Impact of Inoculum Source for *in vitro* and *in situ* Digestion Procedures Performed on Corn Residue and Grass Samples

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Summary and Implications

A study was conducted to assess the effects of inoculum source at time of incubation on neutral detergent fiber digestibility, dry matter digestibility, and organic matter digestibility of corn residue samples. Digestibility of neutral detergent fiber was greater for both grass and corn residue when inoculum source came from steers consuming a high corn residue diet. Digestibility of dry matter and organic matter were not different between grass and corn residue. It is not necessary to maintain two sets of donors for in vitro or in situ procedures involving corn residue. However, donor diet affects neutral detergent fiber digestibility estimates of residue samples. Therefore, when trying to assess energy values using in situ or in vitro techniques, a set of standards with established in vivo digestibility values should be used for adjustment when steers are maintained on a mixed diet.

Introduction

Because forage, grass or residue, plays a major role in most cattle diets, knowing the energy value of forages is critical when estimating feeding values. Whether multiple donor diets are necessary to get accurate digestibility estimates of diverse forage samples such as grass or residue is essential. An interaction of forage type and inoculum source may indicate a need to obtain rumen fluid from donors fed the same forage being tested. A previous study assessed the effects of NDF digestibility using four cannulated steers and found an increase in NDF digestibility for both grass and residue forage types incubated in an inoculum source from steers fed a high residue diet (2016 Nebraska Beef Report, pp. 84-86). However, byproduct use varied with diet, making the impacts of residue or grass less clear.

Therefore, the objective of this study was to evaluate the effects of inoculum source on in vitro and in situ digestibility estimates (IVDMD, IVOMD, and NDF digestibility), when comparing a 70% hay diet and a 70% corn residue diet, to determine if two sets of donor steers would need to be routinely maintained for these procedures.

Procedure

Six ruminally canulated steers were fed, daily at 8 AM, either a mixed diet consisting of 70% brome grass hay and 30% Sweet Bran or a high corn residue diet with 70% conventionally baled stalks and 30% Sweet Bran. Steers were fed at 2% BW on a DM basis. The effects of donor diet were assessed as inoculum source using in vitro techniques and as diet for in situ techniques. There were two periods in a crossover design with two runs per period. Periods were 4 weeks long with a 2 week adaptation and 2 weeks for in vitro and in situ runs. One in vitro run and one in situ run were done in each week of the last two weeks of the period.

Residue samples consisting of 2-row, 8-row, conventional bale, husk and husklage were used for residue forage type. To obtain 2 and 8 row bales a New Holland Cornrower Corn Head was used as previously described (2016 Nebraska Beef Report, pp. 76-78). The husklage was produced with the use of a John Deere 569 round baler that was modified with the Hillco single pass round bale system as previously described (2016 Nebraska Beef Report, pp. 76-78)

Five chopped hays, with known in vivo values were described previously (2016 Nebraska Beef Report, pp. 84-86) and consisted of immature smooth bromegrass (good brome), mature smooth bromegrass (poor brome), immature meadow hay (meadow hay), mature brome hay used in an individual barn feeding system (mature brome), and prairie grass hay (prairie hay). The prairie hay consisted of a mixture of warm and cool season grass species.

All samples were ground through a CT 193 Cyclotec™ Sample Mill using a 2 mm screen for in vitro and a Wiley Mill using a 2 mm screen for in situ. Inoculum for in vitro NDF digestibility was obtained by collecting whole rumen contents from each steer, with three steers per treatment for each run. Each of the strained ruminal fluid samples were then mixed with McDougall's buffer (1:1 ratio) containing 1 g urea / L and incubated for 48 h. This process was repeated in two runs for each period, and steer inoculum source was the experimental unit (n = 8). Three in vitro tubes per experimental unit were averaged for digestibility estimates.

The NDF digestibility of samples was also determined utilizing in situ rumen incubation. Three bags of each sample were placed in the rumen of each of the six steers, with three steers per treatment and 120 bags per steer separated into four time points (n = 8). Individual bags were placed in mesh zipper bags fitted with weights and incubated for 36h, 48h, 60h, and 72h. After the incubation period bags were pulled from steers and placed in a washing machine where they were agitated with water in a washing machine for 1 min and spun for 1 min for five cycles. They were then rinsed with distilled water and stored in the freezer. The Ankom Fiber Analyzer was used to analyze NDF of the remaining residue. This process was repeated in two runs a week apart for each period.

All data were analyzed using the MIXED procedures of SAS. This experiment used a crossover design with two periods and two runs per period. 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 interaction was observed for inoculum source and forage type for IVDMD ($P = 0.99$). There was no interaction between inoculum source and forage type for IVOMD ($P = 0.98$). There was no effect of

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Table 1. Main effect of inoculum source on *in vitro* estimates[1]

	Diet[2]			
	Brome	Residue	SEM	*P*-value
IVDMD, %DM	49.7	50.9	0.79	0.41
IVOMD, %DM	51.5	52.4	0.74	0.25

[1]Averaged across run
[2]Brome diet consists of 70% brome and 30% Sweet Bran; Residue diet consists of 70% stalks and 30% Sweet Bran

Table 2. Interaction of diet and forage type on *in situ* NDF digestibility[1] (%).

	Diet[2]		
Sample[3]	Brome	Residue	*P*-value
2 Row	55.3	58.8	<0.01
8 Row	52.5	56.0	<0.01
Conventional	50.6	54.5	<0.01
Good Brome	53.5	57.0	<0.01
Husk	64.6	71.6	<0.01
Husklage	48.0	51.7	<0.01
Mature Brome	51.1	53.9	0.02
Meadow Hay	58.7	61.5	0.02
Poor Brome	48.7	52.3	<0.01
Prairie Hay	50.4	52.8	<0.01

[1]DMD averaged across run
[2]Brome diet consists of 70% brome and 30% Sweet Bran; Residue Diet consists of 70% stalks and 30% Sweet Bran
[3]Diet x sample $P = 0.19$, SEM=1.2

Table 3. Interaction of diet and incubation time on *in situ* NDF digestibility[1] (%).

	Diet[2]		
Time (h)	Brome	Residue	*P*-value[3]
24	37.7	37.5	0.90
48	49.2	51.7	0.03

[1]NDF digestibility averaged across all forage samples
[2]Brome diet consists of 70% brome and 30% DDGS; Residue Diet consists of 70% stalks and 30% Sweet Bran
[3]Diet x time interaction; $P = 0.11$, SEM= 1.2

Table 4. Main effect of diet on in situ NDF digestibility[1] (%).

	Diet[2]			
	Brome	Residue	SEM	*P*-value
NDF Digestibility	53.3	57.0	0.38	<0.01

[1]NDF digestibility averaged across all forage samples
[2]Brome diet consists of 70% brome and 30% Sweet Ban; Residue Diet consists of 70% stalks and 30% Sweet Bran

inoculum source ($P = 0.41$) for IVDMD or for ($P = 0.25$; Table 1) IVOMD. Forage type had a significant effect (data not shown; $P < 0.01$) showing that different qualities of forage had different IVOMD.

In situ

There was no 3-way interaction observed for forage type by incubation time by diet ($P = 0.85$). There was no interaction for diet by forage type ($P = 0.19$; Table 2). There was an interaction for diet by incubation time ($P = 0.01$; Table 3). Digestibility of NDF was greatest at 36 h for both forage types incubated in steers consuming a residue diet ($P = 0.03$). There was no significant difference between NDF digestibility at 48 h ($P = 0.13$). However, at 60 and 72 h NDF digestibility was greatest for both forage types incubated in steers consuming a residue diet ($P < 0.01$). There was a main effect for incubation time ($P < 0.01$) where NDF digestibility increased over time and was greatest at 72 h (data not shown). There was also a main effect for diet where NDF digestibility was greatest for both forage types when incubated in steers consuming a residue based diet ($P < 0.01$; Table 4).

Conclusions

There was no difference in IVDMD or IVOMD due to inoculum source. The diet of the donor animal did not affect NDF digestibility estimates of corn residue samples. However there was no interaction for forage type and inoculum source or diet. Greater NDF digestibility estimates for *in situ* procedures were observed for both forage types when incubated in a steer consuming a residue diet compared to the brome diet. Maintaining donor animals on different diets to perform these procedures is not necessary; one set of animals on a 30% concentrate diet is sufficient. Therefore, when trying to assess energy values using these techniques, a set of standards should be used for adjustment to account for any variation caused by animal diet.

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