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# Influence of Pre-Collection Diet and Preparation Technique on Nutrient Composition of Masticate Samples

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# Influence of Pre-Collection Diet and Preparation Technique on Nutrient Composition of Masticate Samples

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the objectives of these studies were to evaluate the influence of salivary contamination and squeezing masticate samples on ash, crude protein, NDF, and IVDMD values of samples collected from fistulated cattle.

samples were collected and analyzed for urea nitrogen content. In study 2, three esophageally fistulated cows sampled Sandhills upland range 12 times from May 21 to Aug. 18, 2011. In studies 3 and 4, ruminally fistulated steers were presented with either harvested ground hay or fresh clipped, mid-May vegetative smooth bromegrass. In studies 5 and 6, ruminally fistulated steers grazed two smooth bromegrass pastures during the grazing season from May through Oct. 2011.

## Summary

*Squeezing masticate samples to remove excess saliva skews forage nutrient composition of high quality, vegetative grass. Lower quality grass or harvested hay is less affected. Mastication increases ash content. Pre-collection diet of fistulated animals has no effect on nutrient content of the masticate.*

## Introduction

Fistulated animals have been used extensively to quantify nutrient content of diets consumed by grazing cattle. Unlike clipping, using fistulated cattle accounts for a grazing animal's selectivity and provides a representative sample of grazed forage quality. However, factors such as salivary contamination and sample handling technique could influence how well the masticate represents the actual diet consumed by grazing cattle. Diet masticate samples can be squeezed post-collection to remove excess saliva which decreases the time required for freeze drying. However, this technique could result in a loss of nutrients causing misrepresentation of diet nutrient composition. Therefore,

## Procedure

Six studies were conducted. Ruminally and esophageally fistulated cattle were used to sample vegetative and harvested forages. In studies where ruminally fistulated steers were utilized, steers were held without access to feed overnight then the contents of the rumen were completely evacuated. Following evacuation, steers were either presented with forages of known nutrient composition or allowed to graze for about 30 min. Next, the entire contents (forage and liquid) were collected and finally, the rumen contents previously evacuated were returned. In studies utilizing esophageally fistulated cows, cows were fitted with screen bottom bags after removal of the esophageal plug and were either presented with forages of known nutrient composition or allowed to graze for about 20 minutes. In all studies, masticate samples were then divided and either squeezed by hand until no more saliva could be removed (SQZ), or left un-squeezed (UNSQZ).

In study 1, 12 esophageally fistulated cattle were maintained on either vegetative subirrigated meadow (HI, 24% CP, n = 6) or fed meadow hay in a dry lot (LO, 7.7% CP, n = 6) for eight days prior to the start of the study. Blood

## Results

In study 1, pre-collection diet did not affect ( $P = 0.49$ ) CP content of masticate samples (Table 1). Serum urea nitrogen levels tended to be higher for HI cows ( $27.6 \pm 4.0$  vs.  $23.5 \pm 3.2$  ml/dl; HI vs. LO, respectively;  $P = 0.08$ ). Although a small amount of N is contained in the saliva, it was too small to influence the total nitrogen content of the sample in this instance. Type of forage offered (vegetative grass vs. hay) interacted ( $P = 0.01$ ) with preparation technique for CP, where CP was lost when vegetative grass masticate samples were squeezed ( $20.0$  vs.  $21.5\%$  CP for SQZ vs. UNSQZ, respectively;  $P < 0.05$ ) but there was no difference between squeezed and un-squeezed hay masticate samples ( $7.6$  vs.  $7.6\%$  CP for SQZ vs. UNSQZ, respectively;  $P > 0.05$ ). The pre-ingestion CP value for vegetative grass was 24% and 7.7% for the hay. Type of forage offered (vegetative grass vs. hay) also interacted ( $P = 0.001$ )

(Continued on next page)

**Table 1. Crude protein, NDF, and ash values of squeezed (SQZ) and unsqueezed (UNSQ) vegetative grass and hay masticate samples collected from esophageally fistulated cattle fed high or low levels of CP pre-collection (study 1).**

	High				Low				SE	P-values			
	Hay		Vegetative		Hay		Vegetative			Previous	Type	Process	T x P
	SQZ	UNSQ	SQZ	UNSQ	SQZ	UNSQ	SQZ	UNSQ					
CP	7.5 <sup>d</sup>	7.5 <sup>d</sup>	20.2 <sup>bc</sup>	21.9 <sup>a</sup>	7.6 <sup>d</sup>	7.6 <sup>d</sup>	19.7 <sup>c</sup>	21.0 <sup>ab</sup>	0.5	0.49	< 0.001	0.01	0.01
NDF	68.4 <sup>ab</sup>	64.5 <sup>b</sup>	51.3 <sup>c</sup>	50.8 <sup>c</sup>	72.8 <sup>a</sup>	67.7 <sup>ab</sup>	50.8 <sup>c</sup>	42.7 <sup>d</sup>	2.4	0.89	< 0.001	0.01	0.01
Ash	10.8 <sup>c</sup>	13.0 <sup>b</sup>	18.8 <sup>a</sup>	15.6 <sup>a</sup>	12.1 <sup>c</sup>	14.2 <sup>b</sup>	17.2 <sup>a</sup>	17.5 <sup>a</sup>	0.7	0.39	< 0.001	0.56	0.01

<sup>abc</sup>Means lacking a common superscript letter differ ( $P < 0.05$ ).

with preparation technique for NDF. Squeezing masticate samples increased the NDF content of both forage types but to a greater extent for vegetative grass than for hay (52.2 vs. 44.1% NDF for VEG and 71.0 vs 67.3 for HAY;  $P < 0.05$ ). The pre-ingestion NDF value for vegetative grass was 40% and 66% for the hay. Cell solubles from fresh vegetative grass may go into solution more rapidly than those of the dry hay, possibly accounting for some of the differences observed.

In study 2, squeezing increased NDF content (69.7% vs. 66.0% for SQZ vs. UNSQZ, respectively;  $P < 0.01$ ) and decreased ash content (8.0% vs. 9.0% for SQZ vs. UNSQZ, respectively;  $P < 0.01$ ) but did not impact CP content (9.5% vs. 9.6% for SQZ vs. UNSQZ, respectively;  $P = 0.42$ ; Table 2).

In study 3, harvested ground hay masticate samples, both SQZ and UNSQZ, had significantly increased ( $P < 0.01$ ) CP levels from PRE samples (Table 3). However, in study 4, there was no difference in CP ( $P = 0.20$ ) between the pre-ingested and masticate samples.

In studies 5 and 6, UNSQZ masticated samples collected from fistulated steers had greater CP content ( $P = 0.06$  and  $P < 0.01$ , respectively) compared to SQZ (Table 3). The inconsistency in the effect of squeezing on CP content of the sample among studies utilizing ruminally fistulated steers could be due to increased salivary contamination with mature and grazed forages.

Neutral detergent fiber (as a percent of OM) varied among studies utilizing ruminally fistulated cattle. In study 4, mastication increased ( $P < 0.01$ ) NDF (% of OM) of SQZ and UNSQZ samples by 31.1% and 25.7%, respectively, compared to PRE (Table 3). In studies 5 and 6, squeezing masticate samples significantly increased NDF by 8.2% and 11.7%, respectively compared to UNSQZ. In contrast to the fresh forages, neither mastication nor preparation technique impacted ( $P = 0.17$ ) harvested ground hay NDF content in study 3.

In studies that used ruminally fistulated steers, handling technique had

**Table 2. Crude protein, NDF, and ash values of squeezed (SQZ) and unsqueezed (UNSQ) masticate samples collected from esophageally fistulated cattle grazing Sandhills upland range from May to August (study 2).**

	SQZ	UNSQ	SE	P-value
CP	9.5 <sup>a</sup>	9.6 <sup>a</sup>	0.3	0.42
NDF	69.7 <sup>a</sup>	65.98 <sup>b</sup>	0.008	< 0.0001
Ash	8.0 <sup>a</sup>	9.0 <sup>b</sup>	0.2	< 0.0001

<sup>ab</sup>Means with different superscripts differ ( $P$ -value < 0.01).

**Table 3. Nutrient composition of pre-ingested (PRE) forage, squeezed (SQZ), and un-squeezed (UNSQ) masticate samples collected from ruminally fistulated steers (studies 3 and 4).**

		PRE	SQZ	UNSQ	SEM	P-value
Study 3 <sup>1</sup>	Ash, %	7.71 <sup>b</sup>	8.67 <sup>b</sup>	12.41 <sup>a</sup>	0.36	< 0.01
	CP, %	20.64	18.55	20.15	0.80	0.20
	NDF, %	53.09 <sup>b</sup>	69.58 <sup>a</sup>	66.73 <sup>a</sup>	1.47	< 0.01
	IVDMD, %	66.50 <sup>a</sup>	61.65 <sup>b</sup>	63.42 <sup>b</sup>	0.61	< 0.01
Study 4 <sup>2</sup>	Ash, %	5.98 <sup>c</sup>	7.53 <sup>b</sup>	8.97 <sup>a</sup>	0.32	< 0.01
	CP, %	6.29 <sup>b</sup>	9.16 <sup>a</sup>	9.83 <sup>a</sup>	0.53	< 0.01
	NDF, %	71.58	74.56	72.72	1.06	0.17
	IVDMD, %	53.05	52.95	53.33	0.41	0.79
Study 5 <sup>3</sup>	Ash, %	—	10.74	13.88	0.60	< 0.01
	CP, %	—	15.02	16.81	0.66	0.06
	NDF, %	—	69.76	64.50	1.09	< 0.01
	IVDMD, %	—	54.76	57.79	2.34	0.36
Study 6 <sup>4</sup>	Ash, %	—	12.71	15.28	0.75	0.02
	CP, %	—	15.56	17.16	0.39	< 0.01
	NDF, %	—	69.29	62.06	1.50	< 0.01
	IVDMD, %	—	54.17	56.39	2.17	0.47

<sup>1</sup>Offered freshly clipped, vegetative smooth brome grass of known nutrient composition.

<sup>2</sup>Offered hay of known nutrient composition.

<sup>3</sup>Grazed smooth brome grass pasture.

<sup>4</sup>Grazed smooth brome grass pasture.

no effect ( $P > 0.06$ ) on the IVDMD of masticated diet samples. Similar to NDF and CP, IVDMD differences depend on forage type and maturity. There was no IVDMD difference ( $P = 0.79$ ) between the pre-ingested and masticate samples of the harvested ground hay sample (study 3) which is likely attributable to the increased maturity of the harvested forage (Table 3). However, IVDMD of pre-ingested high quality, vegetative forage samples decreased ( $P < 0.01$ ) with mastication, but with no difference between SQZ and UNSQZ preparation techniques. Mastication of the offered vegetative forage decreased ( $P < 0.01$ ) IVDMD compared to the SQ and UNSQ samples by 7.3 and 4.6%, respectively.

The results of these studies indicate squeezing masticate samples has a large effect on high quality, vegetative grass but a lesser effect on low quality grass or harvested hay. Squeezing diet samples increased the NDF content in all studies, except the harvested ground hay in study 3. Squeezing

also impacted the CP levels of high quality forage but had little effect on CP content of lower quality forage. Mastication increased ash content and ash content was lower in samples that were squeezed compared to unsqueezed samples. Cell solubles are lost with the historical diet sampling methods of screen bottom bags with esophageally fistulated cows and also squeezing the masticate sample. With cell solubles lost, nutrient compositions are skewed. Previous diet did not impact N level of samples. This is the first research to test the effects of squeezing high quality, vegetative masticate samples and further work is warranted in this area.

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