chewing times per kg of NDF or peNDF. Because the nature of NDF of different sources is not similar and it is a non-uniform feed fraction, but iNDF is a uniform feed fraction with zero digestibility [3]. As the iNDF is the functional fiber fraction that influences gut fill, ruminal retention, digestion, and passage dynamics of forages therefore, determination of pdNDF and iNDF is be valuable in feed evaluation to predict digestibility, passage rate, and chewing activity. The results showed that using iNDF as a fibrous index is more sensitive than DM, NDF, and peNDF>1.18, especially in the low iNDF ration (**Table 4**).

Milk yield and composition

The effect of iNDF content and PS on DMI, milk yield and composition is not consistent. Milk yield, FCM, ECM, milk fat and protein yield decreased, but percentage of milk fat and protein increased by increasing of iNDF in rations (**Table 5**). Decreasing iNDF with increasing NFC, starch, and ruminal starch degradation of diets are common approaches to maximize energy intake, milk production, might improve ruminal microbial protein synthesis, and ultimately increase yield of milk and protein [7]. Response for DMI in the lower iNDF (higher NFC or starch) diet is less rumen filling that is a greater limitation to intake. Cows received coarse PS and more NFC had a greater yield of milk, fat, protein, lactose, and SNF. Reducing of forage PS in low iNDF-diets only decreased the SNF content, but in the high iNDF-diets reduced yield of protein, lactose, and SNF of milk. Feeding diets excessive in peNDF may also adversely affect milk production and feed efficiency. Zebeli et al. [20] reported that high-producing dairy cows require sufficient dietary peNDF>1.18 to maintain the milk fat content. The grain fermentability increases the need for peNDF>1.18 to maintain the milk fat content because when peNDF>1.18 contents of ration were 23% [2], 26% [12], and 29% [26] on maize grains, high moisture maize, and a high barley starch diet, milk fat content was lower than 3.4%, respectively. Also Zebeli et al. [21] found that when peNDF>1.18 measures were >21%, increasing peNDF had a negative effect on feed intake and milk yield, but regardless of the source of dietary grain, in early lactating dairy cows that feeding peNDF>1.18 levels of 30-32% does not negatively affect milk yield Zebeli et al. [20]. In this experiment, the peNDF>1.18 intake was in rang of this recommended level. However, by increasing peNDF>1.18 content of diets based on rapidly digested barley starch, Yang and Beauchemin [5] and Silveira et al. [26] found that milk yields decreased due to lowering of DMI but milk fat content and yield, the 3.5% fat-corrected milk, and milk

Table 4. Chewing activity of cows of cows fed the experimental rations with particle size and two level of indegradable NDF.

Items	Experimental Treatments[1]				Mean Standard Error	P-Values		
	LL	FL	LH	FH		PS	iNDF[2]	iNDF*PS
Eating time								
min/day	293.13[a]	262.50[b]	301.88[a]	275.00[b]	4.102	***	*	NS
min/kg of DM	15.02[a]	12.80[b]	14.50[a]	12.90[b]	0.223	***	NS	NS
min/kg of NDF	44.25[a]	37.32[c]	41.69[b]	36.99[c]	0.907	**	NS	NS
min/kg of $peNDF_{>8}$	32.82[b]	42.12[a]	26.72[c]	36.55[b]	1.336	**	*	NS
min/kg of $peNDF_{>1.18}$	10.63[a]	9.39[b]	10.27[a]	9.95[b]	0.174	*	NS	NS
min/kg of pdNDF	10.74[a]	9.55[b]	10.77[a]	9.62[b]	0.224	**	NS	NS
min/kg of iNDF	43.37[b]	37.82[c]	45.12[a]	44.15[ab]	0.979	NS	*	NS
Rumination								
min/day	478.1	463.75	482.5	464.38	5.822	*	NS	NS
min/kg of DM	24.51[a]	22.62[b]	23.18[b]	21.79[c]	0.28	***	**	NS
min/kg of NDF	72.18[a]	66.70[b]	67.49[b]	63.43[c]	0.821	***	***	NS
min/kg of $peNDF_{>8}$	51.53[c]	72.71[a]	43.17[d]	57.56[b]	1.945	***	***	NS
min/kg of $peNDF_{>1.18}$	17.46[a]	16.64[b]	16.62[b]	16.61[b]	0.199	NS	*	NS
min/kg of pdNDF	17.59[a]	17.04[a]	17.40[ab]	16.46[b]	0.21	**	NS	NS
min/kg of iNDF	70.52[bc]	69.26[c]	72.84[b]	75.58[a]	0.921	NS	**	*
Total chewing time								
min/day	771.25[a]	726.25[b]	784.38[a]	739.38[b]	6.77	***	NS	NS
min/kg of DM	37.20[a]	35.01[b]	37.93[a]	35.61[b]	0.363	***	NS	NS
min/kg of NDF	106.41[a]	100.14[b]	104.35[a]	97.97[b]	1.019	***	NS	NS
min/kg of $peNDF_{>8}$	92.95[b]	87.48[c]	101.15[a]	94.96[b]	0.9412	***	***	NS
min/kg of $peNDF_{>1.18}$	28.16[a]	26.07[c]	27.02[b]	26.43[bc]	0.222	***	NS	**
min/kg of pdNDF	28.38[a]	26.68[b]	28.29[a]	26.21[b]	0.245	***	NS	NS
min/kg of iNDF	113.76[b]	108.46[c]	118.41[a]	120.34[a]	1.158	NS	***	**

[a,b,c,d] Means within a row with different superscripts differ ($P < 0.05$).
[1]Four treatments including diet contained: 1) long alfalfa hay and low iNDF (LL), 2) fine alfalfa hay and low iNDF (FL), 3) long alfalfa hay and high iNDF (LH), and 4) fine alfalfa hay and high iNDF (FH)
[2] The NDF concentration was measured using α-amylase and without sodium sulfite [8]

Table 5. Milk yield and composition of cows fed the experimental rations with particle size and two level of indegradable NDF.

Items	Experimental Treatments[1]				Mean	P-Value		
	LL	FL	LH	FH	Standard Error	PS	iNDF	iNDF*PS
Milk yield, Kg/day								
Milk yield	32.12[a]	31.63[a]	29.99[b]	29.72[b]	0.472	NS	***	NS
Fat Corrected milk for 3.5%	32.02[a]	31.13[a]	30.73[b]	30.12[b]	0.429	NS	***	NS
ECM	32.22[a]	31.47[b]	30.63[b]	30.04[bc]	0.465	NS	***	NS
Milk Composition, %								
Fat	3.48[b]	3.40[b]	3.67[a]	3.58[a]	0.101	NS	*	NS
Protein	3.13[ab]	3.16[a]	3.08[b]	3.06[b]	0.049	NS	**	NS
Lactose	4.61	4.59	4.6	4.58	0.068	NS	NS	NS
Solid non-fat	8.09	7.99	8.25	8.16	0.143	NS	NS	NS
Density	33.36	33.74	33.71	33.06	0.472	NS	NS	NS
Milk Composition, Kg/day								
Fat	1.12[a]	1.07[ab]	1.09[b]	1.06[b]	0.031	NS	**	NS
Protein	1.01[a]	1.00[a]	0.92[b]	0.91[c]	0.021	**	***	NS
Lactose	1.48[a]	1.45[ab]	1.38[b]	1.36[c]	0.03	*	***	NS
Solid non-fat	2.60[a]	2.53[b]	2.47[b]	2.45[c]	0.059	**	**	NS
Energy efficiency	1.22[a]	1.12[b]	1.24[a]	1.18[ab]	0.018	**	NS	NS
Protein efficiency	0.31[a]	0.29[a]	0.28[b]	0.27[c]	0.006	*	***	NS

[a ,b, c] Means within a row with different superscripts differ ($P < 0.05$).
[1]Four treatments including diet contained: 1) long alfalfa hay and low iNDF (LL), 2) fine alfalfa hay and low iNDF (FL), 3) long alfalfa hay and high iNDF (LH), and 4) fine alfalfa hay and high iNDF (FH)

energy efficiency increased because of better rumen conditions and higher acetate: propionate ratio. Milk fat content is often used as an indicator of fiber adequacy, rumen health, reflects ruminal acid production, and animal performance. Reduction milk fat was about 0.2% when NFC of rations increased in LL and FL treatments that are not indicator of fiber deficiency in the ration because milk fat reduction about 0.6% in one week could be considered as an indicator of fiber deficiency in dairy cows.

Conclusion

The DM, OM, and NDF intake increased by reducing of the PS. The rumen pH was greater than 6.25 in treatments, however diets low in iNDF allow an increase of ruminal fermentation, higher VFA production, and decline ruminal pH. Reduction in PS increased digestibility of all nutrients except ADF and NFC; and decreased NH_3-N in rumen. The ruminal passage rate was higher in treatment that had fine PS. Using iNDF as an index is more sensitive than DM, NDF, and peNDF>1.18, especially in low iNDF ration. The PS had no effect on chewing time for iNDF but the iNDF content of ration and interaction of iNDF and PS had. Milk yield, milk fat and protein yield decreased but percentage of milk fat and protein increased by increasing of iNDF. Cows received coarse PS and lower iNDF had a greater yield of milk, fat, protein, lactose, and SNF. Reducing of forage PS in the low iNDF-diets only decreased the SNF content, but in the high iNDF-diets reduced yield of protein, lactose, and SNF.

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References

1 Cotanch K, Grant R, Van Amburgh M, Zontini A, Fustini M, et al. (2014) Applications of uNDF in ration modeling and formulation. In proceedings cornell nutrition conference. Cornell University. NY 114-131.

2 Mertens D (1997) Creating a system for meeting the fiber requirements of dairy cows. J Dairy Sci 80: 1463-1481.

3 Zali S, Yansari AT, Sayyadi AJ (2015) Effect of particle size and fragility of corn silage and alfalfa hay on intake, digestibility, performance, and chewing activity of fattening male lambs. Res Rev J Veter Sci 1: 1-10.

4 Yansari AT (2014) Physically effectiveness of beet pulp-based diets in dairy cows as assessed by responses of feed intake, digestibility, chewing activity and milk production. J Anim Physiol Anim Nutr 98: 158-168.

5 Yang W, Beauchemin K (2009) Increasing physically effective fiber content of dairy cow diets through forage proportion versus forage chop length: Chewing and ruminal pH. J Dairy Sci 92: 1603-1615.

6 Huhtanen P, Kaustell K, Jaakkola S (1994) The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim Feed Sci Technol 48: 211-227.

7 NRC (2001) Nutrient requirements of dairy cattle. National Academy press. Washington DC.

8 Van Soest PJ, Robertson J, Lewis B (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74: 3583-3597.

9 Chandler JA, Jewell WJ, Gossett JM, Van Soest PJ, Robertson JB (1980) Predicting methane fermentation biodegrad-ability. J Biotech and Engineering Symp No. 10: 93-107

10 Raffrenato E, Van Amburgh M (2010) Development of a mathematical model to predict sizes and rates of digestion of a fast and slow degrading pool and the indigestible NDF fraction. In proceedings cornell nutrition conference. NY 52-65.

11 Lammers B, Buckmaster D, Heinrichs A (1996) A simple method for the analysis of particle sizes of forage and total mixed rations. J Dairy Sci 79: 922-928.

12 Kononoff P (2002) The effect of forage particle size on cows in early lactation. Ph.D Thesis. The Pennsylvania State University, USA.

13 AOAC (2002) AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. J AOAC Int 85: 1187-1200.

14 Hall M, Larson C, Wilcox C (2010) Carbohydrate source and protein degradability alter lactation, ruminal, and blood measures. J Dairy Sci 93: 311-322.

15 Udén P, Colucci PE, Van Soest PJ (1980) Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J Sci Food Agri 31: 625-632.

16 Yansari AT, Valizadeh R, Naserian A, Christensen D, Yu P, et al. (2004) Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. J Dairy Sci 87: 3912-3924.

17 Heinrichs A, Buckmaster D, Lammers B (1999) Processing, mixing, and particle size reduction of forages for dairy cattle. J Anim Sci 77: 180-186.

18 Yang W, Beauchemin K (2007) Altering physically effective fiber intake through forage proportion and particle length: Chewing and ruminal pH. J Dairy Sci 90: 2826-2838.

19 Tafaj M, Zebeli Q, Baes C, Steingass H, Drochner W (2007) A meta-analysis examining effects of particle size of total mixed rations on intake, rumen digestion and milk production in high-yielding dairy cows in early lactation. Anim Feed Sci Technol 138: 137-161.

20 Zebeli Q, Aschenbach J, Tafaj M, Boguhn J, Ametaj B, et al. (2012) Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J Dairy Sci 95: 1041-1056.

21 Zebeli Q, Mansmann D, Steingass H, Ametaj B (2010) Balancing diets for physically effective fibre and ruminally degradable starch: A key to lower the risk of sub-acute rumen acidosis and improve productivity of dairy cattle. Livestock Sci 127: 1-10.

22 Poorkasegaran S, Yansari AT (2014) Effects of different sources of carbohydrates on intake, digestibility, chewing, and performance of Holstein dairy cows. J Anim Sci Biotechnol 5: 1-11.

23 Oba M, Allen M (2003) Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. J Dairy Sci 86: 195-207.

24 Maulfair D, Heinrichs A (2013) Effects of varying forage particle size and fermentable carbohydrates on feed sorting, ruminal fermentation, and milk and component yields of dairy cows. J Dairy Sci 96: 3085-3097.

25 Tafaj M, Kolaneci V, Junck B, Maulbetsch A, Steingass H, et al. (2005) Influence of fiber content and concentrate level on chewing activity, ruminal digestion, digesta passage rate and nutrient digestibility in dairy cows in late lactation. Asian-Australas J Anim Sci 18: 1116-1124.

26 Silveira C, Oba M, Beauchemin K, Helm J (2007) Effect of grains differing in expected ruminal fermentability on the productivity of lactating dairy cows. J Dairy Sci 90: 2852-2859.