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Technical Note: Collection and preparation techniques change nutrient composition of masticate collected from esophageally fistulated cattle

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ABSTRACT: Two experiments determined effects of collection and preparation techniques on nutrient composition of masticate samples from esophageally fistulated cattle. In Exp. 1, 12 esophageally fistulated cattle were maintained on 2 precollection diets, high CP (24% CP; $n = 6$) or low CP (7.7% CP; $n = 6$), for 8 d. On d 9, the esophageal plug was removed, screen bottom bags were attached, and each cow was offered fresh grass. Immediately after fresh grass sample collection was complete, dry grass (hay) was offered and a sample was collected. Blood samples were collected and analyzed for serum urea nitrogen concentration. Masticate samples of both fresh and dry grass were divided and each was either squeezed by hand until no more saliva could be removed or remained unsqueezed. In Exp. 2, 10 esophageally fistulated cattle were fitted with either screen ($n = 5$) or solid ($n = 5$) bottom collection bags after removal of the esophageal plug and presented grass hay, fresh grass, alfalfa hay, or fresh alfalfa. In Exp. 1, the precollection diet did not affect ($P = 0.49$) CP content of masticate even though serum urea nitrogen tended to be greater ($P = 0.08$) for high- vs. low-CP precollection diets. Forage harvest type offered (fresh vs. hay) interacted ($P = 0.01$) with preparation technique (squeezed vs. unsqueezed) for CP, where CP decreased in squeezed fresh samples ($P < 0.001$) but not in squeezed grass hay samples ($P = 0.98$). In Exp. 2, ingestion greatly increased levels of ash ($P < 0.001$). Crude protein was greater ($P < 0.004$) before ingestion for all samples except grass hay ($P = 0.43$). Levels of NDF were similar before and after ingestion ($P > 0.15$) for all samples except fresh alfalfa, which was greater after ingestion ($P = 0.002$). Ingestion status did not affect in vitro OM disappearance (IVOMD; $P > 0.34$) except for grass hay, which was greater after ingestion ($P < 0.001$). Bag type (screen vs. solid) did not affect ash and NDF ($P > 0.31$), except for fresh alfalfa, which were greater ($P < 0.03$) for solid bottom bags. Bag type did not affect alfalfa CP ($P = 0.71$) but did affect grass CP, which was lower ($P = 0.02$) for solid bottom bags. Bag type did not affect IVOMD ($P > 0.33$). More ($P = 0.01$) fresh forage than hay was recovered through the esophageal opening. Previous diet did not impact masticate samples but squeezing impacted CP levels of high-quality forage and therefore should not be performed. Nutrient values should be reported on an OM basis.

Key words: esophageal diet collection, grazed diets, salivary contamination, sample preparation

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INTRODUCTION

Fistulated animals have been extensively used to quantify nutrient concentration of forage consumed by grazing animals. This method is affected by the grazing animal's diet selectivity that is not accounted for by other methods such as clipped samples (Holechek et al., 1982). However, several factors may affect the degree to which forage masticate samples actually represent grazed animal diets. Salivary contamination and sample preparation technique influences both the organic and inorganic components of grazed forage samples (Hoehne et al., 1967; Acosta and Kothmann, 1978; Coates, 2010). Salivary N concentration depends on N content of the precollection diet

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of fistulated cattle and may, therefore, impact N values of masticate if the collected sample is from a different pasture than where the esophageally fistulated animals are maintained. Even though the preparation technique of squeezing the sample to remove excess saliva is accepted (Hoehne et al., 1967), it could result in a loss of cell solubles and influence the measurement of forage quality. Collection bags with screen bottoms have long been used (Edlefsen et al., 1960; Barth and Kazzal, 1971; Scales et al., 1974) and allow for drainage of excess saliva but nutrients may leach from the forage into the saliva and then be lost when saliva drains from the bag. Forages of different quality may be affected to differing degrees (Coates, 2010). Therefore, the objectives of these studies were to determine effects of 1) precollection diet, 2) squeezing masticate to remove saliva, and 3) collection bag type (screen vs. solid) on the nutrient composition of fresh or dry forage of differing quality collected from esophageally fistulated cattle.

MATERIALS AND METHODS

Institutional Animal Care and Use Committee Approval

With approval of the University of Nebraska Institutional Animal Care and Use Committee (project 921), 2 experiments determined effects of collection and preparation techniques on nutrient quality of masticate samples from esophageally fistulated cattle.

Fistulation Procedure

Esophageal fistulation surgery was performed on 14-mo-old (318-kg) beef heifers (three-fourths Red Angus and one-fourth Simmental) following a procedure similar to that used by Adams et al. (1991). The animal remained in a standing positon while being restrained in a squeeze chute. Xylazine hydrochloride (Rompun; Bayer, Shawnee Mission, KS) was administered (0.11 mg/kg BW) intramuscularly, the surgical site was clipped and scrubbed, a 60-mm incision was made in the skin, the brachiocephalicus and sternocephalicus muscles were separated by blunt dissection, and then a 60-mm incision was made into the lumen of the esophagus. The incised edges of the esophagus and skin were sutured using 2 stitches, and then a convex 89- by 25.4-mm polyethylene coated aluminum cannula with a 65-mm threaded shank measuring 6.35 mm in diameter was inserted into the lumen of the esophagus. The cannula was similar to that depicted in Fig. 3C by Van Dyne and Torrell (1964). The shank on the cannula was put through the center of a conical plug measuring 31 and 25 mm for the large and small ends, respectively, and 51 mm in length and secured with a wing nut. Postoperative care included administration of topical antibiotic (Nitrofurazone Topical Ointment; Vedco Inc., St. Joseph, MO), intramuscular injection (2.2 mL/100 kg BW) of procaine penicillin G (Agri-cillin; Agri Laboratories LTD, St. Joseph, MO), and monitoring rectal temperature, cannula placement, and feed and water intake daily for 5 d. Surgeries were performed at least 2 yr before initiation of the present experiments. The animals were experienced and previously had been used to successfully collect masticate samples.

Experiment 1 Procedures

Experiment 1 determined effects of precollection diet and squeezing masticate samples on nutrient content of harvested grass hay and fresh grass samples. Twelve esophageally fistulated cows were maintained on 2 precollection diets either high or low in CP: 1) grazed vegetative subirrigated meadow (24% CP; $n = 6$) or 2) fed harvested grass hay in a dry lot (7.7% CP; $n = 6$). A description of the grass species found on the subirrigated meadow is given by Volesky et al. (2004). Cows consumed these diets for 8 d before initiation of sample collection. On d 9, cattle were held off feed and water for 12 h and then the esophageal plug was removed, screen bottom bags were attached, and each cow was offered 428 g (DM) vegetative grass (24% CP and 40% NDF), which had been hand harvested from subirrigated meadow immediately before presentation. Collection bags had a round bottom measuring 200 mm in diameter with sides measuring 400 mm. The sides of the bags were constructed of waterproof nylon fabric and the bottom was constructed of nylon mesh with square pores measuring 2 by 2 mm. Two 100-mm slits were placed opposite each other in the top of the bag creating flaps on each side of the neck when placed on the animal. Straps looped around the animal's neck and to a girth strap, holding the bag in place (Van Dyne and Torrell, 1964). All masticate and saliva expelled from the fistula was collected in the bag. After complete consumption of the forage offering (about 15 min), collection bags were removed from the animal, and then masticate samples were removed from the bag and divided in half. Each half was either squeezed by hand until no more saliva could be removed or remained unsqueezed. Most saliva drained out of the screen bottom bag but any remaining with the unsqueezed half was retained. Following fresh grass masticate collection, bags, which had been thoroughly cleaned, were replaced on the animal and each cow was offered 1,032 g (DM) grass hay (7.7% CP and 66% NDF) harvested from subirrigated meadow 10 mo before initiation of the experiment. After all offered hay was consumed, grass hay masticate samples were divided and hand squeezed or not in the same manner as fresh grass masticate samples. The amount of each forage offered was chosen to

ensure forage offered would be completely consumed by the animal. No orts remained in the feed pan for any forage. All masticate and preingested forage samples were immediately frozen and stored at –20°C until lyophilized. Following masticate collection, blood samples were collected via coccygeal venipuncture and analyzed for urea nitrogen content (Broderick and Kang, 1980).

Experiment 2 Procedures

Experiment 2 used 10 esophageally fistulated cows maintained on subirrigated meadow before the start of the experiment to determine the difference in nutrient composition of masticate sample collected using solid and screen bottom collection bags. Cattle were randomly assigned to either solid or screen bottom bag treatments. On d 1 and 4, cattle were held off feed for 12 h and then the esophageal plug was removed. Cattle were then fitted with either solid ($n = 5$) or screen ($n = 5$) bottom collection bags. The same bags used in Exp. 1 were used in Exp. 2. Screen bottom bags were converted to solid bottom bags by adhering waterproof nylon fabric to the bottom such that no liquid was lost from the bag. On d 1, cattle were offered 410 g (DM) grass hay (7.1% CP and 80% NDF) harvested from a subirrigated meadow 10 mo previous to initiation of the experiment and were allowed to completely consume it (about 15 min). Masticate samples were removed, bags, which had been thoroughly cleaned, were placed back on the animal, and cattle were then offered 170 g (DM) fresh meadow grass (15.1% CP and 56% NDF) harvested from a subirrigated meadow immediately before presentation. On d 4, cows were offered 416 g (DM) alfalfa hay (19.5% CP and 49% NDF) harvested 9 mo previous to initiation of the experiment and allowed to completely consume it (about 15 min). Masticate was removed, bags, which had been thoroughly cleaned, were placed back on the animal, and cattle were then offered 109 g (DM) fresh alfalfa (19.1% CP and 40% NDF) harvested immediately before presentation. Preingested forage was randomly subsampled for chemical analysis. The amount of each forage offered was chosen to ensure forage offered would be completely consumed by the animal. No orts remained in the feed pan for any forage. Masticate samples were collected and weighed to calculate percentage of forage offered recovered in the collection bag. All masticate and preingested forage samples were immediately frozen and stored at –20°C until lyophilized. In Exp. 2, no masticate samples were squeezed and all saliva in the collection bag was retained with the sample.

Laboratory Analysis

Samples from Exp. 1 were analyzed for CP, NDF, and ash. Experiment 2 samples were analyzed for CP, NDF, ash, and in vitro OM disappearance (**IVOMD**). Frozen samples were lyophilized using a Vertis Freezemobile 35 XL laboratory lyophilizer (SP Scientific, Gardiner, NY). All samples were ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and analyzed for nitrogen using a Leco FP 2000 combustion nitrogen analyzer (Leco Corp., St. Joseph, MO) and then converted to CP by multiplying by 6.25. Neutral detergent fiber content was determined using the Van Soest et al. (1991) procedure A without the inclusion of amylase or sodium sulfite and was corrected for ash content. In vitro DM disappearance was measured using the Tilley and Terry (1963) method, with the modification of adding 1 g/L of urea to the buffer (Weiss, 1994). Organic matter was determined by placing a dry sample in a combustion chamber for 6 h at 600°C. Ash content of in vitro residues was determined and then used to express in vitro disappearance on an OM basis. Values for CP and NDF were also expressed on an OM basis.

Statistical Analysis

In Exp. 1, data were analyzed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC) as a 2 × 2 × 2 factorial arrangement of treatments in a completely randomized design. The model included pre-experiment diet (low vs. high CP), forage type (grass hay vs. fresh grass), and postcollection processing technique (squeezed vs. unsqueezed) as fixed effects and cow as a random effect. In Exp. 2, data were also analyzed using the mixed procedure of SAS as a 2 × 2 × 2 factorial arrangement of treatments in a completely randomized design. The model included ingestion status (preingested vs. postingested), forage type (fresh grass vs. grass hay), and bag type (screen vs. solid) as fixed effects and cow as a random effect. In both experiments, individual animal was used as the experimental unit and least squares means were separated using the LSD method when there was an overall significant ($P < 0.05$) effect of treatment.

RESULTS AND DISCUSSION

Experiment 1 Results

There were no 3-way interactions ($P > 0.25$) among precollection diet, postcollection processing technique, and forage type (fresh vs. hay) for any measured variable. Likewise, there were no 2-way interactions ($P > 0.26$) between precollection diet and postcollection processing technique or forage type.

Precollection diet did not affect ($P = 0.49$) CP content of masticate samples (Table 1). Serum urea nitrogen levels tended to be greater for cows on the high-CP diet (27.6 ± 4.0 vs. 23.5 ± 3.2 mL/dL for high vs. low

Table 1. Nutrient composition of squeezed (SQZ) and unsqueezed (UNSQZ) grass hay or fresh grass masticate samples collected from esophageally fistulated cows maintained on high- or low-CP precollection diets (Exp. 1)

	High-CP precollection diet				Low-CP precollection diet					*P*-value			
	Hay		Fresh		Hay		Fresh						
Item	SQZ	UNSQZ	SQZ	UNSQZ	SQZ	UNSQZ	SQZ	UNSQZ	SE[1]	Precollection diet[2]	Forage[3]	Process[4]	Forage × process[5]
Ash, % DM	10.8^{c}	11.9^{bc}	18.3^{a}	17.2^{a}	12.1^{bc}	14.2^{b}	17.2^{a}	17.5^{a}	0.7	0.39	<0.001	0.27	0.07
CP, % OM	7.5^{d}	7.5^{d}	20.2^{bc}	21.9^{a}	7.6^{d}	7.6^{d}	19.7^{c}	21.0^{ab}	0.5	0.49	<0.001	0.01	0.01
NDF, % OM	69.2^{ab}	66.9^{b}	53.5^{c}	45.4^{d}	72.8^{a}	67.7^{b}	50.8^{c}	42.7^{d}	2.4	0.89	<0.001	<0.001	<0.001

$^{a–d}$Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Standard error of the simple effect least squares mean ($n = 6$).

[2]Precollection diet: either high (24%) or low (7.7%) CP.

[3]Main effect of forage harvest status: either hay or fresh grass.

[4]Main effect of postcollection processing: either squeezed or unsqueezed.

[5]Interaction of forage harvest status and postcollection processing.

CP, respectively; $P = 0.08$). Although saliva contains only a small amount of N, previous studies demonstrate increased N concentration in masticate samples (Lesperance and Bohman, 1963; Blackstone et al., 1965; Marshall et al., 1967), whereas others indicate it has little effect on N content of masticate (Bath et al., 1956). Weir and Torell (1959) calculated added N attributed to salivary contamination of masticate samples collected from esophageally fistulated sheep would raise the protein content only less than 0.01%. Regardless of whether there was a greater amount of nitrogen present in the saliva of cows on the high-CP diet in the present study, the total amount of salivary contamination was too small to influence the total nitrogen content of the sample.

Type of forage offered (fresh grass vs. grass hay) interacted ($P = 0.01$) with postcollection processing technique (squeezed vs. unsqueezed) for CP content of masticate (Table 1). Crude protein was lower when fresh grass masticate samples were squeezed ($P < 0.001$) but there was no difference ($P = 0.98$) between squeezed and unsqueezed hay masticate samples. The preingestion CP value for fresh grass was 24 and 7.7% for the hay.

Type of forage offered (fresh grass vs. grass hay) also interacted ($P = 0.001$) with preparation technique (squeezed vs. unsqueezed) for NDF. Squeezing masticate samples increased ($P < 0.001$) NDF content of both forage types but to a greater extent for fresh grass than for grass hay. The preingestion NDF value was 40 and 66% for fresh grass and grass hay, respectively. Cell solubles from fresh grass may go into solution more rapidly than those of the dry grass hay, possibly accounting for some of the difference observed. Squeezing did not affect ash content ($P = 0.27$) of either forage type.

Previous research investigating the effects of squeezing masticate samples to prepare them for laboratory analysis demonstrated similar CP content between squeezed and unsqueezed samples (Hoehne et al., 1967). However, the forages used in that research were collected on or after July 28, meaning the forage was mature (not vegetative) and was similar in CP content to the hay used in present study. The results of the present study suggest that squeezing masticate samples had a larger effect on vegetative, high-quality, fresh, low DM content grass than on mature, low-quality, high DM content grass or grass hay. Lower CP level would mean a lower level of soluble CP available to be leached. The effects of squeezing high-quality, fresh vegetative masticate samples has not been well studied and further work is warranted in this area.

Experiment 2 Results

Ash concentration of the masticate sample was much greater ($P < 0.001$) than the preingestion feed offered (Table 2). Even though the DM content of bovine saliva is only about 1% (Bailey and Balch, 1961), the ash content of that DM is quite high (about 85%; Lesperance et al., 1960). The greater ash content after ingestion is in agreement with results reported by Bath et al. (1956), Hoehne et al. (1967), and Barth and Kazzal (1971). The postingestion increase in ash content of forage samples may be accounted for by expressing all chemical components on an OM basis. The addition of minerals by the saliva make samples collected through the esophageal fistula unacceptable for determination of mineral composition of the forage.

Crude protein levels of fresh alfalfa, alfalfa hay, and fresh grass were decreased ($P < 0.004$) by ingestion but CP levels of grass hay were not different ($P = 0.43$) before and after ingestion (Table 2). A lack of difference in grass hay may be a function of the relatively low preingestion CP content compared with the other forages. When there is less initial CP in a forage, there may be less opportunity for loss. Levels of NDF were not different ($P > 0.15$) before ingestion and after ingestion for all samples except fresh alfalfa ($P = 0.002$). Fresh alfalfa contained

Table 2. Nutrient composition of preingested and postingested fresh or dry alfalfa and grass (Exp. 2)

	Fresh		Hay			*P*-value		
	Preingested	Postingested	Preingested	Postingested	SE[1]	Harvest[2]	Ingest[3]	Harvest × ingest[4]
Alfalfa								
Ash, % DM	9.4[c]	17.4[a]	10.6[c]	14.0[b]	0.7	0.21	<0.001	0.01
CP, % OM	21.2	19.3	21.8	19.8	0.5	0.18	<0.001	0.85
NDF, % OM	43.9[c]	49.9[b]	55.3[a]	52.7[ab]	1.5	<0.001	0.17	0.002
IVOMD,[5] %	68.3	68.5	62.0	63.4	1.1	<0.001	0.44	0.61
Grass								
Ash, % DM	13.2	18.0	7.1	10.4	0.8	0.001	<0.001	0.37
CP, % OM	17.5[a]	14.8[b]	7.6[c]	7.8[c]	0.2	<0.001	<0.001	<0.001
NDF, % OM	64.8	62.8	86.1	83.3	1.6	<0.001	0.15	0.81
IVOMD, %	77.8[a]	76.9[a]	55.7[c]	61.1[b]	0.9	<0.001	0.004	<0.001

[a–c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Standard error of the simple effect least squares mean ($n = 10$).

[2]Main effect of forage harvest status: either hay or fresh grass.

[3]Main effect of forage ingestion status: either before or after ingestion.

[4]Forage harvest status × ingestion status interaction.

[5]IVOMD = in vitro OM disappearance.

the least amount of NDF of all forages used in the experiment. Neutral detergent solubles from fresh forages may go into solution more rapidly than those of the dry hay, possibly accounting for some of the difference observed. In general, IVOMD was not affected by ingestion status ($P > 0.34$), except for grass hay ($P < 0.001$). Barth and Kazzal (1971) reported a decrease in IVDMD for tall fescue whereas IVDMD for orchardgrass was similar in preingested and postingested samples (–2.8 vs. –1.0% difference in IVDMD from preingested to postingested samples for tall fescue vs. orchardgrass, respectively). Coates (2010) reported decreased IVDMD, measured via the pepsin-cellulase method, in masticate samples compared with feed samples, especially for grasses of low digestibility. Taken together, these data suggest neutral detergent solubles (which contain most of the CP in a forage sample) may be more labile than NDF and the degree to which masticate samples represent preingested forage may be dependent on initial quality.

Bag type (screen vs. solid) did not affect ($P > 0.31$) masticate ash or NDF except for fresh alfalfa masticate (ash, $P = 0.02$, and NDF, $P = 0.03$), for which levels of both were higher for solid bottom bags. Bag type did not affect ($P = 0.71$) CP concentration (Table 3) of alfalfa masticate samples but resulted in decreased ($P = 0.02$) CP concentration of grass masticate samples when solid bottom bags were used. In vitro DM disappearance was not affected by bag type ($P > 0.33$).

Table 3. Nutrient composition of fresh or dry alfalfa and grass masticate samples collected in screen or solid bottom bags from esophageally fistulated cattle (Exp. 2)

	Fresh		Hay			*P*-value		
Item	Screen	Solid	Screen	Solid	SE[1]	Harvest[2]	Bag[3]	Harvest × bag[4]
Alfalfa								
Ash, % DM	14.5[b]	20.8[a]	13.5[b]	14.5[b]	1.3	0.04	0.02	0.07
CP, % OM	19.4	19.2	19.9	19.7	0.7	0.44	0.71	0.99
NDF, % OM	47.4	53.1	52.8	52.7	2.4	0.05	0.03	0.40
IVOMD,[5] %	70.0	66.5	63.1	63.7	1.9	0.02	0.37	0.34
Grass								
Ash, % DM	18.3	17.6	9.7	11.1	1.5	<0.01	0.81	0.51
CP, % OM	15.0	14.6	8.0	7.6	0.2	<0.01	0.02	0.88
NDF, % OM	64.3	61.2	83.7	82.8	2.9	<0.01	0.39	0.76
IVOMD, %	77.6	76.2	59.5	62.6	1.6	<0.01	0.48	0.25

[a,b]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]Standard error of the simple effect mean ($n = 5$).

[2]Main effect of forage harvest status: either hay or fresh grass.

[3]Main effect of collection bag: either screen or solid bottom.

[4]Forage harvest status × collection bag interaction.

[5]IVOMD = in vitro OM disappearance.

Table 4. Amount of fresh or dry alfalfa and grass offered to esophageally fistulated cows recovered in collection bag (Exp. 2)

	Fresh		Hay			*P*-value		
Item	Alfalfa	Grass	Alfalfa	Grass	SE[1]	Harvest[2]	Forage[3]	Harvest × forage[4]
Recovery, % DM	68.2	63.8	53.1	48.8	0.1	0.01	0.43	0.99
Recovery, % OM	74.5	66.4	55.1	50.4	0.1	0.01	0.31	0.79

[1]Standard error of the simple effect mean (n = 10).

[2]Main effect of harvest status: either hay or fresh grass.

[3]Main effect of forage type: either alfalfa or hay.

[4]Harvest status × forage interaction.

Traditionally, screen bottom bags have been used to collect diet samples (Edlefsen et al., 1960; Barth and Kazzal, 1971). These bags allow for drainage of saliva, which reduces the weight of the bag resulting in more natural grazing behavior and reduced drying time in the laboratory. In a review of the literature, Van Dyne and Torrell (1964) concluded that leaching of nutrients from esophageal masticate samples was not a concern but all the research they cited was conducted with low-quality feeds. Acosta and Kothmann (1978) reported differences between medium-quality bermudagrass samples collected using solid and screen bottom bags and attributed the difference to nutrient leaching. In high-quality forages, nutrients may leach from the sample when combined with saliva and then be lost when the saliva drains from the bag (Holechek et al., 1982). Results of both Exp. 1 and Exp. 2 demonstrate that soluble CP in high-quality forage is subject to leaching. Intuitively, it seems solid bottom collection bags would be preferable to screen bottom bags when collecting high-quality forage masticate samples because potentially leached nutrients are not lost, even though results of the present study suggest bag type does not make a large difference.

Forage type (fresh vs. hay) influenced the amount of the forage recovered through the esophageal fistula (P = 0.01; Table 4). Barth and Kazzal (1971) found DM recoveries from fescue and orchardgrass pastures (66% DM fresh vegetative vs. 67 and 68% DM for fescue and orchardgrass, respectively) to be similar to fresh vegetative values in the present study. Blackstone et al. (1965) showed recovery was related to the size of the opening of the esophageal fistula; smaller openings consistently yielded lower amounts of sample whereas sheep with larger fistula yielded larger amounts of sample. One primary problem with smaller openings is that the opening may become plugged (Holechek et al., 1982) and forage sample may bypass the opening but saliva may be expelled. Low recoveries suggest masticate samples may not always be representative. Hence, it is crucial that proper care is taken when surgeries are performed to ensure a proper fistula opening size so recovery may be optimized. For this experiment, care was taken during surgery to achieve consistent esophageal openings among all cattle used and the amount of time between plug removal from the fistula and sample collection was minimized in an effort to prevent the opening from closing down. However, inherent animal-to-animal differences were impossible to completely eliminate and may have affected results.

Crude protein concentration in the diet cattle were maintained on before sample collection did not impact N level of masticate samples. Squeezing the samples impacts CP levels of high-quality forage but has little effect on lower-quality harvested forage. Squeezing increases NDF content of both high-quality and low-quality forage. Masticate samples should not be squeezed to remove excess saliva because nutrients, particularly neutral detergent solubles, will be lost. These data suggest that forage masticate samples collected through the esophageal fistula may underestimate the amount of CP and neutral detergent solubles present in high-quality forages but are representative of mid- or low-quality forages. Masticate samples appear to adequately represent the levels of NDF and IVOMD of forages sampled. Due to increased levels of ash, all values should be reported on an OM basis.

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