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Effects of Alfalfa Particle Size on Ensalivation Rate, Chewing Efficiency, and Functional Specific Gravity of Particulate Matter in Hereford Steers

Research Article

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ABSTRACT

Six ruminally fistulated Hereford steers (body weight=414±13 kg) were used in a switch back design to determine whether two particle sizes of alfalfa hay (18.75 and 4.65 mm theoretical cut length) influenced salivary secretion during eating. The experiment carried out in two 26-d periods, with 11-d of adaptation to ration, followed by 5 d for determining the level of voluntary feed intake, 7-d for adaptation to feeding to 90% of voluntary feed intake, and 3-d for measurements. Saliva secretion was measured during the morning meal by rumen evacuation technique at 35 minute after feeding through the rumen fistula of each steer. Coarse and fine alfalfa had the same chemical composition. The geometric mean of coarse and fine alfalfa particles during the eating time decreased by 40.62 and 45.53%, respectively. Reduction of particle size increased the functional specific gravity of alfalfa hay and particulate matter. In addition, the gas associated with particles had similar trend to the functional specific gravity. Production of saliva in milliliters (P=0.001), per kg of dry matter intake (P<0.0001) and per each kilogram of neutral detergent fiber (NDF) intake (P<0.0001) were affected by alfalfa particle size. Saliva production was higher in coarse alfalfa treatment. Reduction of alfalfa particle size reduces the ability of alfalfa forage as a physically effective fiber source in feeding of ruminant by decreasing the ability to saliva secretion.

KEY WORDS chewing, dairy cow, eating behavior, salivation.

INTRODUCTION

Saliva production is very important to ruminants because increased saliva secretion will lead to increased buffering capacity in the rumen. Mertens (1997) and Grant (2010) outlined that the most methods relate to feed's effectiveness by its ability to stimulate chewing activity and saliva secretion in the cow. Saliva secretion is different in resting and eating time. In resting, saliva secretion is constant throughout the day, but significantly increase when the cow is eating or ruminating. Maekawa *et al.* (2002a) and Maekawa *et al.* (2002b) found that salivary secretion during eating among studies was variable that might be due in part to animal variation and feed characteristics. Several studies have shown that increasing forage particle size is a means of increasing chewing activity and saliva secretion in cows (Mertens, 1997; Teimouri Yansari *et al.* 2004; Teimouri Yansari and Pirmohammadi, 2009; Hall and Mertens, 2017). Generally, increasing forage particle size will increase eating activity, may increase ruminating and chewing activity. Beauchemin *et al.* (2008) found that a longer forage particle size does not increase the rate of secretion of saliva; rather it decreases eating rate, allowing more saliva to be secreted per unit of dry matter intake

(DMI). However, some studies have reported that the amount of saliva secreted during eating in cows ranged from 166 to 253 g/min (Bailey, 1961; Cassida and Stokes, 1986; Maekawa *et al.* 2002b; Beauchemin *et al.* 2003, Beauchemin *et al.* 2008; Bowman *et al.* 2003). The forages differed in eating rate as g of dry matter (DM) per minute or g of NDF per minute. Beauchemin *et al.* (2008) found that ensalivation was greatest for straw (7.23 g of saliva/g of DM) and similar for barley silage, alfalfa silage, and alfalfa hay (4.15, 3.40, and 4.34 g of saliva/g of DM, respectively). However, the effects of feed characteristics such as particle size did not well quantified on rate of saliva secretion yet.

Feed characteristics such as particle size, DM, and NDF content affect salivary production during eating by affecting the eating rate. Slower eating rate and greater time spent for eating may help prevent ruminal acidosis by increasing the total daily salivary secretion in ruminant. Beauchemin *et al.* (2008) and Zali *et al.* (2015) stated that any variation in feedstuffs that decreases eating rate will increase the amount of saliva secreted per unit of DMI and type of forage could have an impact on eating rate due to different levels of NDF and differences in fragility. In addition, increasing ration DM will also tend to decrease eating rate (Beauchemin *et al.* 2008).

Therefore, a practical way to increase chewing activity, saliva secretion, and rumen buffering is to increase forage particle size. However, forages vary in particle size or physically effective neutral detergent fiber (peNDF) content and the extent to which they promote chewing and saliva secretion in ruminant have not been well quantified (Hall and Mertens, 2017). The objectives of this study were to determine whether rate of salivation during eating differs for different particle size of alfalfa hay.

MATERIALS AND METHODS

Animal and feeding

Six fistulated Hereford steers (body weight=414±13 kg) were used in a switch back design to evaluate whether particle size of alfalfa hay influenced salivary secretion during eating.

The experiment carried out in two 26-d periods, with 11-d of adaptation to ration, followed by 5 d of to achieve to level of voluntary feed intake, 7-d for adaptation to feeding to 90% of voluntary feed intake, and 3-d for measurements. The steers were housed in individual stalls. During the experiment, cows were housed in tie-stalls and fed twice daily at 09^{00} and 21^{00} with alfalfa hay. Water and mineralized salt were available for all cows over the experiment.

Hay preparation and particle size distribution

Alfalfa harvested at early flowering (about 15%), and dried. Individual bales were chopped with a forage field harvester (Jaguar # 62, Class Company, Germany) to 18.75 and 4.65 mm theoretical cut length (TCL) for preparation of two different particle sizes.

The geometric mean and the standard deviation of geometric mean in each type of alfalfa were determined as reported by American Society of Agricultural and Biological Engineers (2007).

Chemical composition

The two types of alfalfa analyzed for DM, organic matter (OM), crude protein (CP), ether extract (EE) (AOAC, 2005), neutral detergent fiber (NDF; using heat stable α-amylase but without the use of sodium sulfite), acid detergent fiber (ADF), lignin (Van Soest *et al.* 1991), and ash. The non fiber carbohydrate (NFC) was calculated by 1000 - (CP (g/kg) + NDF (g/kg) + Ash (g/kg) + EE (g/kg)) (NRC, 2001).

Functional specific gravity measurements

The functional specific gravity (FSG) of two alfalfa types were measured using 100 mL pycnometers (Wattiaux *et al.* 1992a; Wattiaux *et al.* 1992b; Wattiaux *et al.* 1993; Teimouri and Pirmohammadi, 2009). The samples (1.5 g) were incubated for 24 h in pycnometers and their FSG were measured at 12 and 24 h after incubation (Table 3). All the measurements were made at 39.0 ± 0.5 ˚C. The mixed rumen fluid from two steers fed only alfalfa were collected prior to feeding and rinsed with 8 layers of cheese cloth, centrifuged at 30000 × g, for 10 min and the supernatant with density 1.0039 ± 0.0024 g/mL, used as the hydration solution. Sodium azide (0.50 g/L) and penicillin G (25000 units/L) were added to the hydration solution to prevent microbial growth.

Measurement of saliva secretion

Saliva secretion was measured during the morning meal by rumen evacuation technique at 35 minutes after feeding through the rumen fistula of each steer (Bailey, 1961). The rumen particulate matter was poured in a 20 L bucket, which kept under a carbon dioxide gas in a large bath of 39 ˚C.

A specific amount of experimental diets was given to the animal for 35 minutes. During this time, animals did not have access to water. After 35 minutes, the rumen was completely discharged by rumen evacuation technique and its digesta weighed, and about 1000 g of sample was taken to determine the moisture content and particle size distribution.

To determine the amount of particle size reduction during the eating, samples of digesta were sieved using a wet sieving method in three replicates to determine the particle size distribution (Table 2). The amount of saliva added to feed (ensalivation rate, g/g of DM) was calculated as the difference in moisture content between the feed and the digesta. The ensalivation rate was expressed on the basis of fiber (g/g of NDF) by correcting for the NDF content of the feed. Salivation rate (g/min) was calculated for each collection by dividing the quantity of saliva by the duration of the collection period. The values were averaged over the meal within animal and day to calculate the amount of saliva secreted per minute during the consumption of forage (Table 3).

Statistical analysis

Using a complete randomized design with two treatments in three replications, data were analyzed by PROC general linear method (GLM) of SAS (2002). Means were separated using Duncan's multiple range tests with an alpha level of 0.05.

RESULTS AND DISCUSSION

Chemical composition

Coarse and fine alfalfa had the same chemical composition (Table 1), but their particle size distribution was different and there was a significant difference between the geometric mean and their geometric standard deviation (Table 2). The contents of CP, NDF and ADF of two types of alfalfa did not differ significantly; therefore, it seems that the differences observed in animal responses result in differences in the size of alfalfa particles.

Particle size distribution

Reduction of alfalfa particle reduced the geometric means and their standard deviation. On the other hand, reducing the size of the particles resulted in a significant decrease in the residual materials on the upper sieves (Table 2). The reduction of alfalfa particles significantly decreased the residual content of the 19 and 12.7-mm sieves, but the remaining materials on the 3, 6, 3.96, and 1.18- mm sieves were significantly increased (Table 2). The geometric mean of alfalfa particles decreased significantly with particle size reduction, while the standard deviation of geometric mean of two different alfalfa sizes did not have a significant difference (Table 2).

The particle size of foodstuffs decreased significantly during the initial chewing (P<0.0004; Table 2). When using coarse and fine alfalfa, the percentage of cumulative frequency of particles under the 19, 12.7, 6.3, 3.96, and 1.18-mm sieves were 96.79, 84.75, 42.73, 16.6, and 5.59; 99.07, 96.65, 82.33, 36.16, and 8.12%, respectively. However, the cumulative frequency of particles under 19, 12.7, 6.3, 3.96, and 1.18- mm sieves for rumen particulate matter for coarse and fine alfalfa were 99.95, 85.98, 78.62, 56.49, and 25.04; 100, 100, 97.50, 76.55, and 36.16%, respectively. Initial chewing caused an increase in the cumulative frequency of particles under 19, 12.7, 6.3, 3.96, and 1.18- mm sieves in both experimental treatments. Initial chewing caused a significant decrease in the geometric mean of coarse and fine alfalfa (P<0.0001; Table 2). Grant (2010) reported that chewing during feed consumption reduced the proportion of particles larger than one millimeter by about 50% in sheep. The feed particles were chewed during consumption until it reached a point where the masticated matter were easily swallowed (Grant, 2010). In this experiment, the geometric mean of coarse and fine alfalfa particles during chewing decreased by 40.62 and 45.53 percent, and the percentage of the cumulative frequency of particles under each sieves increased after chewing, because chewing reduced particle size and cause more particles to pass through the top sieves and accumulate on the lower ones.

Functional specific gravity measurements

The FSG was significantly different between two types of alfalfa. Reduction of particle size increased the FSG of alfalfa hay and in particulate matter, too.

In addition, the gas associated with particles had similar trend to the FSG. However, the true specific gravity of alfalfa hay and particular matter were similar. Particle size and specific gravity accounted for 28 and 59% of the variation in mean retention time, respectively (Kaske and Engelhardt, 1990). Wattiaux *et al.* (1992a), Wattiaux *et al.* (1992b), Wattiaux *et al.* (1993) and Bhattai and Firkins (1995) found that particles with a specific gravity < 1.2 are likely to float above, and those > 1.5 are likely to sink below the reticulo-omasa1 orifice. In addition, Wattiaux *et al.* (1992a), Wattiaux *et al.* (1992b) and Wattiaux *et al.* (1993) found that dried forages had specific gravity between 0.6 to 1.0 and specific gravity of their particles is profoundly altered by exposure to ruminal conditions. The results of current experiment confirmed reduction in particle size of alfalfa hay has led to an increase in specific gravity of alfalfa hay and ruminal particulate, which in turn increases the passage rate of particulate matters from the reticulorumen, decreases the rumen's retention time, decreases the rumen mat consistency, and ultimately decrease effectiveness of alfalfa hay in ruminants.

Saliva secretion

Production of saliva in milliliters (P=0.001), per kg of DMI (P<0.0001) and per each kilogram of NDF intake (P<0.0001) were affected by alfalfa particle size.

Table 1 Dry matter and chemical composition of alfalfa hay with two different particle sizes

Items	Alfalfa hay	
	Coarse	Fine
Dry matter (%)	92.73±1.23	92.68±1.04
Chemical composition (% of dry matter)		
Neutral detergent fiber	43.78±1.01	44.02±0.93
Acid detergent fiber	37.20±0.98	37.23±0.87
Lignin	12.84±0.43	12.90±0.35
Non fiber carbohydrate	33.01±1.12	33.08±1.01
Crude protein	16.83±0.36	16.89±0.40
Ether extract	1.55±0.12	1.57±0.11
Ash	4.83±0.21	4.44±0.19

Table 2 The distribution of particle size and some physical characetristics of two types of alfalfa hay used in experiment and chewed matter in rumen after 35 minutes

Items	Coarse alfalfa hay[1]		Fine alfalfa hay[1]	
	Alfalfa particle	Particulate matter	Alfalfa particle	Particulate matter
The proportion of materials that retained on different sieves (% of DM)				
19 mm	3.21[a]	0.05[A]	0.93[b]	0.00[B]
12.7 mm	12.04[a]	1.10[A]	2.42[b]	0.00[B]
6.3 mm	42.02[a]	20.23[A]	14.32[b]	2.25[B]
3.96 mm	26.13[b]	22.13	46.17[a]	21.20
1.18 mm	11.01[b]	31.45[B]	28.16[a]	40.39[A]
Pan	5.59	25.04[B]	8.12	36.16[A]
Geometric mean (mm)	6.50[a]	2.64[A]	3.80[b]	1.73[B]
Standard deviation of geometric mean (mm)	0.42	0.41	0.48	0.32

[1] The difference between feed particle (coarse and fine) and particulate matter after 35 mim chewing for coarse and fine treatments were presented with lower and uppercase letter, respectively.

Saliva production was higher in coarse alfalfa treatment (Table 4). Saliva secretion is stimulated by feed consumption and rumination (Van Soest, 1994). The feed consumption rate is of paramount importance in the saliva-feed buffering capacity. The faster consumption rate, at the maximum saliva secretion rate, reduces saliva secretion per kilogram of DMI. The total amount of saliva secretion depends on the amount of time of feed consumption and the rumination time. In this experiment, coarse alfalfa consumer cows had less consumption rate of alfalfa than cows consuming fine alfalfa, so they spent more time for eating and seemed to produce more saliva. Cassida and Stokes (1986) estimate that dairy cows secrete 150, 177 and 300 mL per minute saliva during resting, eating, and rumination time. Henry (2001) reported that saliva secretion was 28.84 and 263.3 mL/min when using two different sizes of barley silage with a hypothetical cut length of 18.75 and 4.65 mm, respectively. However, the amount of DMI and NDF intake decreased significantly with the reduction of particle size, the salivation rate per kilogram of DM and NDF when coarse alfalfa was used was significantly greater. The characteristics of forages (i.e., DM content, NDF content, and particle size) influenced the amount of saliva secreted during meals by affecting eating rate and duration of meals, rather than salivation rate (g/min).

A slower eating rate led to longer meals, and therefore, greater ensalivation of feed (g of saliva/g of DM) and more saliva secreted during the meal. Using cardial collection technique, Beauchemin *et al.* (2008) found that salivation rate (213 g/min) during forage meals was not affected by source of forage, despite differences in moisture content, fiber content, and particle size. In addition, a mean salivation rate ranging from 166 to 253 g/min was reported by Bailey (1961), Cassida and Stokes (1986), Maekawa *et al.* (2002b), Beauchemin *et al.* (2003), Beauchemin *et al.* (2008) and Bowman *et al.* (2003). Bailey (1961) found that during eating, the amount of saliva added to foods such as hay was much greater than was added to the same weight of other foods such as concentrates. The amount of saliva added to a given weight of food depends on the rate at which the saliva is being secreted and the rate at which the food is being swallowed; a change in the rate of swallowing could be brought about by increase in either the amount of food in each bolus or in the rate at which the boluses are swallowed. Beauchemin *et al.* (2008) found that forage type had no effect on salivation rate, the eating characteristics of meals depended upon the forage consumed. On a fresh, dry matter, and NDF basis, silages were consumed 6 to 7 times, 2.5 times, and 2 times faster than hay and straw, respectively.

Table 3 The functional specific gravity, true specific gravity, and the gas associated with particle in two types of alfalfa hay that used in experiment and particulate matter in rumen after 35 minutes

Items	Coarse alfalfa hay		Fine alfalfa hay		SEM	P-value
	Alfalfa particle	Particulate matter	Alfalfa particle	Particulate matter		
Functional specific gravity	0.745[d]	0.987[b]	0.824[c]	1.021[a]	0.012	< 0.0001
True specific gravity (kg/L)	1.449	1.462	1.449	1.466	0.213	0.8321
Gas associated with particles (mL/g)	0.4305[a]	0.2918[b]	0.1963[c]	0.2973[b]	0.058	< 0.0001

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

Table 4 The effects of particle size of alfalfa hay on ensalivation

The rate of ensalivation	Coarse alfalfa hay	Fine alfalfa hay	SEM	P-value
mL per minute	260.31[a]	249.32[b]	3.562	0.0021
Litter per kg DMI	1.305[a]	0.986[b]	0.121	< 0.0001
Litter per kg NDF intake	2.978[a]	2.243[b]	0.124	< 0.0001

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

The ability of cattle to consume silage NDF quicker than hay or straw NDF was likely due to the shorter particle size of the silages, although fragility of the fiber may also have been a factor (Iwaasa *et al*. 1996). Eating rate declines with increasing NDF concentration for long forages (Beauchemin, 1991).

Chopping, as in the case of ensiled feeds, increases eating rate because the reduction in feed particle size before feeding reduces the need for subsequent mastication of the NDF by the cow (Beauchemin, 1991). However, the forage sources differed in eating rate (g of DM/min), which led to differences in ensalivation of forages (g of saliva/g of DM and g of saliva/g of NDF).

Beauchemin *et al*. (2008) reported that ensalivation (g of saliva/g of DM) was greatest for straw (7.23) and similar for barley silage, alfalfa silage, and alfalfa hay (4.15, 3.40, and 4.34 g/g of DM, respectively). Higher ensalivation of straw could be accounted for by its higher NDF content; ensalivation of NDF (g of saliva/g of NDF) was actually greatest for long-stemmed alfalfa hay (12.4) and similar for the other chopped forages (8.9). It seems that salivation during eating appears to be about 1.3 to 2-times higher than resting salivation based on the resting salivation rates reported by others (107 mL/min by Maekawa *et al*. 2002b; 138 mL/min by Bowman *et al*. 2003; 151 mL/min by Cassida and Stokes, 1986). Thus, as was discussed by Beauchemin *et al*. (2008), increasing the daily time spent eating either through increased meal frequency or by longer meals (by reducing the rate of intake during meals), or feeding coarse hay would be beneficial in terms of increasing total salivary secretion.

CONCLUSION

The geometric mean of coarse and fine alfalfa particles during chewing decreased by 40.62 and 45.53%, respectively. The percentage of cumulative frequency of the substances below each sieves increased. By reducing of particle size, saliva production decreased by DM and NDF. Particle size and time after feeding had a significant effect on FSG and gas content with solid contents, but no significant effect was observed on the FSG of liquid and solid mass in solid and ruminal fluid.

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