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# Effects of moisture content or particle size on the *in situ* degradability of maize silage and alfalfa haylage in lactating dairy cows

Yang Zou<sup>1</sup>, Shuangzhao Dong<sup>1</sup>, Yun Du, Shengli Li, Yajing Wang, Zhijun Cao\*

State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

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## ABSTRACT

A study using four Holstein cows with ruminal cannulas was conducted to evaluate the degradability of different moisture content or particle size of maize silage and alfalfa haylage. The maize silage (MS; 20-mm length) and alfalfa haylage (AH; 40-mm length) samples were wet (wet maize silage, MSW; wet alfalfa haylage, AHW), dried (dried maize silage, MSD; dried alfalfa haylage, AHD), or ground to pass through a 2.5-mm screen (dried ground maize silage, MSG; dried ground alfalfa haylage, AHG). Samples were incubated in the rumen for 2, 6, 12, 24, 36, 48, and 72 h. Cows were fed *ad libitum* and allowed free access to water. High moisture content treatment of MSW expressed a lower rinsing NDF and ADF degradability at 2 h ( $P < 0.05$ ) compared with dried samples (MSD and MSG). Different moisture content and particle size had a significant impact ( $P < 0.05$ ) on the NDF degradability at 72 h, ADF degradability at 36, 48, and 72 h, and ruminally degradable ADF. All of the highest values were observed in small particle size and low moisture content AHG treatment. Based on this study, sample processing, such as drying and grinding, should be considered when evaluating nutritive values of forages.

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## 1. Introduction

Forage comprises a large quantity of the dairy rations. Because forages stored in silo could increase the original protein, NDF and ADL content (Fairbairn et al., 1992; Han et al., 2004) after microbe fermentation, maize silage and alfalfa haylage, which are widely used on dairy farms (Dash et al., 1973), are substantially different from other forages. Maize silage and alfalfa haylage are the only forages for which moisture is released when silages are exposed to the air, which may affect the ruminal degradation and nutritional value. Researchers have concluded the mechanical grinding of forage replaces chewing and digestion for animals (Emanuele and

Staples, 1988), and differences in ruminal fermentation caused by grinding or cracking play an important role in the digestive process of animals. Firkins et al. (1986) suggested ground hay produces a greater surface area than chopped hay, which leads to more rapid fermentation and greater ease in passing through the reticuloomasal orifice, inducing more feed intake (Troelsen and Campbell, 1968) and decreasing saliva flow and ruminal pH (Beauchemin et al., 1997; Norgaard, 1983). Meanwhile, cows in a study on feeding alfalfa haylage consumed more feed without improving milk production (Kononoff and Heinrichs, 2003). Some studies on forage degradability compared alfalfa, rhizome peanut, bermudagrass, limpograss (Emanuele and Staples, 1988), perennial peanut, annual peanut, cowpea, and pigeonpea (Foster et al., 2011). Nylon bag disappearance methods has been adopted as a convenient, rapid and stable standard method in estimating feed degradation kinetics (Ørskov et al., 1980; Huntington and Givens, 1997). Only one study performed in dairy cows mentioned nylon bag degradability in response to sample processing of fresh, dried, and ground maize silage (Valentin et al., 1999). Therefore, this experiment was conducted to evaluate the effects of different moisture content or different particle size (wet, dried, or dried grounded) of maize silage and alfalfa haylage on the ruminal

\* Corresponding author.

E-mail address: caozhijun@cau.edu.cn (Z. Cao).

<sup>1</sup> These authors contributed equally to this work.

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degradability of NDF, ADF and CP in Holstein lactating cows using the nylon bag technique.

## 2. Materials and methods

Cows were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching (FASS, 2010).

### 2.1. Animals and diets

Four multiparous Holstein cows fitted with permanent ruminal cannulas with similar age, BW, parity and day in milk (DIM) were used to measure the effects of different pre-treatments of corn silage and alfalfa haylage on ruminal nutrient degradability. Cows were fed a 3.5 maintenance total mixed ration (TMR) formulated according to the dairy nutrient requirement and feeding standard (NY/T-2004, third edition) in equal portions at 08:30 and 20:30 on an *ad libitum* basis. The baseline diet (50.67% DM, 9.04% CP, 15.54% NDF, 10.04% ADF, and 2.23% EE) consisted of 43.7% corn silage, 10.9% alfalfa hay, and 45.4% concentrate (as fed basis). Water was available for *ad libitum* consumption. Cows were milked three times daily at 06:00, 13:10 and 19:40.

### 2.2. Sample preparation

Maize silage (MS) and alfalfa haylage (AH) were obtained from Beijing Sino Farm (Shunyi, Beijing, China) and Modern Farming (Ma'anshan, Anhui, China). Grab samples were taken from several sites of the silo faces. All samples were subjected to the following six treatments: 1) Wet maize silage (MSW), approximately 20-mm chop length; 2) Sample MSW were dried in a forced air oven at 65°C for 24 h, and air was equilibrated to formulate dried samples, which was called dried maize silage (MSD); 3) Sample MSD, ground to pass through a 2.5-mm screen to produce dried ground maize silage (MSG); 4) Wet alfalfa haylage (AHW), approximately 40-mm chop length; 5) Sample AHW were dried in a forced air oven at 65°C for 24 h, and air was equilibrated to formulate dried samples called dried alfalfa haylage (AHD); 6) Sample AHD, ground to pass through a 2.5-mm screen to produce dried ground alfalfa haylage (AHG).

### 2.3. Rumen incubation, sample collection and analysis

Rumen incubations were carried out according to Herrera-Saldana et al. (1990). Nylon cloth (Guangda Hengyi Co., Beijing, China) with a pore size of 40 µm was used to prepare bags with an inner size of 25 cm × 35 cm. Nineteen grams DM of maize silage and 30 g of alfalfa haylage were, respectively, placed in bags in all four cows; wet samples (80 g, 23.96% DM of maize silage and 37.97% DM of alfalfa haylage) were adjusted according to DM so that there were 19 or 30 g dry matter contained in the bag. Seven small nylon bags of each treatment were prepared for each cow. All sets of small nylon bags were placed into a larger nylon mesh bag (32 cm × 40 cm) at the same time point and were then placed in the ventral sac of the rumen in the reverse order of the incubation time point at 2, 6, 12, 24, 36, 48, and 72 h; after incubation, all bags were removed simultaneously. The 0 h samples were not placed in the rumen, but they were soaked and rinsed as described below. Removed bags were placed in cold tap water immediately after removal from the rumen, and they were washed by hand until the water was clear. After washing, the bags were dried in a forced air oven at 65°C for 48 h, air equilibrated and weighed. Residues from the bags were pooled within time and treatment, finely ground by mortar and pestle to pass through a 1-mm screen and retained in

sealed containers to determine the NDF, ADF and CP. Feeds were analyzed for nitrogen according to Kjeldahl (AOAC, 1990), and thereafter, CP was determined by the total nitrogen (N) × 6.25. The NDF and ADF contents were residual portions after rinsing according to Van Soest et al. (1991).

### 2.4. Calculations and statistical analysis

*In situ* degradation curves of NDF, ADF and CP were fitted to the model (Ørskov and McDonald, 1979), degradation percentage =  $a + b(1 - e^{-K_d t})$ , where  $a$  was the rapidly degraded fraction (%),  $b$  the remaining slowly degraded fraction (%), and  $K_d$  the constant rate of degradation of the  $b$  fraction (%/h). The  $b$  fraction and  $K_d$  were generated by the DUD method using the NLIN Procedure of SAS (1999). Rumen-degraded NDF (RDNDF), rumen-degraded ADF (RDADF) and rumen-degraded CP (RDP) were calculated for an outflow rate ( $K_p$ ) of 0.031/h using the following equation:  $RDNDF/RDADF/RDP = a + b \times K_d / (K_d + K_p)$ .

The above equation was applied to each component tested NDF, ADF, CP. Data were analyzed using the GLM procedure for ANOVA in SAS. Significant mean value differences were evaluated by Duncan's multiple-range test. A significance level of  $P < 0.05$  was used.

## 3. Results and discussion

Feed intake for the four cows used in the present study averaged 40.77 kg/d.

As two important forages used in dairy rations, maize silage and alfalfa haylage had different performances in ruminal degradation. Singh et al. (1972) detected microorganisms that digest legumes prior to grasses in rumen. Also, Troelsen and Campbell (1968) examined the omasum from sheep and discovered the digestion of alfalfa hay was shorter and broader than that of grass hay, which means alfalfa hay could enter the omasum more easily than grasses.

Degradability can be influenced by many factors, including grinding, sample size (Emanuele and Staples, 1988; Kim et al., 1996), origin of grains (Galyean et al., 1981; Waldo, 1973), pore size of *in situ* bags (Nocek and English, 1986), and basal diet fed to the animal (Loerch et al., 1983). The size of the particle has been suggested as a key factor that affects digestion rates (Emanuele and Staples, 1988). There were no significant differences ( $P > 0.05$ ) in the degradability, rate of degradation and kinetic parameters for NDF, ADF and CP in maize silage with different moisture content or particle size (Table 1, Table 2), but high moisture content treatment of MSW expressed a lower rinsing NDF and ADF degradability at 2 h ( $P < 0.05$ ) compared with dried samples (MSD and MSG) in the current study. Additionally, a comparison of fresh, dried and ground maize silage on the nylon bag degradability in dairy cows indicated that grinding of maize silage could increase the soluble fraction (Valentin et al., 1999), which was inconsistent with our results. This variation may be related to the differences in the physical structure induced by grinding; thereafter, the mechanical breakdown of cell walls accelerates colonization and attack of bacterial and protozoa in the rumen (Bauchop, 1981; Latham et al., 1978).

All of the variations in the CP degradability of alfalfa haylage (Table 3, Table 4) were insignificant ( $P > 0.05$ ), which might be because the samples were prepared in a lab without leaf loss. However, different moisture content or different particle size of alfalfa haylage did not affect the nylon bag NDF or ADF disappearance during the early hours of incubation, but they did influence the longer fermentation periods. When alfalfa haylage was finely grounded, the fiber structure was destroyed, increasing the surface area of particles and allowing an easily attack with microbes (Galyean et al., 1981; Emanuele and Staples, 1988). Besides, the particle size of MSG and AHG (2.5 mm) is much larger than the pore

**Table 1**  
Degradability and rate of degradation of NDF, ADF and CP in maize silage (MS) of different moisture content or different particle size incubated *in situ*.

Item	Treatments			SEM	P-value
	MSW	MSD	MSG		
<b>NDF</b>					
2 h	13.72	16.68	14.48	0.72	0.31
6 h	14.76	18.85	16.80	1.30	0.49
12 h	18.90	21.51	16.51	1.49	0.45
24 h	36.37	36.18	28.57	2.11	0.27
36 h	40.96	41.91	39.91	2.19	0.94
48 h	43.40	42.69	40.80	2.45	0.80
72 h	48.58	55.20	47.31	1.90	0.28
<b>ADF</b>					
2 h	12.16 <sup>b</sup>	14.58 <sup>ab</sup>	16.17 <sup>a</sup>	0.64	0.09
6 h	15.04	15.15	18.18	1.28	0.57
12 h	17.48	19.68	20.81	2.01	0.79
24 h	36.27	35.75	32.11	2.50	0.77
36 h	41.54	41.39	51.57	2.11	0.22
48 h	44.54	45.21	43.30	2.88	0.97
72 h	50.06	54.43	50.95	2.15	0.68
<b>CP</b>					
2 h	36.53	32.76	37.31	0.97	0.18
6 h	36.37	34.74	34.95	1.23	0.83
12 h	39.05	35.03	33.18	1.26	0.24
24 h	46.99	43.02	35.05	2.47	0.23
36 h	44.50	45.20	38.38	2.75	0.32
48 h	53.50	44.09	38.89	4.10	0.43
72 h	49.90	52.25	44.77	3.21	0.66

MSW = wet maize silage; MSD = dried maize silage; MSG = dried ground maize silage.

<sup>a,b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

size of the nylon bag (40  $\mu\text{m}$ ), variations according to particle size was not due to the loss of DM from the nylon bag. In this experiment, sample moisture content and particle size had a significant impact on the NDF degradability at 72 h and ADF degradability at 36 h ( $P < 0.05$ ), and the highest value corresponded to small particle size and low moisture content AHG treatment. Additionally, treatment AHG showed significantly higher values than treatments AHW and AHD in ADF degradability at 48 and 72 h and in ruminally degradable ADF ( $P < 0.05$ ); therefore,  $K_d$  exhibited the opposite

**Table 2**  
Degradation variables of NDF, ADF and CP in maize silage (MS) of different moisture content or different particle size incubated *in situ*.

Item	Treatments			SEM	P-value
	MSW	MSD	MSG		
<b>NDF</b>					
a <sup>1</sup>	8.23 <sup>b</sup>	13.65 <sup>a</sup>	10.77 <sup>ab</sup>	0.87	0.10
b <sup>2</sup>	51.05	58.50	59.05	4.74	0.74
$K_d$ <sup>3</sup>	2.99	1.69	1.99	0.34	0.32
RDNDF <sup>4</sup>	31.16	34.18	29.81	1.19	0.39
<b>ADF</b>					
a <sup>1</sup>	7.14	9.82	11.40	0.96	0.24
b <sup>2</sup>	56.18	56.72	57.39	4.83	0.99
$K_d$ <sup>3</sup>	2.79	2.17	2.65	0.39	0.80
RDADF <sup>4</sup>	31.41	33.14	33.04	1.43	0.85
<b>CP</b>					
a <sup>1</sup>	33.45	30.31	31.60	0.81	0.31
b <sup>2</sup>	21.93	25.52	35.66	4.14	0.45
$K_d$ <sup>3</sup>	5.01	3.41	0.66	0.87	0.21
RDP <sup>4</sup>	44.26	42.22	38.03	1.67	0.38

MSW = wet maize silage; MSD = dried maize silage; MSG = dried ground maize silage.

<sup>a,b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Soluble part (%).

<sup>2</sup> Slowly degradable part (%).

<sup>3</sup> Rate of the slowly degradability fraction (%/h).

<sup>4</sup> Ruminally degradable portion (%).

**Table 3**  
Degradability and rate of degradation of NDF, ADF and CP in alfalfa haylage (AH) of different moisture content or different particle size incubated *in situ*.

Item	Treatments			SEM	P-value
	AHW	AHD	AHG		
<b>NDF</b>					
2 h	24.62	27.76	27.81	0.84	0.30
6 h	31.81	30.85	30.97	1.75	0.97
12 h	34.59	37.28	34.73	0.86	0.39
24 h	45.33	44.71	47.06	1.23	0.73
36 h	50.79	46.23	51.89	1.41	0.27
48 h	51.08	52.75	55.96	0.98	0.18
72 h	53.68 <sup>b</sup>	56.54 <sup>ab</sup>	59.42 <sup>a</sup>	0.72	0.03
<b>ADF</b>					
2 h	20.61	21.50	23.91	1.16	0.54
6 h	21.76	23.48	25.84	0.93	0.27
12 h	27.87	30.69	28.01	0.97	0.44
24 h	40.93	37.46	42.44	1.18	0.26
36 h	41.71 <sup>ab</sup>	39.26 <sup>b</sup>	46.68 <sup>a</sup>	0.99	0.04
48 h	46.19 <sup>b</sup>	47.66 <sup>b</sup>	53.10 <sup>a</sup>	0.92	0.03
72 h	49.20 <sup>b</sup>	51.75 <sup>b</sup>	57.05 <sup>a</sup>	0.65	<0.01
<b>CP</b>					
2 h	45.23	46.25	48.28	1.45	0.71
6 h	51.51	52.57	49.49	1.46	0.69
12 h	54.96	58.06	55.13	1.06	0.44
24 h	69.33	71.41	69.61	1.09	0.76
36 h	72.29	74.27	72.34	1.24	0.76
48 h	72.25	74.86	71.76	0.97	0.37
72 h	73.55	77.25	74.65	0.97	0.33

AHW = wet alfalfa haylage; AHD = dried alfalfa haylage; AHG = dried ground alfalfa haylage.

<sup>a,b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

tendency ( $P < 0.05$ ). As CP was degraded in the rumen easier than NDF and ADF (Miao et al., 2007), indicating the fiber digestion requires a more effective fermentation time, this might explain the differences in 36 to 72 h of incubation. Frikins et al. (1986) reported an increase in the total ruminal digestible NDF caused by particle size reduction of grass hay in steers based on restricted feeding. However, it is difficult to compare our results with his because of the lack of information on alfalfa haylage.

**Table 4**  
Degradation variables of NDF, ADF and CP in alfalfa haylage (AH) of different moisture content or different particle size incubated *in situ*.

Item	Treatments			SEM	P-value
	AHW	AHD	AHG		
<b>NDF</b>					
a <sup>1</sup>	20.76	25.65	24.13	1.20	0.28
b <sup>2</sup>	34.93	36.48	41.28	1.31	0.18
$K_d$ <sup>3</sup>	4.94	3.10	3.28	0.39	0.17
RDNDF <sup>4</sup>	41.96	42.82	44.57	0.67	0.31
<b>ADF</b>					
a <sup>1</sup>	14.86	19.33	18.77	0.99	0.19
b <sup>2</sup>	36.53	44.25	46.60	1.80	0.11
$K_d$ <sup>3</sup>	4.36 <sup>a</sup>	2.38 <sup>b</sup>	2.71 <sup>b</sup>	0.33	0.04
RDADF <sup>4</sup>	35.75 <sup>b</sup>	36.62 <sup>b</sup>	40.04 <sup>a</sup>	0.61	0.04
<b>CP</b>					
a <sup>1</sup>	40.25	41.17	41.56	1.63	0.82
b <sup>2</sup>	34.77	37.05	34.52	1.78	0.82
$K_d$ <sup>3</sup>	6.30	5.99	5.47	0.46	0.77
RDP <sup>4</sup>	63.29	65.54	63.37	0.67	0.35

AHW = wet alfalfa haylage; AHD = dried alfalfa haylage; AHG = dried ground alfalfa haylage.

<sup>a,b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Soluble part (%).

<sup>2</sup> Slowly degradable part (%).

<sup>3</sup> Rate of the slowly degradability fraction (%/h).

<sup>4</sup> Ruminally degradable portion (%).

#### 4. Conclusion

Different moisture content or different particle size altered the ruminal degradability, rate of degradation, or degradation variables of maize silage and alfalfa haylage. Therefore, sample factors of moisture content and particle size that is drying and grinding should be considered when determining *in situ* ruminal degradation of maize silage and alfalfa haylage.

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