Effects of Different Inoculum Used for *In Vitro* and *In Situ* Digestion Procedures Performed on Corn Residue Samples

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Summary

An in vitro and in situ study was conducted to assess the effects of donor diet at time of incubation on NDF digestibility of corn residue samples. Residue samples had greater NDF digestibility when animals were on a similar diet (i.e. a high corn residue diet). Digestibility of NDF was greater when time of incubation increased. The diet of the donor steer had greater impact as time of incubation increased implying that the animal's diet, and incubation time, has an impact on the true values of NDF digestibility.

Introduction

Increased corn production has resulted in increased corn residue available as a feed source for cattle. Traditionally corn residue has been viewed as a low quality forage source due to its relatively low digestibility. Parts of the corn residue have different digestibility values, with stalk and cob being the least digestible, but composing the largest portion (60.11%) of the plant (*2012 Nebraska Beef Report*, pp 11–12).

With increasing progress in corn harvesting methods there has been a shift in which parts of corn residue are being baled as feed. An improvement in feed efficiency was found when corn residue was harvested using a John Deer 569 round baler with the Hillco single pass round bale system, compared to conventional harvesting methods (*2015 Nebraska Beef Report*, pp 42–44). Further, feed efficiency was improved by harvesting 2 rows of stalks plus tailings using the Cornrower system compared to raked and baled stalks (*2016 Nebraska Beef Report* pp 81–83). This improvement in feed efficiency is attributed to a higher ratio of leaf and husk to cob and stalk in the residue. Knowing the energy value of corn residue is critical when feeding.

One of the most important feed assays to assess the energy of forages is the neutral detergent fiber digestibility (NDF digestibility). *In vitro* and *in situ* procedures are traditionally carried out using an inoculum retrieved from a donor on a 30% concentrate diet to evaluate feed. However, the diet fed to the donor may impact NDF digestibility estimates. Therefore, the objective of this study was to evaluate the effects of different donor diets on *in vitro* and *in situ* digestibility when compared to a 30% concentrate diet.

Procedure

Four ruminally canulated steers were utilized in this study comparing two forage diets. A mixed diet consisting of 70% brome and 30% dry distillers grains (DDGS) was fed to two steers and a high corn residue diet with 70% stalks and 30%

Table 1. Simple effects of incubation time and donor diet on in vitro NDF digestibility of different forages[a] (%).

Sample[c]	24 h			48 h		
	Mixed[b]	Residue	*P*-Value	Mixed	Residue	*P*-Value
2Row	41.15	40.36	0.86	57.90	57.62	0.95
4Row	33.84	33.10	0.87	47.27	48.73	0.74
6Row	37.87	35.56	0.60	48.60	51.55	0.50
8Row	36.33	35.67	0.88	47.83	49.24	0.75
Cob	40.30	38.84	0.75	49.86	53.17	0.45
Conventional	31.30	32.75	0.74	41.55	44.84	0.45
Good Brome	46.21	48.63	0.58	63.06	65.88	0.52
Husk	43.99	44.79	0.85	59.99	61.06	0.81
I-barn Hay	37.58	36.15	0.74	47.37	51.67	0.32
Leaf	36.87	41.36	0.30	48.07	51.70	0.40
Meadow Hay	47.88	41.30	0.13	53.90	58.66	0.27
Poor Brome	32.17	33.95	0.68	41.84	45.25	0.43
Prairie Hay	27.54	26.84	0.87	38.26	39.57	0.76
Stalk	34.69	36.24	0.73	43.47	44.67	0.78

[a]NDF digestibility averaged across run
[b]Mixed diet consists of 70% brome and 30% DDGS; Residue diet consists of 70% stalks and 30% Sweet Bran
[c]Sample × time × diet; [a]SEM = 3.0; *P* = 0.99; LSD = 0.04

Table 2. Main effects of diet fed to donors on in vitro NDF digestibility[a] (%).

Time (h)	Diet[b]		SEM	*P*-value[c]
	Mixed	Residue		
24	37.68	37.54	1	0.90
48	49.21	51.69	1	0.03

[a]NDF digestibility averaged across all forage samples
[b]Mixed diet consists of 70% brome and 30% DDGS; Residue diet consists of 70% stalks and 30% Sweet Bran
[c]Diet × time interaction; *P* = 0.11

Table 3. Simple effects of incubation time and donor diet on in situ NDF digestibility of different forages[a] (%)

Sample[c]	28 h			36 h			48 h		
	Mixed[b]	Residue	*P*-Value	Mixed	Residue	*P*-Value	Mixed	Residue	*P*-Value
2Row	31.04	39.21	0.02	46.73	47.90	0.74	54.44	59.76	0.13
4Row	28.08	32.02	< 0.01	40.03	39.87	0.03	47.67	49.15	< 0.01
6Row	28.68	33.72	< 0.01	42.64	42.63	0.14	49.73	50.72	< 0.01
8Row	19.20	26.33	< 0.01	35.52	39.19	< 0.01	41.95	46.31	< 0.01
Cob	15.65	21.74	< 0.01	28.65	31.02	< 0.01	37.57	40.90	< 0.01
Conv.	29.77	33.59	< 0.01	39.77	38.60	0.03	43.02	42.38	< 0.01
Husk	36.36	46.39	0.42	48.05	46.09	0.97	59.43	62.10	0.93
Leaf	42.96	46.88	0.29	53.37	52.45	0.12	54.31	57.54	0.12
Stalk	19.58	23.03	< 0.01	25.68	30.47	< 0.01	30.11	33.65	< 0.01

[a]NDF digestibility averaged across replication
[b]Mixed diet consists of 70% brome and 30% DDGS; Residue diet consists of 70% stalks and 30% Sweet Bran
[c]Sample x time x diet; [a]SEM = 2.0; *P* = 0.99; LSD = 0.04

Table 4. Main effects of diet fed to donors on in situ NDF digestibility[a] (%)

Time (h)	Diet[b]		SEM	*P*-value[c]
	Mixed	Residue		
28	27.92	33.66	2	< 0.01
36	40.00	40.91	2	0.45
48	46.47	49.17	2	0.02

[a]NDF digestibility averaged across all forage samples
[b]Mixed diet consists of 70% brome and 30% DDGS; Residue diet consists of 70% stalks and 30% Sweet Bran
[c]Diet × time interaction; *P* = 0.01

Sweet Bran was fed to the remaining two steers.

Residue samples consisting of 2-row, 4-row, 6-row, 8-row, conventional bale, leaf, husk, stalk and cob were evaluated *in situ* and *in vitro* using inoculant from both sets of steers. A New Holland Cornrower Corn Head was used to obtain bales with 2, 4, 6, and 8 rows. The Cornrower head uses an attachment to cut the stems and blow them into a windrow between the wheels of the combine. The straw spreader is disengaged, allowing for the exiting residue to fall onto the windrow of stalks. The number of rows being cut at once can be adjusted from 0 to 8. The residue exiting the combine includes all of the cobs, a majority of the husks, some leaves, and some of the upper 1/3 portion of the stems. The 8 row bale includes all of the stalk material, thus, may be equivalent to conventionally baled stalks. Conventionally baled stalks from another single field were also used.

Five chopped hays, with known in vivo NDF digestibility values were used as non-corn residue samples to compare effects of inoculum with other forage types. The hays were immature smooth bromegrass (good brome), mature smooth bromegrass (poor brome), low quality brome or immature meadow hay (meadow hay), mature brome hay used in individual feeding (mature hay), and prairie grass hay (prairie hay).The prairie hay consisted of a mixture of warm and cool season grass species. Inoculum for *in vitro NDF digestibility* was obtained by collecting whole rumen contents from each steer, with two steers per treatment, strained through 4 layers of cheesecloth. Each of the strained ruminal fluid samples were then mixed with McDougall's buffer (1:1 ratio) containing 1 g urea/L. Residue and non-residue samples of 0.5 g were weighed into a 100 mL tube where 50 mL of one of the two inoculum, was added to each tube. All 11 samples were tested with each inoculum from each individual steer, to determine the effects of each diet for each sample. This process was repeated in three runs, and steer inoculum source was the experimental unit (n = 6). Three in vitro tubes per experimental unit were averaged for digestibility estimates. Test tubes were placed in a water bath at 101°F and incubated for 24 or 48 hours. Fermentation was ended by removing tubes from the water bath and placing them in the freezer immediately. The runs were performed at one week intervals, beginning one week after donor steers started their respective diets. Tubes were later thawed in a 101°F for 10 minutes and evaluated for NDF content to estimate NDF digestibility. Tubes were poured into a 600 mL beaker and rinsed with NDF solution added up to 150 mL total volume. The beaker was brought to a boil on a hot plate and allowed to reflux for one hour. The beaker content was then filtered through Whatman 541 filter paper, rinsed with distilled water, and dried in a 100°C oven for six hours.

The NDF digestibility of the corn

residue was also determined utilizing *in situ* rumen incubation. Residue samples were weighed (1.25 g) into small (5 × 10 cm) *in situ* bags. Three bags of each sample were placed in the rumen of each of the four steers, with two steers per treatment and 81 bags per steer separated into three time points (n = 4). Individual bags were placed in a mesh zipper bags fitted with weights and incubated for 28 h, 36 h, and 48 h. After the incubation period bags were pulled from the animal and rinsed with distilled water. After rinsing the bags, Ankom analysis was conducted to analyze NDF of the remaining residue. This process was repeated with two runs performed at 48 hour intervals.

All data were analyzed using the MIXED procedures of SAS (SAS Inst., Inc., Cary, N.C.). The effects of run, diet, time, and sample were examined. Diet by time and diet by time by sample interactions were also tested.

Results

In vitro

No 3-way interaction was observed for time by sample by diet ($P = 0.99$). There are no interactions for time by sample ($P = 0.79$) or diet by sample ($P = 0.99$; Table 1). There was a tendency for an interaction for diet by time ($P = 0.11$) where diet significantly ($P = 0.03$) affected NDF digestibility at 48 h, but not at 24 h (Table 2). Both residue samples and non-residue samples were found to have greater NDF digestibility when inoculum from the residue diet was used compared to that of the mixed diet at 48 hours (Table 2). There was an effect of run ($P < 0.01$), and an effect of time ($P < 0.01$) illustrating that runs are variable. Run 1, 2, and 3 had average NDF digestibility values of 41.07%, 48.27%, and 42.74% across both diets and all samples.

In situ

No 3-way interaction was observed for time by sample by diet ($P = 0.99$; Table 3). There was an effect ($P < 0.01$) for diet across *in situ* runs. Average NDF digestibility was greater for residue samples when the donor was fed a high corn residue diet (Table 4). There was an effect of run ($P < 0.01$), and an effect of time ($P < 0.01$) again demonstrating variability between run. At 28 and 48 hours, diet impacted NDF digestibility ($P < 0.01$). However, there was no effect of diet fed to donor at 36 hours ($P = 0.45$). As expected, average NDF digestibility for all samples increased with time, regardless of diet fed to the donor steer.

As anticipated, the husk and leaf had the greatest average NDF digestibility for *in situ* (0.52, 0.45) and *in vitro* (0.50, 0.51), respectively. The stalk and cob followed with smaller average NDF digestibility for *in situ* (0.27, 0.29), respectively. Similarly stalk and cob had smaller average NDF digestibility for *in vitro* (0.40, 0.46), respectively.

This study shows that the diet of the donor animal does affect NDF digestibility estimates of corn residue samples. However, when trying to assess energy values using these techniques a set of standards should be used for adjustment.

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