

## Effect of Particle Size and Fragility of Corn Silage and Alfalfa Hay on Intake, Digestibility, Performance, and Chewing Activity of Fattening Male Lambs

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### Research Article

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Implications: The differences in particle size, fragility and digestibility may explain the variation in chewing response not explained by particle size alone. The results showed that unfragile DM was the best predictor of chewing activity and combination of particle size and fragility in rations can estimate its effectiveness well.

#### ABSTRACT

Particle size, digestibility, and fragility of ration influence physically effectiveness of fiber but their correction procedures and effects in fattening lambs have not been studied well. The experiment evaluated the effects of these factors on effectiveness and performance of fattening lambs. Using three cannulated Zel ewes, DM, NDF and CP ruminal degradation was determined. Also, after 240h ruminally incubation, indigestible (iNDF) and potential digestible NDF (pdNDF) were determined. Ration NDF, iNDF and pdNDF were 34.28% DM, 58.31 and 41.69% of NDF, respectively. Using a complete randomize design, 24 Iranian Zel male fed ad libitum twice daily at 0830 and 2030 h over 90 days with a basal concentrate plus: 1) Short corn silage with long alfalfa hay (SLC), 2) Long corn silage with short alfalfa hay (LSC), and 3) Short corn silage with short alfalfa hay (SSC) as treatments. On days 30, 60, and 90, the lambs were weighed and at day 90 were slaughtered. Reduction of particle size reduced the fragility index, increased DMI, ADG, reduced digestibility of NDF, eating and ruminating time. The intake of DM, peNDF $>8$  and peNDF $>1.18$ , fragile DM and NDF, and unfragile DM and NDF explained 74.23, 87.06, 69.45, 74.24, 9.11, 94.41 and 62.81% of variation in chewing activity, respectively. Unfragile DM was the best predictor of chewing. Using peNDF $>1.18$  intake and fragile DM in a linear model explained maximum (95.41%) of variation in chewing activity. Therefore, combination of particle size and fragility in rations can estimate its effectiveness because there is a positive relationship between NDF digestibility and fragility and NDF fragility may be more useful in adjusting peNDF values than a direct measure of digestibility.

### INTRODUCTION

The physically effectiveness NDF (peNDF) is the fraction of NDF that stimulates chewing, forms ruminal mat, resistances to rumen escape, and measures by dry sieving as the fraction of particles retained on the 1.18-mm sieve multiplied by the feed NDF<sup>[1]</sup>. A major assumption of the peNDF system was that forage particle size explains all of the variation in chewing response, but this assumption is not always true because forages with similar particle length may elicit substantially different chewing times per kg of NDF. Some characteristics such as digestibility<sup>[2]</sup>, forage fragility and stem brittleness<sup>[1]</sup>, and functional specific gravity<sup>[3]</sup> may explain the variation in chewing response not explained by particle size.

Fragility is defined as the relative rate at which forage is reduced in particle size during chewing or some laboratory simulation of chewing action. It related to lignin concentration, digestibility, and anatomical differences among forages such as cell wall thickness<sup>[4]</sup>. Consequently, digestibility of forage cell wall may be predictive of its fragility. Based on composition with resistance to comminution and voluntary intake in sheep, Minson<sup>[5]</sup> categorized grasses as high (81%), medium (72%), and low (56%) digestible, and found NDF digestibility has a positive and negative correlation with fragility and chewing activity, respectively; therefore, the NDF digestibility may be more useful in adjusting peNDF values than a direct measure of fragility.

Grant <sup>[6]</sup> found that the 24 h NDF digestibility explains about 60% of the variation in forage fragility, the potential exists to combine a “fragility factor” related to NDF digestibility with the physically effective factor (pef) that derived by sieving to arrive at a superior value to predict cow chewing response. Adjusting a pef value based on fragility or NDF digestibility, determination of pdNDF and iNDF is be valuable in feed evaluation <sup>[7]</sup>. Due to lack information about fragility, variability of rumen degradation for NDF and the influence thereof on animal performance <sup>[6]</sup>, research about fragility, iNDF, pdNDF, determination of their effects is critical for effective ruminant feeding. Therefore, the objectives of this experiment were to ccombination of particle size and digestibility or fragility of rations to estimate the effectiveness and its relation to performance of fattening of male lambs that fed with alfalfa hay and corn silage in different particle length.

## MATERIALS AND METHODS

### Particle distribution and physically effectiveness

Particle size distributions were determined by dry sieving using 19, 8, and 1.18 mm Penn State Particle Separator sieves. The geometric mean (GM) and its SD was calculated <sup>[8]</sup>. The pef of rations were determined as the proportion of DM retained on 19 and 8 mm sieves (pef>8; <sup>[9]</sup>) and on 19, 18, and 1.18 mm sieves (pef>1.18; <sup>[10]</sup>). The pef>8 and peNDF>1.18 were calculated by multiplying the NDF of each portion on each sieve on pef>8 and pef>1.18, respectively.

### Fragility of rations

Dried sample placed in a ball mill that loaded with ceramic balls, sieved, and the pef prior (pefi) and following ball milling (pefBM) were determined. Fragility determined using following equation: (pefi – pefBM)/pefi x 100% <sup>[2]</sup>.

### Ruminal degradation and indigestible NDF

The ruminal degradation determined with in situ method, using three ruminal cannulated Zel ewes (two years old, BW=30.5 ± 1.8 kg). The sheep was fed a TMR containing 50% chopped alfalfa hay, 25% wheat straw, and 25% barely grain plus mineral/vitamin supplement according to their requirements. Three grams of sample in four replications were weighed in sealed nylon bags (6 cm × 7.5 cm, polyamide, 26% porosity 40± 10 μ pore size) and incubated in the rumen for 0, 1, 3, 6, 12, 24, 36, 48 h. The kinetics of DM, CP, and NDF disappearance was estimated by the nonlinear regression procedure of <sup>[11]</sup> using the model of  $Y=a + b(1-\exp(-Kd*t))$ ; where, a, soluble fraction (%); b, slowly digestible fraction (%); Kd, fractional rate of disappearance (per h); and t,=time of incubation (h) <sup>[12]</sup>.

Also, using three ruminal cannulated ewes, five grams of sample were weighed in sealed nylon bags (7 cm × 8 cm, polyamide, with 15 ± 2 μ pore size), incubated in the rumen for 240 h <sup>[13]</sup>. Residual of samples were analyzed for NDF <sup>[14]</sup>. The pdNDF was calculated (pdNDF=NDF–iNDF; <sup>[7]</sup>). Also, using 2.4 × ADL equation, the iNDF2.4 was calculated <sup>[15]</sup>.

### Lambs performance

Using a complete randomize design, 24 Iranian Zel male sheep (BW=31.5 ± 2.32 kg) fed with a basal concentrate plus: 1) Short corn silage with long alfalfa hay (SLC), 2) Long corn silage with short alfalfa hay (LSC), and 3) Short corn silage with short alfalfa hay (SSC) as treatments. Sheep were housed in individual 1.5 × 1.2 m cages, fed ad libitum twice daily at 0830 and 2030 h while water and mineral supplement were available during the experiments. Orts were weighed daily in each pen prior to feeding at 0830 h. Diets were formulated with SRNS version 1.9.5105. Ration had 29.14, 6.66, 2.5, 20.82, 29.18, 9.95, 0.08, and 1.67% of barely grain, beet pulp, soybean meal, alfalfa hay, corn silage, wheat bran, salt, and DCP, respectively. Early bloom alfalfa hay from a single load was used as a forage source. A half of the load was chopped by a chopper equipped with a 30 × 35 mm screen size considered as long particles. The other half was chopped while the screen size was reduced to 20 mm and considered as short particles. The corn silage that uptake from silo was considered as long particles. Small particles of corn silage were provided daily by a chopper equipped with a 20 mm screen pore size. Base on initial weights that determined using the average of weights taken on two consecutive days, the lambs allotted to 3 treatments in eight replications. On days 30, 60, and 90, following a 12 h rest period, the lambs were individually weighed. The average daily gain (ADG), DMI, and feed efficiency were determined. Total collection of feces was carried out for all sheep over days 50 to 55. On day 50, lambs were visually monitored for chewing activity (eating, ruminating and resting) in 5-minute intervals during two 24 h.

At day 90, lambs were slaughtered after a 12 h fasting period with no water restriction. Carcass dressing was determined. Carcasses were hung and refrigerated for 24 h at 4 °C. Chilled carcass weights were determined 24 h after slaughter, back fat and loin-eye area were measured, and internal fat was estimated. Fat in the rumen, intestines and kidneys was removed manually and determined as percentage of slaughter weight.

### Laboratory analyses

Feeds, rations, and feces samples were dried at 55 °C, ground through a Wiley mill (1-mm screen), analyzed for DM, OM, Kjeldahl N, ether extract, Starch <sup>[16]</sup>, NDF, ADF, lignin <sup>[14]</sup>; using heat resistance alpha amylase when sodium sulphate was removed) and ash at 605 °C. NFC was calculated by 1000- (CP (g/kg)+NDF (g/kg)+Ash (g/kg) +EE (g/kg)) (Table 1).

### Statistical analysis

Using a complete randomized design with three treatments, data were analyzed by PROC GLM of SAS® [11]. Means were separated using Duncan's multiple range tests with an alpha level of 0.05. Also, using PROC REG of SAS® [11] the relationship between some measurements were investigated.

## RESULTS

### Chemical composition, particle size and fragility

The feeds represented a wide range of chemical composition (**Table 1**). The iNDF of barley grain, beet pulp, soybean meal, alfalfa hay, corn silage, and wheat bran were 572.1, 599.5, 600.2, 235.7, 482.5 g/kg of NDF, respectively. The rations had different particle size but similar ingredient ratios, NDF(34.28%), CP(15.75%), iNDF(16.32%), NFC(40.28%), starch (31.55%), and pdNDF (52.39% NDF). Diets contained 50% forage to achieve the targeted forage NDF concentration.

**Table 1.** Chemical compositions (%) of ingredients and ratios (%) of experimental rations.

Items	Barley	Beet	Soybean	Alfalfa	Corn	Wheat	Salt	DCP	Experimental Ration
	grain	pulp	meal	hay	silage	bran			
Ash	2.40	8.45	6.70	9.00	3.00	6.90	100	100	6.63
CP	13.20	8.73	43.80	18.82	11.85	17.1	...	...	15.75
Ether extract	2.20	0.48	1.10	2.50	3.00	4.40	...	...	3.06
Lignin	1.56	4.13	2.50	12.21	11.00	5.88	...	...	6.64
NDF	18.10	35.80	15.79	42.00	48.40	48.45	...	...	34.28
iNDF240	8.86	20.48	4.67	22.21	10.23	24.61	...	...	16.32
iNDF2.4	3.74	9.91	6.00	29.30	26.4	14.11	...	...	15.94
ADF	7.21	26.31	11.03	33.22	28.7	14.21	...	...	25.43
Non fiber carbohydrate	64.10	46.54	32.61	27.68	33.75	23.15	...	...	40.28
Starch	53.93	2.43	6.31	25.24	28.43	14.43	...	...	31.55

The particle distribution varied among the rations for the upper, middle and lower screens and for the bottom pan ( $P < 0.0001$ ). Geometric mean, its SD, pef, and peNDF were different ( $P < 0.0001$ ). The GM of particle in treatment 1, 2 and 3 were 17.31, 7.63, and 0.98 mm, respectively. Also, regardless to determination method, treatment 1 had the highest pef and peNDF in comparison to others. Consequently, there is variability among the measured pef obtained using several methods. The  $peNDF > 8$  were 0.99, 0.94, and 0.45% lower than the  $peNDF > 1.18$  values in treatments 1, 2, and 3, respectively.

Initial and after milling pef and fragility index of rations were different (**Table 2**). The fragility index in SSC was lower than others treatments, also LSC had the highest index. The fragility index in SSC was 18.15; therefore reduction of forage particle size decreased fragility. Treatment SLC had lower fragility than LSC that confirmed reducing of silage particle size decreased the fragility more than alfalfa. However, inclusion of long corn silage with short alfalfa hay in treatment LSC resulted in maximum of fragility.

**Table 2.** Particle sizes distribution of experimental diets using of the Penn State Particles Separator.

Items	Treatments <sup>1</sup>			SEM	P-value
	1	2	3		
Particle that remained on different sieves (% of dry matter)					
19-mm	97.74 <sup>a</sup>	77.93 <sup>b</sup>	23.59 <sup>c</sup>	0.008	<0.01
8-mm	0.82 <sup>c</sup>	3.48 <sup>b</sup>	5.14 <sup>a</sup>	0.005	<0.01
1.18-mm	0.82 <sup>c</sup>	5.55 <sup>b</sup>	34.56 <sup>a</sup>	0.007	<0.01
Pan	0.62 <sup>c</sup>	13.04 <sup>b</sup>	36.71 <sup>a</sup>	0.010	<0.01
Geometric mean <sup>2</sup> (mm)	17.31 <sup>a</sup>	7.63 <sup>b</sup>	0.98 <sup>c</sup>	0.007	<0.01
SD of geometric mean <sup>2</sup> (mm)	1.63 <sup>a</sup>	1.44 <sup>b</sup>	1.55 <sup>a</sup>	0.234	0.05
pef>8 <sup>3</sup>	98.26 <sup>a</sup>	81.41 <sup>b</sup>	28.73 <sup>c</sup>	0.008	<0.01
pef>1.18 <sup>3</sup>	99.38 <sup>a</sup>	86.96 <sup>b</sup>	63.29 <sup>c</sup>	0.010	<0.01
peNDF>8 <sup>4</sup> (%)	33.79 <sup>a</sup>	27.91 <sup>b</sup>	9.85 <sup>c</sup>	0.008	<0.01
peNDF>1.18 <sup>4</sup> (%)	34.10 <sup>a</sup>	29.81 <sup>b</sup>	21.70 <sup>c</sup>	0.003	<0.01
Fragility <sup>5</sup>					
Initial physical effective factor	99.38 <sup>a</sup>	86.96 <sup>b</sup>	63.29 <sup>c</sup>	0.010	<0.01
After milling physical effective factor	70.38 <sup>b</sup>	51.76 <sup>c</sup>	56.80 <sup>b</sup>	0.008	<0.01
Fragility index	29.22 <sup>b</sup>	40.45 <sup>a</sup>	18.15 <sup>c</sup>	0.001	<0.01

a, b, c Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Three treatments were rations including: 1) Short corn silage with long alfalfa hay, 2) Long corn silage with short alfalfa hay, 3) Short corn silage with short alfalfa hay.

<sup>2</sup>Particle size geometric mean and its SD for chopped hay and rations determined as recommended by ASAE [8].

<sup>3</sup>The physically effective factor determined as the proportion of DM retained on two (pef>8; [9]) and three sieves of the Penn State particle separator (pef>1.18; [10]).

<sup>4</sup>The peNDF>8 and peNDF>1.18 were calculated by multiplying NDF of each ration on each sieve on pef>8 and pef>1.18, respectively.

<sup>5</sup>The fragility of feeds were determined based on methods that outlined by [2].

### Ruminally degradability and indigestible NDF

For alfalfa, potential and rate of DM, CP, and NDF degradability were 59.38, 0.055; 64.91, 0.061; and 44.11 and 0.037% and %/h, respectively. Corn silage had 70.11, 0.044; 41.56, 0.047; and 42, 0.033% and %/h potential and rate of DM, CP, and NDF degradability, respectively. The ration had 74.52, 69.62, and 54.92% potential degradable DM, CP and NDF, respectively (**Table 3**).

**Table 3.** Ruminal dry matter, crude protein, and neutral detergent fiber degradability of different experimental rations after incubation in the rumen of Zel ewes using nylon bag technique.

	Alfalfa hay	Corn silage	Total mixed ration	SEM	P-value
<b>DM</b>					
Soluble fraction (%)	18.64 <sup>c</sup>	23.45 <sup>a</sup>	26.11 <sup>b</sup>	0.369	<0.01
Slowly degraded fraction (%)	40.74 <sup>c</sup>	46.66 <sup>b</sup>	48.41 <sup>a</sup>	0.835	<0.01
Degradable fraction (%)	59.38 <sup>c</sup>	70.11 <sup>b</sup>	74.52 <sup>a</sup>	1.005	<0.01
Undegradable fraction (%)	40.62 <sup>a</sup>	29.89 <sup>b</sup>	25.48 <sup>c</sup>	1.004	<0.01
rate of degradation (%/h)	0.055 <sup>a</sup>	0.044 <sup>b</sup>	0.056 <sup>a</sup>	0.002	<0.01
<b>CP</b>					
Soluble fraction (%)	24.96 <sup>a</sup>	22.00 <sup>b</sup>	25.26 <sup>a</sup>	0.485	<0.01
Slowly degraded fraction (%)	39.95 <sup>b</sup>	19.56 <sup>c</sup>	44.36 <sup>a</sup>	0.414	<0.01
Degradable fraction (%)	64.91 <sup>b</sup>	41.56 <sup>c</sup>	69.62 <sup>a</sup>	1.872	<0.01
Undegradable fraction (%)	35.09 <sup>b</sup>	58.44 <sup>a</sup>	30.38 <sup>c</sup>	0.863	<0.01
rate of degradation (%/h)	0.061 <sup>a</sup>	0.047 <sup>b</sup>	0.049 <sup>b</sup>	0.004	<0.01
<b>NDF</b>					
Soluble fraction (%)	10.49 <sup>b</sup>	12.52 <sup>a</sup>	11.11 <sup>b</sup>	0.260	<0.01
Slowly degraded fraction (%)	33.62 <sup>b</sup>	29.48 <sup>c</sup>	43.81 <sup>a</sup>	0.190	<0.01
Degradable fraction (%)	44.11 <sup>b</sup>	42.00 <sup>c</sup>	54.92 <sup>a</sup>	0.213	<0.01
Undegradable fraction (%)	55.89 <sup>b</sup>	58.00 <sup>a</sup>	45.08 <sup>c</sup>	0.214	<0.01
rate of degradation (%/h)	0.037 <sup>b</sup>	0.033 <sup>c</sup>	0.041 <sup>a</sup>	0.007	<0.01

a, b, c means within a row with differing superscripts are significantly different ( $P < 0.05$ ).

The values of iNDF240 and iNDF2.4 are presented in **Table 1**. The value of iNDF240 in barely grain, corn silage, wheat bran and ration were greater than iNDF2.4, but in soybean meal, alfalfa hay, and beet pulp were greater lower.

### Intake, daily gain and carcass conformation

In the first, second, and final 30 days and over the experiment DMI were different among treatments ( $P < 0.0001$ ). Reducing the particle size increased DMI, so SSC had the highest DMI. Also, DMI in SLC was less compared to LSC. The average DMI for SLC, LSC, and SSC diets were 1.45, 1.53, and 1.57 kg/day, respectively. Based on average DMI and iNDF of diets, the lambs fed SLC, LSC, and SSC diet consumed 0.236, 0.249, and 0.256 kg/day; and 0.758, 0.800, and 0.821 kg/day iNDF and pdNDF, respectively. Digestion of DM, OM, NDF, and NFC affected by particle length of forage but for EE and CP was similar among treatments.

The ADG of lambs at days 1 to 30 and 61 to 90 were different (**Table 4**). In days 1 to 30, treatment SLC had the highest gain that was higher in comparison to treatment LSC and similar to SSC. Within days 61 to 90 the lowest and highest lambs gain was observed in SLC and SSC, respectively, but statistical analysis showed no significant difference between SLC and LSC and also between LSC and SSC. However, the overall ADG of lambs between treatments were similar. While lambs fed the SSC diet had significantly higher DMI than fed the LSC and SLC diets, their ADG were significantly higher. Feed conversation was significantly higher in SSC than SLC at days 1 to 30, 31 to 60, 61 to 90, and during the study but there were only significantly differ at days 31 to 60 and 61 to 90 among SSC and SLC. Also, feed conversation was different at days 61 to 90 among LSC and SLC.

**Table 4.** Average dry matter intake, daily gain of lambs, feed conversation ration, and Carcass decomposition in sheep that fed three experimental rations.

Days	Treatments <sup>1</sup>			SEM	P-value
	1	2	3		
<b>Dry matter intake (g/day)</b>					
1to 30	1395.2 <sup>b</sup>	1394.72 <sup>b</sup>	1473.64 <sup>a</sup>	21.221	<0.01

31 to 60	1446.04 <sup>c</sup>	1501.60 <sup>b</sup>	1533.64 <sup>a</sup>	12.142	<0.01
61 to 90	1497.09 <sup>b</sup>	1686.80 <sup>a</sup>	1695.47 <sup>a</sup>	19.518	<0.01
Total period	1446.11 <sup>c</sup>	1527.71 <sup>b</sup>	1567.58 <sup>a</sup>	19.534	<0.01
Daily gain of lambs (g/day)					
1 to 30	201.67 <sup>a</sup>	188.34 <sup>b</sup>	189.33 <sup>ab</sup>	6.049	<0.01
31 to 60	205.67	211.67	198.33	8.371	0.23
61 to 90	208.33 <sup>b</sup>	216.67 <sup>ab</sup>	221.67 <sup>a</sup>	11.033	0.04
Total	205.22	205.56	203.11	10.211	0.54
Feed conversation					
1 to 30	6.93 <sup>b</sup>	7.40 <sup>ab</sup>	7.78 <sup>a</sup>	0.349	<0.01
31 to 60	7.03 <sup>b</sup>	7.09 <sup>b</sup>	7.73 <sup>a</sup>	0.371	0.03
61 to 90	7.18 <sup>b</sup>	7.78 <sup>a</sup>	7.65 <sup>a</sup>	0.333	<0.01
Total	7.05 <sup>b</sup>	7.43 <sup>ab</sup>	7.72 <sup>a</sup>	0.212	0.03
Carcass decomposition					
Hot carcass weight (kg)	18.250 <sup>b</sup>	18.600 <sup>b</sup>	19.600 <sup>a</sup>	0.206	0.02
Cold carcass weight (kg)	18.050 <sup>b</sup>	18.050 <sup>b</sup>	19.150 <sup>a</sup>	0.466	<0.01
Dressing (%)	55.13 <sup>a</sup>	53.30 <sup>b</sup>	54.16 <sup>ab</sup>	0.695	0.02
Leg weight (kg)	2.283	2.060	2.393	0.079	0.35
Wristband weight (kg)	1.596	1.507	1.702	0.035	0.22
Neck weight (kg)	1.595.00	1.398	1.166	0.427	0.48
Tail weight (g)	231.00	202.50	471.00	321.284	0.52
Order weight (g)	1704.50 <sup>a</sup>	1040.00 <sup>b</sup>	955.00 <sup>b</sup>	20.123	<0.01
6-11 rib weight (g)	1201.00	866.50	1120.50	119.519	0.56
Before 6 rib weight (g)	897.00	767.00	1005.50	61.543	0.40
Carcass fat (g)	441.50 <sup>a</sup>	393.32 <sup>b</sup>	341.50 <sup>c</sup>	12.65	<0.01

a, b, c Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Three treatments were rations including: 1) Short corn silage with long alfalfa hay, 2) Long corn silage with short alfalfa hay, 3) Short corn silage with short alfalfa hay.

Hot and cold carcass weight between treatment LSC and SLC were similar but differ from treatment SSC. Increased carcass weight of lambs fed SSC diet was due to increased DMI and digestibility of DM and NFC (**Table 5**). Dressings were different between treatments. There were no difference in leg, wristband, neck, tail, and 6-11th rib weight among treatments, but different in order weight and carcass fat observed. Lambs in treatment SLC had the highest carcass fat in comparison to LSC and SSC treatments. Also, carcass fat in LSC was significantly less than SSC treatment.

**Table 5.** Body weight, dry matter and nutrients intake, and digestibility.

Items	Treatments <sup>1</sup>			SEM	P-value
	1	2	3		
Intake					
BW (kg)	40.68	39.83	42.76	2.350	0.67
DM (g/day)	1471.84 <sup>b</sup>	1686.80 <sup>a</sup>	1695.47 <sup>a</sup>	4.539	<0.01
NDF (g/day)	504.55 <sup>b</sup>	578.24 <sup>a</sup>	581.21 <sup>a</sup>	1.559	<0.01
NDF (g/LW <sup>0.75</sup> )	3149 <sup>b</sup>	36.59 <sup>a</sup>	35.12 <sup>ab</sup>	0.789	<0.01
peNDF>8 <sup>1</sup> (g/day)	495.76 <sup>a</sup>	470.73 <sup>b</sup>	166.98 <sup>c</sup>	1.428	<0.01
peNDF>1.18 <sup>2</sup> (g/day)	501.43 <sup>a</sup>	502.73 <sup>a</sup>	367.72 <sup>b</sup>	1.553	<0.01
pdNDF(g/day)	264.34 <sup>b</sup>	302.95 <sup>a</sup>	304.51 <sup>a</sup>	0.851	<0.01
iNDF (g/day)	240.20 <sup>b</sup>	275.29 <sup>a</sup>	276.70 <sup>a</sup>	0.741	<0.01
Fragile DM (g/day)	430.07 <sup>b</sup>	492.88 <sup>a</sup>	495.42 <sup>a</sup>	1.326	<0.01
Unfragile DM (g/day)	1324.41 <sup>c</sup>	1452.90 <sup>b</sup>	1589.98 <sup>a</sup>	4.071	<0.01
Fragile DM (%)	10.02 <sup>b</sup>	13.87 <sup>a</sup>	6.22 <sup>c</sup>	1.326	<0.01
Unfragile DM (%)	89.98 <sup>b</sup>	86.13 <sup>c</sup>	93.78 <sup>a</sup>	1.326	<0.01
Fragile NDF (g/day)	147.43 <sup>b</sup>	233.90 <sup>a</sup>	105.49 <sup>c</sup>	0.473	<0.01
Unfragile NDF (g/day)	357.12 <sup>b</sup>	344.34 <sup>c</sup>	475.72 <sup>a</sup>	1.090	<0.01
Fragile NDF (%)	29.22 <sup>b</sup>	40.45 <sup>a</sup>	18.15 <sup>c</sup>	3.234	<0.01
Unfragile NDF (%)	70.78 <sup>b</sup>	59.55 <sup>c</sup>	81.82 <sup>a</sup>	4.321	<0.01
Digestibility (%)					
DM	68.83 <sup>b</sup>	67.66 <sup>c</sup>	69.14 <sup>a</sup>	0.021	<0.01
Organic matter	67.89 <sup>b</sup>	67.21 <sup>c</sup>	74.21 <sup>a</sup>	0.016	<0.01
NDF	46.59 <sup>a</sup>	45.57 <sup>b</sup>	44.49 <sup>c</sup>	0.013	<0.01
EE	48.06	48.34	49.01	0.498	0.81
CP	72.11	73.15	72.68	0.542	0.88

NFC	85.15 <sup>b</sup>	84.96 <sup>b</sup>	86.34 <sup>a</sup>	0.087	< 0.01
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a, b, c Means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Three treatments were rations including: 1) Short corn silage with long alfalfa hay, 2) Long corn silage with short alfalfa hay, 3) Short corn silage with short alfalfa hay.

## Chewing activity

The data of eating, rumination and chewing time, and all per day and per gram of DM, NDF, peNDF>8, peNDF>1.18, pdNDF, iNDF, apparent digestible DM and NDF, fragile and unfragile DM and NDF, and their index were presented in **Table 6**. Ewes fed SLC and SSC spent less time for eating and ruminating compared to those fed LSC. In all treatments, the lambs spent relatively more time and effort on rumination than on eating. Eating, rumination and total chewing time were over than 217, 406, and 620 min/day, respectively. When lambs fed on diets with coarse hay eating, rumination and total chewing time were longer (min/day, min/g DM, NDF, pdNDF, iNDF, apparent digestible DM and NDF, fragile and unfragile DM and NDF intake, and the index as min per 100 g DM and NDF, but reduction of corn silage or alfalfa hay and both of them reduce these measures even more reduction observed when SSC treatment was used. In contrary, when lambs fed SLC diets eating, rumination and total chewing time were shorter (min/g of peNDF>8, peNDF>1.18 intake, feed efficiency of DM and NDF (g/min). In this study, reductions in eating, ruminating, and chewing times as min/kg of DM and NDF were observed for lambs that fed SLC and SSC treatments in comparison to LSC.

**Table 6.** Chewing activity of sheep fed total mixed diets containing different alfalfa and corn silage particle size.

Items	Treatments <sup>1</sup>			SEM	-value
	1	2	3		
Eating time (min/day)	260.00a	240.00b	217.50c	1.443	<0.01
Rumination time (min/day)	427.50a	415.00b	406.25c	1.450	<0.01
Chewing time (min/day)	687.50a	655.00b	623.75c	1.910	<0.01
Eating time minute per gram of					
DM	0.18a	0.14b	0.13c	0.006	<0.01
NDF	0.52a	0.41b	0.37c	5.404	0.99
peNDF>8	0.525b	0.510b	1.302a	0.007	<0.01
peNDF>1.18	0.520b	0.477c	0.591a	0.004	<0.01
pdNDF	0.98a	0.79b	0.71c	0.007	<0.01
iNDF	1.08a	0.87b	0.79c	0.008	<0.01
Apparent digestible DM	0.28a	0.21b	0.19c	0.002	<0.01
Apparent digestible NDF	1.11a	0.91b	0.84c	0.008	<0.01
Fragile DM	0.60a	0.49b	0.44c	0.004	<0.01
Fragile NDF	1.76b	1.03c	2.06a	0.012	<0.01
Unfragile DM	2.89a	2.79b	2.32c	0.001	<0.01
Unfragile NDF	0.73a	0.70b	0.46c	0.016	<0.01
Eating index (min/100 g DM)	17.67a	14.23b	12.83c	0.012	<0.01
Eating index (min/100 g NDF)	51.54a	41.51b	37.42c	0.298	<0.01
Rumination time minute per gram of					
DM	0.29a	0.25b	0.24b	0.025	<0.01
NDF	0.85a	0.72b	0.70b	0.007	<0.01
peNDF>8	0.86b	0.88b	2.43a	0.007	<0.01
peNDF>1.18	0.85b	0.82c	1.10a	0.004	<0.01
pdNDF	1.62a	1.40b	1.33b	0.007	<0.01
iNDF	1.18a	1.51b	1.47b	0.008	<0.01
Apparent digestible DM	0.45a	0.36b	0.35c	0.002	<0.01
Apparent digestible NDF	1.82a	1.57b	1.57b	0.008	<0.01
Fragile DM	0.60a	0.49b	0.44c	0.004	<0.01
Fragile NDF	2.06a	1.76b	1.03c	0.016	<0.01
Unfragile DM	0.20a	0.16b	0.14c	0.004	<0.01
Unfragile NDF	0.73a	0.70b	0.46c	0.020	<0.01
Rumination index (min/100 g DM)	29.05a	24.60b	23.96b	0.134	<0.01
Rumination index (min/100 g NDF)	84.75a	71.77b	70.00b	0.389	<0.01
Total chewing time minute per gram of					
DM	0.47a	0.39b	0.37c	0.414	<0.01
NDF	1.36a	1.13b	1.07c	0.007	<0.01
peNDF>8	1.39b	1.39b	3.73a	0.002	<0.01
peNDF>1.18	1.37b	1.30c	1.70a	0.006	<0.01
pdNDF	2.60a	2.16b	2.05c	0.007	<0.01
iNDF	2.86a	2.38b	2.54c	0.011	<0.01



Apparent digestible DM	0.74a	0.58b	0.53c	0.002	<0.01
Apparent digestible NDF	2.93a	2.49b	2.41c	0.012	<0.01
Fragile DM	0.60a	0.49b	0.44c	0.004	<0.01
Fragile NDF	1.76b	1.03c	2.06a	0.012	<0.01
Unfragile DM	0.20a	0.17b	0.14c	0.001	<0.01
Unfragile NDF	0.73a	0.70b	0.46c	0.004	<0.01
Chewing index (min/100 g DM)	46.72a	38.83b	36.79c	0.186	<0.01
Chewing index (min/100 g NDF)	136.29a	113.28b	107.32c	0.543	<0.01

a, b, c Means within a row with different superscripts differ ( $P < 0.05$ ).

1 Three treatments were rations including: 1) Short corn silage with long alfalfa hay, 2) Long corn silage with short alfalfa hay, 3) Short corn silage with short alfalfa hay.

## DISCUSSION

### Fragility

Grant <sup>[6]</sup> outlined that a fragility value of 100%, highly fragile forage, would equate to complete reduction of particle size to less than 1.18 mm, and a fragility value of 0, very tough forage, would reflect no reduction in particle size upon ball milling (pefi=pefBM). Particle size reduction is necessary to pass and the limiting process in clearing of indigestible particles, especially iNDF from the rumen and rumination plays a major role in this process. There is difference in ration fragility due to changing in particle size, therefore as particle size; fragility must be accounted for in models to predict ruminal retention, passage rate, digestibility, and chewing activity.

### Ruminally degradability

About 44 and 65% of NDF and CP of alfalfa was ruminal degradable, respectively. Broderick and Buxton <sup>[17]</sup> found that in 19 cultivars of alfalfa CP can vary ranged from 15 to 19 % DM and more than 80% of protein was ruminally degradable. Paya et al. <sup>[18]</sup> found that for Iranian alfalfa, potential degradable and degradability rate for DM and CP were 48.3, 3.40; 58.4, and 3.75 % and %/h, respectively. The *in situ* rate of degradability of CP for alfalfa was considerably high (0.061%/h). In this study, the rate of degradability of CP for corn silage was higher. Paya et al. <sup>[18]</sup> found that for corn silage potential degradable and degradability rate of DM were 73.9 and 3.4 % and %/h, respectively. Corn silage is the main source of NDF and energy, but its NDF has a variable rumen degradability, which influences its energetic value, intake, and effectiveness. Corn silage has 27.7 ± 4.9 % of DM starch and concentrations and ruminal fermentability of NDF and starch are highly variable across corn silage hybrids that must be considered when formulating diets <sup>[19]</sup>. In this study, alfalfa, corn silage and ration had 28.43, 25.24, and 31.55 % starch. Digestibility of DM and NDF for corn silage is positively related to high concentrations of starch and sugar, which are almost completely digestible in ruminants <sup>[20]</sup>. The degradable NDF of corn silage was lower than alfalfa because the starch in ensiled corn is rapidly degraded in the rumen that depresses fibre digestion.

### Indigestible NDF

The value of iNDF240 in corn silage and ration were greater than iNDF2.4 but in alfalfa hay was lower than iNDF2.4. The *in situ* method, although determines the pdNDF of feeds, may be biologically biased because of lower microbial activity inside the bags than in surrounding digesta, and inflow and outflow of particles. However, the NRC <sup>[21]</sup> did not use the *in situ* method as its basis for NDF digestibility of forages because NDF is a non-uniform feed fraction; it contains multiple pools that digest predictably as a function primarily of lignification <sup>[4]</sup>. Cotanch et al. <sup>[22]</sup> suggested that determination of iNDF should be included in routine forage and feed analysis because it is a uniform feed fraction with no digestibility. In this experiment, the iNDF240 of corn silage in this study (102.3 g/kg DM) was 3.7 times of its iNDF2.4, and was 23% higher than 84 g/kg DM that determined after 288 h ruminally incubation by Huhtanen and Jaakkola <sup>[23]</sup>. For alfalfa, iNDF (222.1 g/kg DM) was 30% lower than its iNDF2.4. Cotanch et al. <sup>[22]</sup> found that two type of alfalfa (low and high digestibility) had 36.7, 7.1, and 15.7; 44.5, 7.5, and, 18.5 % of NDF, lignin, and iNDF, respectively. Although Van Soest <sup>[4]</sup> outlined that extent and nature of lignification of forage cell walls control its NDF digestibility, which is a function of various factors, such as forage species, maturity, and number of harvest, latitude, and climate. The results showed that iNDF or pdNDF may not be very accurately predicted from lignin, because it is not a uniform chemical entity of cell wall. One goal of this experiment was iNDF determination, explaining feeding and ruminating behavior using particle size and iNDF because iNDF is the functional fiber fraction that influences ruminal retention, gut fill, digestion, passage dynamics, physical effectiveness, and ultimately it can be used to estimate DMI, explain feeding and ruminating behavior, especially when chemical composition (NDF, ADL, and ADF) are similar.

### Intake and lambs performance

Effects of TMR particle size on DMI and performance is unclear. Sometimes the conflicting results are experienced because response of particle size might be due to digestibility, functional specific gravity, rumen retention time, forage: concentrate ratio, type of forage and its proportion, type of ingredients, the amount of non-forage fiber components, and the used processing <sup>[3]</sup>. This situation is a challenge where forage is accompanied by appreciable amounts of rapidly degradable carbohydrates in TMR because

ruminal pH may drop so quickly that any advantage of reducing the forage particle size in promoting higher intake and productivity is unfavorably negated. In this experiment, treatments were arranged to investigate whether the forage particle size and/or their fragility significantly effect on intake, chewing activity, and performance. Intake response when reducing particle size is usually positive, with the magnitude depending upon the extent of particle size reduction as well as the type and digestibility of the forage fed. The NRC <sup>[24]</sup> expected DMI for maintenance is 1.05 kg/day. Daily DMI and ADG were higher than reported by Cannas et al. <sup>([24]; 1111.0 ± 312 and 189 ± 103.7g/day, respectively)</sup>. Different effects when consumed diets with different particle sizes have been reported. Improved characteristics of silage as a response to chopping increased DMI <sup>[25]</sup>. Reduction silage particle size, increased DMI, performance, BCS of pregnant and lactating ewes, ewe BW after lambing, lamb birth weight, ADG of suckling lambs, also DMI and ADG in finishing lambs <sup>[26,27]</sup>. Our results were in agreement by Fitzgerald <sup>[26]</sup>. In contrast, Helander <sup>[27]</sup> reported that DMI of lambs was not increased by chopping silage. Diets with short particles especially when poor quality or high fiber diets were fed it increases DMI, intake of digestible nutrients, rumen VFA concentrations <sup>[10]</sup>, decreases the filling effects and increases ruminal passage rate <sup>[4]</sup>, but has less impact on intake when well-balanced rations are fed. Lack of effect of chop length on DMI has been observed also in earlier studies by others feeding diets containing more than 50% concentrate <sup>[28]</sup>. The increased ADG and lack of increase in DMI was possibly due to higher feed efficiency as a result of more efficient rumination by the lambs fed chopped silage.

As DMI increased the iNDF and pdNDF intake increased that suggests that DMI of the lambs in this experiment were not limited by NDF, iNDF and pdNDF levels of rations. Although the NDF is the best single chemical predictor of DMI, and the filling effect <sup>[1]</sup>, but its filling effect depends on particle size, fragility, passage rate, digestion <sup>[4]</sup>, the quantity and sources NDF and iNDF <sup>[29]</sup> in growing lambs. Without differences in gain, differences in intake showed that lambs adjusted their intake according to the level of diet iNDF and at the same level of NDF with a lower iNDF, the lambs fed higher intake <sup>[29]</sup>. At equal levels of iNDF, similar intake was observed. To test this assumption, Hogue <sup>[29]</sup> found no differences on DMI of lambs were fed diets with low iNDF (11%) from different sources (beet pulp, corn gluten feed, wheat midds, alfalfa meal, or oat hulls). When diet iNDF increased from 15 to 27% in oat hull diets, DMI decreased and iNDF intake stabilized, but in soy hull diets where the iNDF increased to a maximum of 20%, with 51% NDF, DMI continued to increase linearly, along with proportional increases in NDF and iNDF. Lamb feeding with soy hulls, neither NDF nor iNDF appeared to limit feed intake. In this experiment, diets had equal NDF (34.28 %) and iNDF (16.32 %) however it seems that others characteristics may be impact.

Hogue <sup>[30]</sup> found that lambs ADG were different between that lambs that fed different levels of forage NDF, but at one level of forage NDF (15 or 25 or 35 % DM), there was no significant difference on ADG. It seems that reduction of particle size increases particles surface areas, which promotes rapid fermentation by ruminal bacteria and, therefore, generally yields more energy to the lambs fed a smaller particles. It is likely that the supplementation of concentrate enhanced feed efficiency resulting in increased ADG without increased DMI <sup>[26,27]</sup>. One possible explanation for the different responses to chopping silage is the different silage characteristics especially digestibility in the two different size. The higher silage digestibility when small particle fed to lamb is thought to have caused the higher ADG lambs fed chopped <sup>[31]</sup>.

Digestibility of NDF will become one of the most important feed characteristics in coming feed/ration formulation systems. Reduction of particle size reduced apparent digestibility of NDF and in contrary increased for NFC. Reduction in total tract ADF and NDF digestibility has also been observed with fine chopping of alfalfa hay and silage that due to faster passage rate and less ruminal mean retention time available for microbial digestion. Also, reduction of particle size reduced rumination and total chewing activity resulting in depressed saliva secretion and rumen pH, and reduced cellulolytic activity, and consequently depression in ADF and NDF digestibility <sup>[3]</sup>. It is well-known that increasing grass silage digestibility increases forage intake and performance of ruminants <sup>[31]</sup>. Chopping grass silage, and decreasing silage particle length, from long (250-370 mm) to intermediate (70-120 mm) and further to short (5-20 mm) increased DMI and performance in sheep. Chopping silage increased feed intake and daily ADG of finishing lambs <sup>[26]</sup>. However, Keady and Hanrahan <sup>[31]</sup> showed no clear effects on silage intake or ADG of finishing lambs when fed maize silage containing 3 or 28% starch of DM.

The effects of particle size on carcass characteristics were rarely studied. Fluharty et al. <sup>[32]</sup> found that altering alfalfa hay particle size had no effect on hot carcass weight and yield grade. Al-Saiady et al. <sup>[33]</sup> reported that hot carcass weight and percentage of separable lean from the 9-11th rib joint increased as the particle length of alfalfa hay contained in the diets decreased. Also, they reported that dressing percentage and separable fat percent (assumed to be subcutaneous and intermuscular fat) from lambs fed varying particle lengths of alfalfa hay did not differ. The most likely reason for this would be increased nitrogen retention and associated increases in energy available for growth as opposed to the lambs fed the long hay diet. Lambs fed a long hay diet had higher NDF digestibility than those lambs fed diets which contained shorter particle lengths, but the net effects on DM digestibility, TDN, and digestible CP were negligible.

## **Chewing activity**

Chewing activity is the first mechanism to reduce particle size in feed, influence both the nature of the digestion and passage rate, depend on forage particle sizes, NDF, feed quality and amount eaten <sup>[10]</sup>. The mastication efficiency could be measured as time spent chewing per unit of intake. Differences in chewing activity per kg DM has been found to differentiate between breed, body size, physiological state, and level of production, suggesting that animals with a higher intake capacity need shorter time to eat and ruminate per unit of ingested feed. The value of chewing activity in this experiment is consistent with that found by



Helander [27]. As lambs fed to rations with similar NDF these results may be attributed to differences in the particle size. Mertens [1] proposed that chewing corrected for DMI (equal to or greater than 30 min/kg of DM) as a criterion for physical effectiveness of forages for dairy cattle. Sheep spend between nine and 16 times longer than cows in eating, and ruminate per one kg of DM because they are smaller animals, their chewing activity is less powerful, have to grind the particles more finely than cattle [4]. With these limitations, intake is limited in sheep more than in cattle by the particle size, NDF and iNDF and filling effects of rations. Three rations had equal NDF with similar nature because above 70% of their NDFs came from the corn silage and alfalfa that differ in particle size, therefore the results of the experiment due to changes in particle size or in others physical properties such as digestibility and fragility. Smaller forage particles stay in the rumen for a shorter time, consequently they are less available for microbial digestion and the effect is decreased digestibility, particularly with regards to fiber digestion [3]. Chewing activity is drastically reduced when hay is ground to less than 3 mm mean particle length [3,10]. Feed particle size and NDF are more reliable indicators of chewing activity than the NDF of the forage alone. In particular, diets containing high peNDF have a higher stimulatory effect on mastication than diets with finely chopped forage.

## CONCLUSION

Our assumption was forage fragility and digestibility may explain the variation in chewing response not explained by particle size. The intake of DM, peNDF>8 and peNDF>1.18, fragile DM and NDF, and unfragile DM and NDF explained 69.28, 88.18, 72.23, 69.29, 11.67, 92.77 and 65.75% of variation in eating time, respectively therefore, unfragile DM was the best predictor (eating time = 469.07-0.158 × unfragile DM; P<0.0001). Using intake of peNDF>8 and fragile NDF in a linear model explained maximum (94.5%) of its variation (Eating time=211.69+0.153peNDF>8-0.187 × fragile NDF; R2=0.945; P=<0.0001). The intake of DM, peNDF>8 and peNDF>1.18, fragile DM and NDF, and unfragile DM and NDF explained 67.58, 67.49, 50.79, 67.59, 3.87, 77.85, and 45.39% of variation in rumination, respectively; as unfragile DM was the best predictor (Rumination=531.79-0.079 × unfragile DM; P=0.0001). When intake of peNDF>1.18 and fragile DM were used to predict rumination, in a linear model achieved maximum R2 (Rumination=346.98-0.20peNDF>1.18-0.15 × fragile DM; R2=0.78; P=0.0011). The intake of DM, peNDF>8 and peNDF>1.18, fragile DM and NDF, and unfragile DM and NDF explained 74.23, 87.06, 69.45, 74.24, 9.11, 94.41 and 62.81% of variation in total chewing activity, respectively (**Table 7**). Therefore, unfragile DM was the best predictor of total chewing activity (Total chewing activity=1000.86-0.237 unfragile DM; P<0.0001). Using intake of peNDF>1.18 and fragile DM in a linear model explained maximum (95.4%) of its variation (Total chewing activity=446.26+ 0.59 × peNDF>1.18- 0.382 × fragile NDF; R2= 0.954; P=<0.0001). Using intake of peNDF>1.18 and DMI in a linear model explained maximum (95.41%) of variation in total chewing activity (Chewing activity=797.27-0.151 × DMI+0.224 × peNDF>1.18; R2=0.954; P=<0.0001). Our hypothesis was to determine if the fragility factor would be of any practical use in adjusting pef values to better predict the animal eating time, rumination time, and chewing response that is the ultimate bioassay for the peNDF system. Therefore, combination of peNDF>8 and NDF, peNDF>1.18 and DM fragility, and DMI and peNDF>1.18 in rations can estimate eating, rumination, and chewing time of lambs. Although there is a positive relationship between NDF digestibility and fragility; it appears that fragility may be more useful in adjusting peNDF values than digestibility; however, more research is needed to remove ambiguities and recommend acceptable correct manner.

**Table 7.** Equations<sup>1</sup> for linear regression of response of eating, rumination and chewing time parameters to different dietary factors in lambs fed experimental rations.

Chewing parameters (Y)	Dietary factor <sup>2</sup> (X)	Parameter estimates				Model statistics	
		Intercept	SE Intercept	Slope	SE Slope	RMSE <sup>2</sup>	R <sup>2</sup>
Eating time (min)							
	DMI (g/day)	470.13	48.74	-0.143	0.030	10.864	0.693
	peNDF>8(g/day) <sup>3</sup>	199.76	4.96	1.654	0.192	6.738	0.882
	peNDF>1.18(g/day) <sup>4</sup>	129.62	21.64	0.236	0.047	10.314	0.723
	Unfragile DM(g/day) <sup>5</sup>	469.07	20.35	-0.158	0.014	5.269	0.928
	Unfragile NDF(g/day) <sup>5</sup>	335.26	22.181	-0.245	0.056	11.471	0.658
Rumination time (min)							
	DMI (g/day)	541.40	27.47	-0.077	0.017	6.122	0.676
	peNDF>8(g/day)	397.34	4.51	0.794	0.174	6.131	0.675
	peNDF>1.18(g/day)	365.33	15.88	0.110	0.034	7.543	0.508
	Unfragile DM(g/day)	531.79	19.55	-0.079	0.013	5.061	0.779
	Unfragile NDF(g/day)	460.06	15.37	0.112	0.039	7.95	0.456
Total chewing time (min)							
	DMI (g/day)	1011.53	66.48	-0.220	0.041	14.819	0.742
	peNDF>8(g/day)	597.09	7.73	2.449	0.299	10.503	0.871
	peNDF>1.18(g/day)	495.51	33.59	0.350	0.073	16.135	0.695
	Unfragile DM(g/day)	1000.86	26.66	-0.237	0.018	6.904	0.944
	Unfragile NDF(g/day)	795.32	34.425	-0.357	0.087	17.802	0.628

<sup>1</sup>Only significant relationships are shown (P<0.05).

<sup>2</sup>RMSE = Root mean square error.

<sup>3</sup>The physically effective factor determined as the proportion of DM retained on two (pef>8; [9]) and three sieves of the Penn State particle separator (pef>1.18; [10]).

<sup>4</sup>The peNDF>8 and peNDF>1.18 were calculated by multiplying NDF of each ration on each sieve on pef>8 and pef>1.18, respectively.

<sup>5</sup>The fragility of feeds were determined based on methods that outlined by Cotanch et al. [2].

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