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Comparison of *In Vivo* Digestibility to *In Vitro* Digestibility of Five Forages Fed to Steers

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Summary

Eight crossbred yearling steers were used in a Latin rectangle design to determine the in vivo digestibility of five different forages. Feed intakes were higher when steers were fed forages with higher IVDMD. In vivo digestibility of the hay used in this trial was highly correlated to in vitro *digestibility. On average, in vitro DMD was 5.4 percentage units higher than in vivo digestibility. Including these five hay samples as standards for in vitro analysis allows researchers to compare samples analyzed across in vitro runs. It also allows researchers to adjust the in vitro DMD to in vivo DMD, which allows for more accurate ration formulation and animal response prediction.*

Introduction

Previous research indicates *in vitro* DMD of forages is highly correlated with *in vivo* digestibility. Including a set of samples within each *in vitro* run which has known *in vivo* digestibilities allows researchers to adjust *in vitro* digestibility of forages to *in vivo* values using regression equations generated from the standards. It has been shown that the regression equations differ within plant type (C3, C4 and legumes) and the same samples run in different laboratories also differ. This is due to a number of factors which include donor animals, diets fed to donor animals, and differences in analytical techniques. *In vitro* runs analyzed in different runs cannot be compared equally because of run variability. Adjusting the *in vitro* results using the equations generated from the standards (with known *in vivo* digestibility) allows researchers to compare estimates from different *in vitro* runs. With these adjustments, forage samples with different species composition, such as grasses vs. legumes, can also be compared because each sample has been adjusted accordingly. The objective of this experiment was to determine the *in vivo* digestibility of five different hay samples and to use these samples as standards in *in vitro* DM digestibility procedures and make comparisons between *in vivo* and *in vitro* digestibility.

Proceedure

This experiment used eight crossbred yearlings in a 5x5 Latin rectangle with five periods and five diets. Diets consisted of five chopped hays including mature brome grass (MBrome), immature brome grass (IBrome), mature alfalfa (MAlf), immature alfalfa (IAlf), and prairie (Prairie). Prairie hay consisted of a mixture of warm season grasses. All hay was chopped on one day, through a 4 inch screen using a tub grinder at the beginning of the trial, mixed, and stored on concrete in an enclosed building. Periods consisted of a 16-day adaptation period followed by a five-day collection period. During the adaptation period steers were fed at ad libitum intake for the first 10 days.

The following six days steers were fed at 95 % of ad libitum intake to minimize feed refusals and reduce variation in measurements of digestion. Steers remained on the restricted DMI throughout the collection period. Steers initially weighed 710 lbs and gained an average of 55 lbs throughout the trial.

Hay samples were taken daily during the last eight days of each period, composited and a sub-sampled for lab analysis. If necessary feed refusals were also collected the last eight days of each period for analysis. Steers were fitted with fecal collection bags during the collection period to measure total fecal output. Bags were emptied and feces weighed and sub-sampled twice daily (7:00 am and 4:00 pm). All feed samples and fecal samples were dried in a 60°C forced air oven and ground through a Wiley mill (1mm screen) for analysis.

In Vitro dry matter digestibility (IVDMD) analysis was conducted on the five hay samples and replicated six times. The IVDMD values from each run were regressed against the *in vivo* DMD. The slopes of each regression line were compared. Differences between regression equations were also tested. Total protein was determined as well as degradable intake protein (DIP) and undegradable intake protein (UIP) using in situ mobile bag technique *(2005 Nebraska Beef Report*, pp. 25-27) using two ruminally and duodenally fistulated Holstein steers.

Results

In Vitro

Crude protein of the diets were 7.9, 7.5, 9.3, 16.3, and 17.6% for Prairie, MBrome, IBrome, MAlf, and IAlf, respectively (Table 1). UIP ranged from 10.1% (% of total CP) for IAlf to 37.2 % for the MBrome (Table 1). Total Tract indigestible protein (TTIDP) follows the same pattern as the UIP (5.0, 8.0, 14.8, 15.3, and 16.6 for IAlf, Malf, IBrome, MBrome, and Prairie, respectively). Digestibility of UIP fraction was highest for MAlf (62.4%) and lowest for IBrome (34.0%). UIP digestibility of these forages are lower than the NRC assumed 80%. These UIP digestibilities agree with results from Haugen et al., (*2005 Nebraska Beef Report,* pp. 25-27) who reported UIP digestibilities lower than the NRC

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estimates. There was a wide range in IVDMD between the different hays as well (50.2, 51.2, 56.4, 50.9, and 60.6 % for Prairie, MBrome, IBrome, MAlf, and IAlf, respectively). As digestibility of the hay increased so did DMI ($P<0.001$; Table 1). Intakes were highest when steers were fed either of the alfalfa hays and lowest when fed mature grass hay. There were no differences in intake within the three grass hays or within the two alfalfa hays. This would be expected as it is well documented that cattle intakes increase when fed a highly digestible forage (Figure 1) compared to forages that are lower in digestibility, presumably, due to increased rate of passage.

In vitro DMD and OMD were higher for IAlf than the other four hays (Table 2). Unlike the in vivo DMD data, the IBrome hay was similar ($P>0.05$) to the MBrome and the MAlf and Prairie was similar to the IAlf hay.

In Vivo

In vivo DM digestibility was significantly higher ($P<0.001$)for the IBrome and the IAlf (62.2 and 66.5%, respectively) compared to the other three hays (56.5, 58.1, and 55.5% for MAlf, MBrome, and Prairie, respectively) (Table 2). There were no differences ($P=0.74$) between the Prairie, MBrome, and the MAlf hay. Organic matter digestibility followed the same pattern as DMD, with IBrome and IAlf having greater (P<0.001) digestibility than the other three hays. Neutral detergent fiber digestibility followed the same pattern as DMD and OMD. There were no differences between IBrome and IAlf, but they were significantly higher than Prairie, MBrome, and MAlf.

Regression analysis indicated no significant difference ($P=0.99$) between the slopes of the regression lines (Figure 1). However, there was a difference ($P=0.04$) between the six different runs. This difference between the runs demonstrates the need for standards to adjust *in vitro* values in order make comparisons to *in vivo* digestibility and between different forages. The differences between the *in vitro* runs could be attributed to rumen fluid from

Table 1. Chemical composition of the experimental hays.

	Diet				
Variable	Prairie	MAlf[a]	MBrome[b]	IBrome[c]	IAlf[d]
CP, %	7.9	16.3	7.5	9.3	17.6
IVDMD, %	52.8	58.6	54.5	60.1	67.1
NDF, %	68.3	67.9	69.6	66.7	60.5
ADF,%	43.4	43.7	43.7	40.0	35.2
UIP, %	27.9	14.9	37.2	22.6	10.1
TTIDP, %[e]	16.6	8.0	15.3	14.8	5.0
UIPD,%[f]	40.1	62.4	58.9	34.0	46.0

[a]Mean Mature Alfalfa Hay
[b]Means Mature Brome Grass Hay
[c]Means Immature Brome Grass Hay
[d]Means Immature Alfalfa Hay
[e]Total tract Indigestible Protein
[f] Lower tract UIP Digestibility

Table 2. *In Vivo* **and** *In Vitro* **digestibility of five different hays fed to yearling steers.**

	Diet					Statistics	
Variable	Prairie	MAlf[1]	MBrome[2]	IBrome[3]	IAlf[4]	SEM	P-value
In Vivo							
DMI, lb	11.9^{ce}	14.7^{ad}	13.0^{be}	13.6^{abc}	16.1^{d}	0.6	<0.01
DMD, %	50.2^{cf}	50.9^{ad}	51.2^{be}	56.4^{abc}	60.6^{def}	1.6	<0.01
OMD, %	55.5^{be}	56.5^{ac}	58.1^{d}	62.2^{ab}	66.5^{cde}	1.4	<0.01
NDFD, %	47.1^{cf}	47.0^{ad}	45.2^{be}	57.0^{abc}	53.7^{def}	2.3	<0.01
In Vitro							
DMD, %	52.8^{be}	52.9^{ac}	53.9^{d}	59.1^{ab}	63.9^{cde}	1.6	0.02
OMD, %	49.8^{acd}	54.5^{b}	57.9^{c}	62.4^{a}	64.2^{bd}	2.0	0.03
NDFD, %	43.8^{bd}	43.4^{ac}	48.6	54.0^{ab}	51.5^{cd}	1.6	0.03

[1]Mean Mature Alfalfa Hay
[2]Means Mature Brome Grass Hay
[3]Means Immature Brome Grass Hay
[4]Means Immature Alfalfa Hay
[abcdef] Means with like superscripts differ significantly ($P<0.001$)

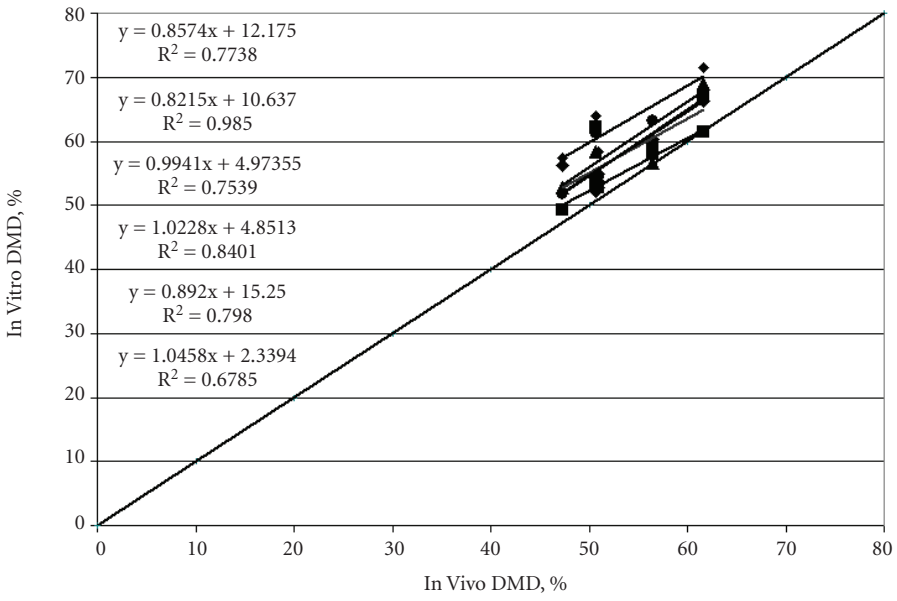

Figure 1. Regression analysis of *in vivo* **vs.** *in vitro* **digestibility.**

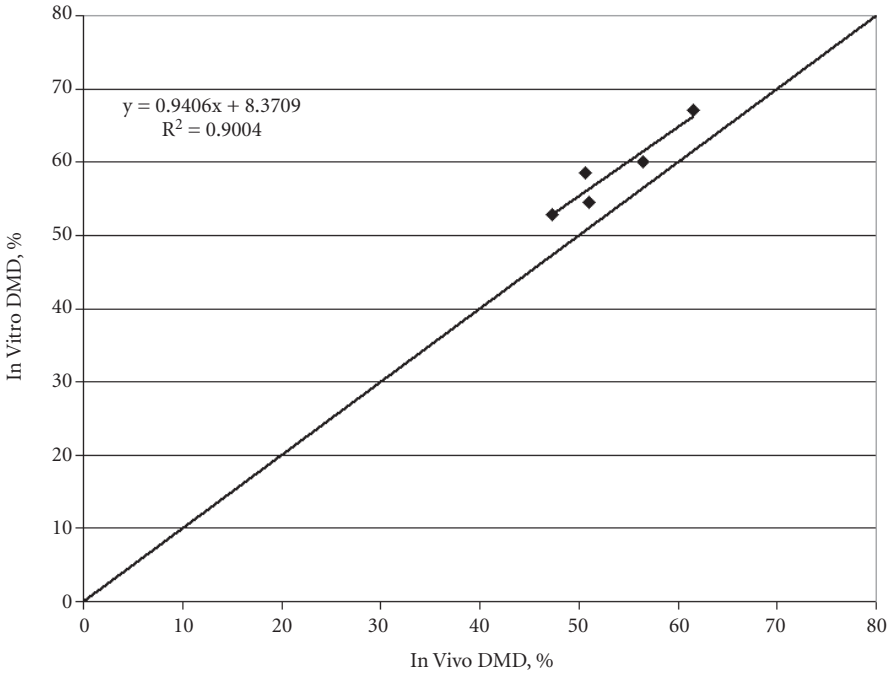

Figure 2. Regression analysis of the average of all six *in vitro* runs. Each point represents the average of each of the five different hay samples.

donor animals, differences in technicians, and the handling of rumen fluid prior placing in the tubes. However, in vitro (test tube) and in vivo (in the animal) digestibilities had good agreement, and were significantly correlated (r = 0.82 to 0.99). When the six runs were averaged (Figure 2) together IVDMD was 5.4 percentage units higher than *in vivo* DMD. This equates to an 8% difference between *in vivo* and *in vitro* digestibility.

Implications

Including these five hay samples with *in vitro* DMD analyse as standards will allow prediction of *in vivo* digestibility for new forages. This is important in research settings where a large number of samples are collected and cannot be included within the same *in vitro* run. Samples can be analyzed at different times and the adjustment allows us to compare different runs.

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