CONFIDENTIAL

DEVELOPMENT OF A DATABASE TO CHARACTERIZE THE EFFECT OF AMAFERM ON CORN SILAGE, HAYLAGE AND HAY NDF DIGESTIBILITY IN DAIRY CATTLE

Final Report

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Investigator: James E. Nocek, Ph.D.

Sponsor: BioZyme Incorporated
St Joseph, MO

OBJECTIVES:

Develop a database that characterizes:

1. Extent and limited rate of ruminal digestion of DM and NDF from corn silage, haylage and hay
2. Determine the effect of Amaferm (10 ml) on extent and limited rate of ruminal digestion of DM and NDF from corn silage, haylage and hay.
3. Correlate original chemical parameters (DM, CP, NDF, dNDF30 and lignin) to digestion parameters to identify possible predictive relationships.

PROCEDURES:

Approximately 75 samples each of corn silage, haylage and hay of various NDF concentrations and digestibilities were retained by Cumberland Valley Analytical Services personnel. A portion of the sample (about 100g) was sent to Spruce Haven Farm and Research Center (SHFRC), Auburn, NY.

Two lactating ruminally canulated cows (40-120 DIM) were used to determine rumen digestibility. Cows were fed a diet containing approximately 50:50 forage to concentrate and the forage portion of the diet was approximately 50:35:15 corn silage: haylage:hay (DM basis, appendix table 1and 2). One cow received a control diet (0ml) with no Amaferm, and the other cow received the same diet with 10ml of liquid Amaferm.

Standardization of cows: Only two cows were used because of the limited amount of sample available to in situ analysis. First cutting orchard grass hay was used as the standard ingredient. Adequate amounts were cut into pieces of less than .75 inches, motored and pestle, and refrigerated. In order to identify any cow differences samples (as described below) were suspended in the rumen of both cows for three determinations before introduction of amaferm and again at the end of the forage testing to characterize any potential difference in DM disappearance.

Corn silage, haylage and hay samples were prepared for in situ digestibility determination (see below). Rumen bag residence times with be 12, 24 and 36h. Although these time points are not extensive, they allowed the determination of 12, 24 and 36h rumen residuals for both DM and NDF, as well as a simple linear digestion rate. Once in situ procedures were conducted, linear regression was conducted to determine the "0hr " intercept for both DM and NDF. This process was conducted with all samples in each the designated control or amaferm control after it was determined there were no cows difference (see "results") The Amaferm cow received a 21d rumen acclimation to Amaferm prior to in situ experimentation. Amaferm (10ml) was administered via rumen canula twice daily (5ml am and pm).

Sample Preparation for In situ determinations:

- All forage samples were processed in the same manner as follows: Forage samples were placed on a mortar and pestle type apparatus to undergo mimicked mastication in the cow (ten concussions then mixing, repeated 3 times).

- The entire sample from each forage type was equally sub sampled into 2 equal aliquots for in time within cow in situ determinations.

- For each sample a 12, 24 and 36h sample was prepared. At each time, one polyester bag (porosity 53microns, 3x9") was filled with approximately 5g of dry forage sample. All samples were prepared at the same time and returned to a freezer (-20F). A 0hr bag was prepared which was prepared in the same fashion as other time points.

- One rumen canulated cow was used per treatment for all in situ determinations

- Bags (12, 24 and 36h) were removed from the freezer, brought to room temperature and suspended in warm water for 10-15 minutes prior to placement into the rumen of the canulated cow at 6 am (36h), 6pm (24h), 6am (12h) and all bags were retrieved at 6pm the next day.

- Upon removal from the rumen, bags were rinsed clean of rumen contents and placed thru a "rinse cycle" of a washing machine. The 0 hr bag was not suspended in the rumen and was placed through the rinse cycle.

- All bags were dried at 80F in a forced air oven and weighted.

- Residues (0.12, 24 and 36h) were removed from the bags and sent to CVAS for NDF analyses.

- From this information, 0, 12, 24 and 36h DM and NDF residuals/digestibility and Kd were calculated for relative comparative purposes. The same "0 hr" value from each forage was used for both control and treated regression, and represented that material which is 53 micron filterable and water soluble.

- All original forage samples were received from CVAS and analyzed by CVAS for the "CNCPS" chemical nutrient profile according to the client. Within these analysis were the parameters of DM, CP, NDF, in vitro 30 hr dNDF, and lignin. Subsequent to rumen suspension, residues were sent back to CVAS for analysis of the afore mentioned parameters. These analyses were not conducted in the same time frame as the original, however, the same analytical procedures were used. The original chemical results were used to identify any possible relationships and or predictive potential to digestion parameters by the backward stepwise regression technique of SAS.

RESULTS

Rumen Dry Matter Digestibility:

Corn Silage: Time zero DM residue for CS were the same for Control (0ml) and 10ml Amaferm: 69.2% (Table 1). There was no affect of Amaferm on CS DM digestibility at 12 or 24h, however, the 36h residue was lower for 10 vs. 0ml Amaferm dose. This contributed to the higher (7.1%, P<.01) Kd DM for 10 vs. 0ml (Figure 1 and 2).

Hay: Amaferm had no effect on DM digestibility at 12h of ruminal incubation, however, the 24h residue was lower (P<04, higher digestion) and a numerically lower residue for the 36h residue with Amaferm supplementation. In addition, Amaferm supplementation resulted in a 12.8% increase in digestion rate (Figures 3 and 4).

Haylage: For all rumen residual time points, Amaferm decreased (P<.01) rumen residual DM of haylage. However, the rate (Kd) of haylage DM digestion was not affected (P=.32) by treatment (Figures 5 and 6).

Rumen NDF Digestibility:

Corn Silage: At 12 and 24h of ruminal incubation, there was no difference in residual DM between Control and Amaferm. However at 36h, Amaferm supplementation resulted in a decrease (P<.01) of residual NDF by 8.2%. This translated into an increased (P<.01) rate of CS NDF digestion associated with Amaferm supplementation (15.2 % increase, Figure 7,8 and 13).

Hay: Amaferm supplementation had little affect Hay NDF digestion. However, at 24h of ruminal residence time, residual NDF was lower (P<.05). There was no effect of Amaferm on rumen digestion rate of hay NDF (Figure 9,10 and 14).

Haylage: As with DM, Amaferm supplementation resulted in a reduction (P<.01) of residual haylage NDF at 12, 24 and 36h by 13.3, 12.6 and 10.0% respectively. The linear NDF rate of digestion was 16.6% higher (P<.01) for Amaferm supplementation (Figure 11,12 and 15).

Stepwise regressions:

Backward stepwise regressions were conducted among specific chemical parameters to identify their contribution to the prediction of in situ ruminal digestion parameters. The equation intercepts and significant (P<.10) prediction coefficients are listed for corn silage, hay and haylage in table 3, 4 and 5 respectively. For example, the equation that would be used to predict the 36h NDF residue for haylage when fed with Amaferm would be = [.5563 + .0022(DM%) - .0125(CP%) - .0029(NDF%) + .0277(lignin%)]x

100. For corn silage, the most consistant significant predictive chemical parameter for the different NDF ruminal digestion parameters was NDF concentration (Table 3). Lignin, as well as crude protein became a factor in the later digestion points. Hay possessed a variety of factors that influenced NDF digestion (Table 4). Dry matter concentration was consistent for control but not when Amaferm was fed. Crude protein entered the equation several times for both control and Amaferm. Dry Matter, NDF and lignin were key variables in the haylage NDF residue and rate prediction (Table 5). It is interesting that in vitro dNDF30 was a significant variable in relatively few times for any of the forages.

Relationship Between NDF concentration and digestibility:

Table 6 illustrates the relationship between NDF concentration in the original sample and ruminal insitu DM and NDF digestibility at 12, 24 and 36h. For Corn Silage, the relation between NDF and dDM was significant ($P<.01$) for all hours with coefficients ranging from .54 to .44. As NDF increased in corn silage, there was a tendency for dNDF to decrease. These same tendencies were evident for each time point with the inclusion of Amaferm, however, only 12h was significant for NDF vs. dNDF and the significant positive nature of dNDF vs. dDM was greater with Amaferm.

For Hay and Haylage, the same general trends were present. The relationship between NDF and dDM, dDM and dNDF was positive and significant ($P<.01$), regardless of Amaferm supplementation. The relationship between NDF and dNDF was variable and non significant at all time points, regardless of Amaferm supplementation.

CONCLUSION:

1. Amaferm supplementation (10g/d) increased 36h corn silage DM and NDF digestion (2.2 and 5.0 percentage units respectively) and rate of DM and NDF digestion (7.1 and 15.2 % respectively) compared to control (0g/d).

2. For Hay, Amaferm supplementation increased the 24h DM and NDF extent of digestion (2.9 and 3.0 percentage units), with a tendency ($P=.10$) for increasing the rate of DM digestion (12.8%), with no effect on NDF rate.

3. Amaferm supplementation increased digestion for both DM and NDF at 12, 24 and 36h (7.1, 5.0 and 3.2 percentage units for DM and 10.3, 8.0 and 5.2 percentage units respectively). The rate of DM digestion was not affected by Amaferm supplementation, however supplementing Amaferm increased (16.6%) the rate of NDF digestion compared to control.

4. Stepwise regression analyses demonstrated that forage NDF concentration was typically a significant chemical variable in predicting ruminal digestion. However, DM was usually significant with haylage.

5. There was positive relationship between NDF vs. dDM, and dDM vs. dNDF for all forages, however, there was no consistent relationship between NDF and dNDF for any forages evaluated.

Table 1. Effect of Amaferm fed at 10ml/d on Ruminal Dry Matter Indigestibility of Corn Silage, Hay and Haylage

	Control		Amaferm(10ml)		
Corn Silage	Mean	SEM	Mean	SEM	P<
n	75		75		
0h[1]	69.2	1.2	69.2	1.2	-
12h	48.7	0.8	49.5	1	0.43
24h	39.1	0.6	38.4	1	0.45
36h	33.4	0.6	31.2	1	**0.02**
Kd %/h					
0-36 h	.98	0.03	1.05	0.03	**0.01**
Hay					
n	75		75		
0h[1]	76.0	.64	76.0	.64	-
12h	40	0.88	40.2	1	0.86
24h	34.7	1.1	31.8	1	**0.04**
36h	29.6	0.9	28.2	1	0.22
Kd %/h					
0-36 h	.436	0.02	.492	.02	0.1
Haylage					
n	75		72		
0h[1]	76.5	.82	76.5	.82	-
12h	50	1.3	42.9	2	**0.01**
24h	38.2	1	33.2	1	**0.01**
36h	31.6	0.9	28.4	1	**0.01**
Kd %/h					
0-36	1.22	0.03	1.32	0.02	**0.32**

[1]0hr was the same for both Control and Amaferm treated forage.

Table 2. Effect of Amaferm fed at 10ml/d on Ruminal NDF Indigestibility of Corn Silage, Hay and Haylage

	NDFResidual				
	Control		Amaferm		
CornSilage	Mean	SEM	Mean	SEM	P<
n	75		75		
0h [1]	95.0	1.3	95.0	1.3	-
12h	85.4	1	87.3	1	0.91
24h	70.5	1	68.7	1.3	0.29
36h	60.5	1.1	55.5	1.3	**0.01**
Kd, %/h					
0-36	1.05	0.035	1.21	0.046	0.01
Hay					
n	73		75		
0h[1]	94.4	1.2	94.4	1.2	-
12h	76.3	1.3	77.5	1.1	0.5
24h	69.5	1.1	66.5	1	**0.05**
36h	60.6	1.1	59.9	1.1	0.66
Kd, %/h					
0-36 h	1.43	.04	1.44	.037	.95
Haylage					
n	75		72		
0h[1]	95.2	1.6	95.2	1.7	-
12h	77.1	1.3	66.8	2.5	**0.01**
24h	63.4	1.1	55.4	1	**0.01**
36h	52.5	1	47.3	1	**0.01**
Kd, %/h					
0-36 h	1.26	0.037	1.47	.032	0.01

[1]0hr was the same for both Control and Amaferm treated forage

NDF concentration for the mean of samples within each forage type.

Forage	Mean ± SD
Corn Silage	42.1 + 4.8
Hay	38.6 + 4.6
Haylage	45.5 + 9.1

Table 3. Stepwise regressions[1] among specific chemical parameters used to predict in situ ruminal residues and rates of NDF digestion for Control and Amaferm (10ml) treated corn silage

			Control			
	Intercept	DM	CP	NDF	dNDF30[2]	Lignin
Residuals						
0 hr	1.126			-0.0041		
12 hr	0.943			-0.0029		
24 hr	0.878			-0.0048		
36 hr	0.782		-0.011	-0.0048	-0.0006	0.035
Kd						
0-36 hr	-0.01025		-0.00043			0.001

			Amaferm			
	Intercept	DM	CP	NDF	dNDF30	Lignin
Residuals						
0 hr	1.126			-0.0041		
12 hr	1.149			-0.0072		
24 hr	0.7727			-0.0043		0.0207
36 hr	0.529		-0.0073			0.022
Kd						
0-36 hr	-0.0189			0.00016		

[1]Coefficient(s) listed for each chemical entity significantly (P<.10) contribute to the prediction of the each ruminal parameter by backward stepwise elimination procedures of SAS.

[2] In vitro

Table 4. Stepwise regressions[1] among specific chemical parameters used to predict in situ ruminal residues and rates of NDF digestion for Control and Amaferm (10ml) treated hay

			Control			
	Intercept	DM	CP	NDF	dNDF30[2]	Lignin
Residuals						
0 hr	0.538	0.0054		0.0045		
12 hr	0.2788	0.0048		0.0059		
24 hr	0.599	0.0027	-0.0103			0.0158
36 hr	0.4389	0.0031	-0.007			0.0134
Kd						
0-36 hr	-0.0069	-0.00009		-0.0001		0.00037

			Amaferm			
	Intercept	DM	CP	NDF	dNDF30	Lignin
Residuals						
0 hr	1.315			-0.0052		
12 hr	0.697				-0.0021	0.0094
24 hr	0.959		-0.0145			
36 hr	0.905		-0.0159			
Kd						
0-36 hr	-0.0037		-0.0005		0.00042	

[1]Coefficient(s) listed for each chemical entity significantly (P<.10) contribute to the prediction of each ruminal parameter by backward stepwise elimination procedures of SAS.
[2] In vitro

Table 5. Stepwise regressions[1] among specific chemical parameters used to predict in situ ruminal residues and rates of NDF digestion for Control and Amaferm (10ml) treated haylage

	Control					
	Intercept	DM	CP	NDF	dNDF30[2]	Lignin
Residuals						
0 hr	0.538	0.0054		0.0046		
12 hr	0.279	0.0048		0.0059		
24 hr	0.599	0.0027	-0.0103	0.0158		
36 hr	0.4387	0.0031	-0.0074			0.0134
Kd						
0-36 hr	-0.0068	-0.0009		-0.0001		0.00037

	Amaferm					
	Intercept	DM	CP	NDF	dNDF30	Lignin
Residuals						
0 hr	0.538	0.0054		0.0046		
12 hr	0.218	0.005		0.0057		
24 hr	0.4655	0.0025	-0.0072			0.0162
36 hr	0.5563	0.0022	-0.0125	-0.0029		0.0277
Kd						
0-36 hr	-0.0173					0.0049

[1]Coeffcient(s) listed for each chemical entity significantly (P<.10) contributed to the prediction of the each ruminal parameter by backward stepwise elimination procedures of SAS.

[2] In vitro

Table 6. Correlation coefficients (r) between NDF concentration in the original sample and DM and NDF In situ ruminal digestibility at 12,24 and 36h

	Control						Amaferm					
	NDF vs. dDM	P<	NDF vs. dNDF	P<	dNDF vs. dDM	P<	NDF vs. dDM	P<	NDF vs. dNDF	P<	dNDF vs. dDM	P<
Corn Silage												
12h	0.54	**.01**	-0.19	ns	0.29	**.01**	0.44	**.01**	-0.3	**.01**	0.57	**.01**
24h	0.44	**.01**	-0.36	**.01**	0.56	**.01**	0.47	**.01**	-0.2	ns	0.72	**.01**
36h	0.46	**.01**	-0.22	ns	0.69	**.01**	0.54	**.01**	-0.12	ns	0.71	**.01**
Hay												
12h	0.63	**.01**	0.004	ns	0.59	**.01**	0.63	**.01**	-0.13	ns	0.59	**.01**
24h	0.74	**.01**	0.17	ns	0.75	**.01**	0.65	**.01**	0.23	**.05**	0.76	**.01**
36h	0.65	**.01**	0.19	ns	0.81	**.01**	0.7	**.01**	0.12	ns	0.78	**.01**
Haylage												
12h	0.67	**.01**	0.11	ns	0.76	**.01**	0.78	**.01**	0.04	ns	0.6	**.01**
24h	0.75	**.01**	-0.01	ns	0.62	**.01**	0.73	**.01**	-0.09	ns	0.59	**.01**
36h	0.69	**.01**	-0.11	ns	0.61	**.01**	0.64	**.01**	-0.21	ns	0.58	**.01**

Appendix Table 1. Ingredient composition of experimental TMR.

Ingredient	Lactation TMR
	--------- % dry matter basis -------------
Corn silage	26.5
Hay crop silage	19.1
Western Hay	8.4
Corn meal	26.0
Soybean meal (49%)	4.0
Roasted Soybeans	4.0
Beet Pulp	2.8
SHF Lactation Mix	9.2

Appendix Table 2. Nutrient composition of experimental diet and Ingredients.

Nutrient	Lactation Diet
	% DM basis
Dry Matter, %	52.7
Crude protein, %	17.7
NE_L (Mcal/lb)	1.78
NDF (%)	31.9
NSC (%)	40.9
Fat (%)	4.5
Calcium, %	.92
Phosphorous, %	.38
Magnesium, %	.35
Potassium, %	1.0
Sulfur, %	.21
Sodium, %	.40
Chloride, %	.37
Iron, ppm	111
Selenium, ppm	.30
Cobalt, ppm	.48
Iodine, ppm	.58
Zinc, ppm	64
Copper, ppm	24
Manganese, ppm	51

Appendix table 3. Standardized hay in situ DM digestibility: cow effects
(In situ DM residues at 12, 24 and 36h of residence time)

	Rumen Residual DM			Cow Effect
Initial	**Control Cow**	**Amaferm Cow**	**SEM**	**P<**
n	3	3		
12h	50.2	49.8	0.41	NS
24h	37.9	39.1	0.46	NS
36h	28.3	28.1	0.49	NS
Final				
12h	49.9	49.7	0.47	NS
24h	38.4	38	0.51	NS
36h	28.2	28.3	0.36	NS
Time Effect				
12h	NS	NS		
24h	NS	NS		
36h	NS	NS		

Hay is orchard grass with an NDF%=42.7%

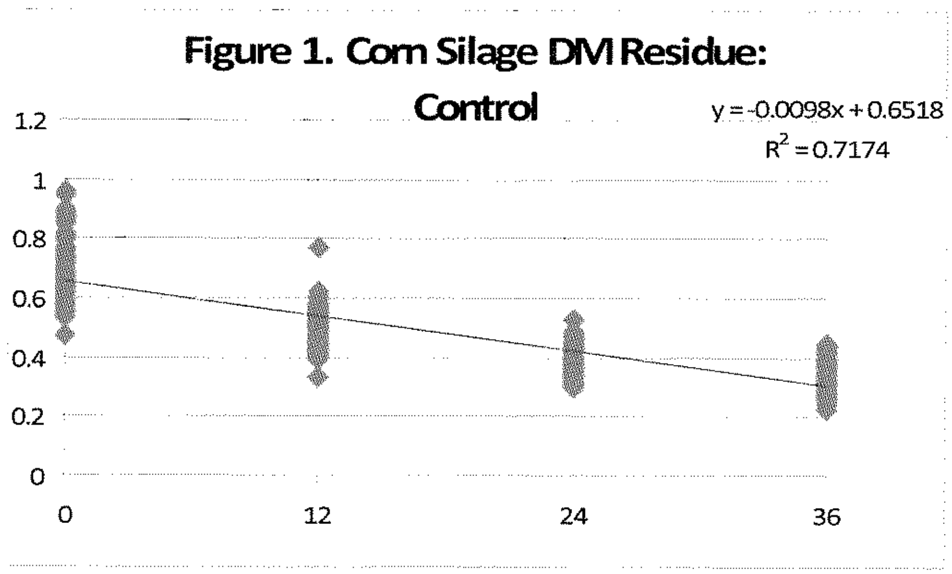
Figure 1. Corn Silage DM Residue:
Control
y = -0.0098x + 0.6518
R2 = 0.7174
1.2
1
0.8
0.6
0.4
0.2
0
0
12
24
36

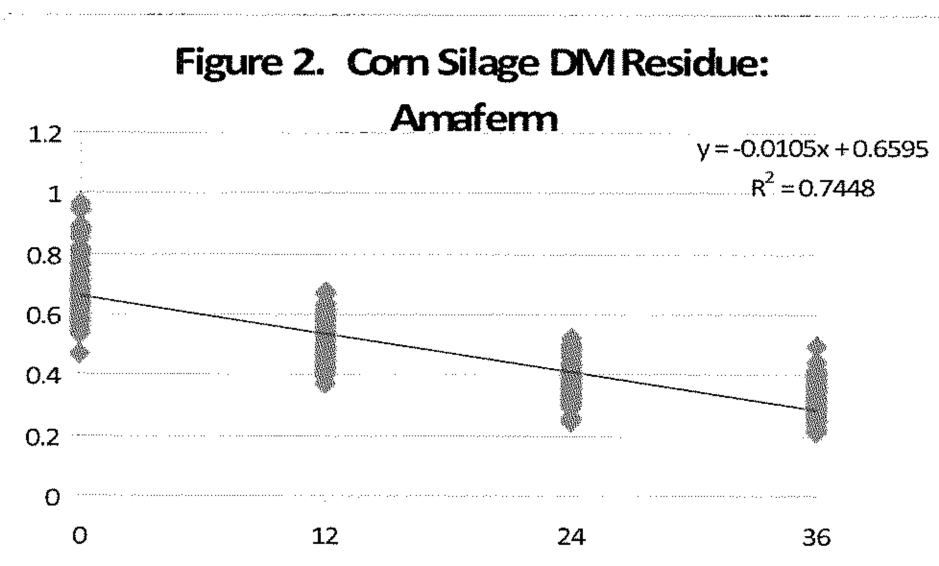
Figure 2. Corn Silage DM Residue:
Amaferm
y = -0.0105x + 0.6595
R2 = 0.7448
1.2
1
0.8
0.6
0.4
0.2
0
0
12
24
36

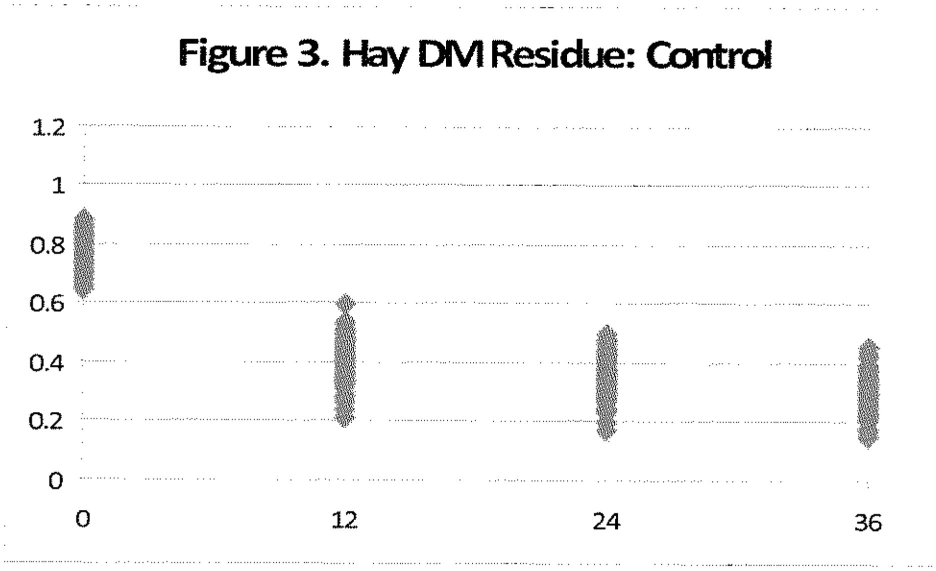
Figure 3. Hay DM Residue: Control
1.2
1
0.8
0.6
0.4
0.2
0
0
12
24
36

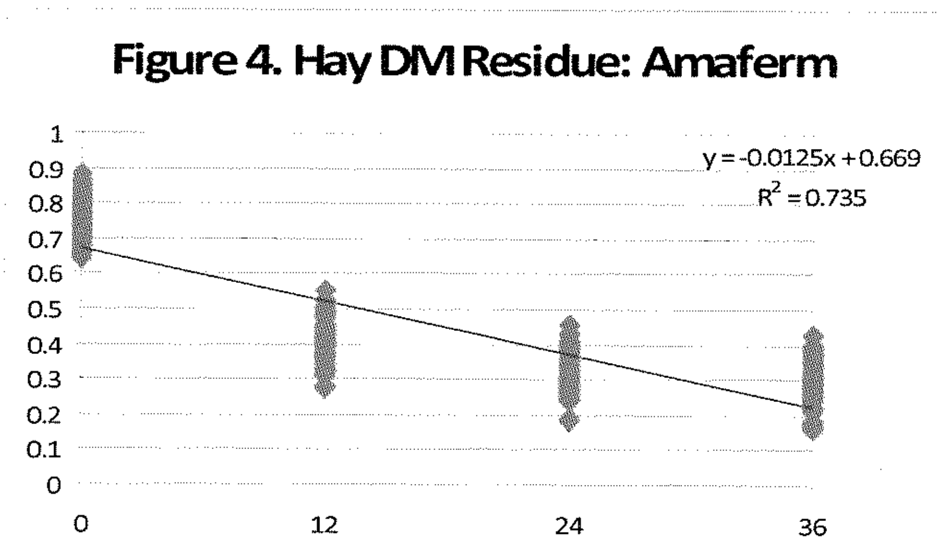
Figure 4. Hay DM Residue: Amaferm
y = -0.0125x + 0.669
R2 = 0.735
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
12
24
36

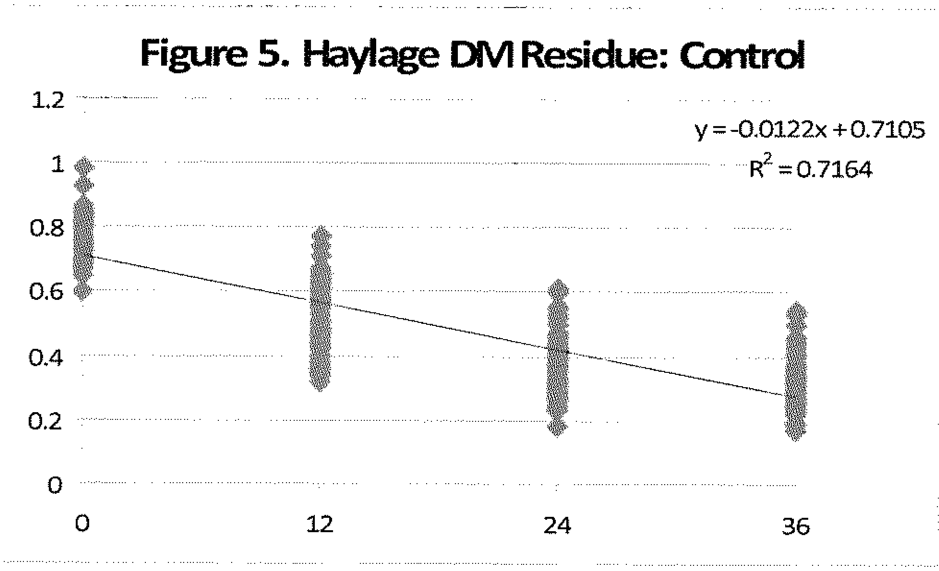
Figure 5. Haylage DM Residue: Control
y = -0.0122x + 0.7105
R² = 0.7164
1.2
1
0.8
0.6
0.4
0.2
0
0
12
24
36

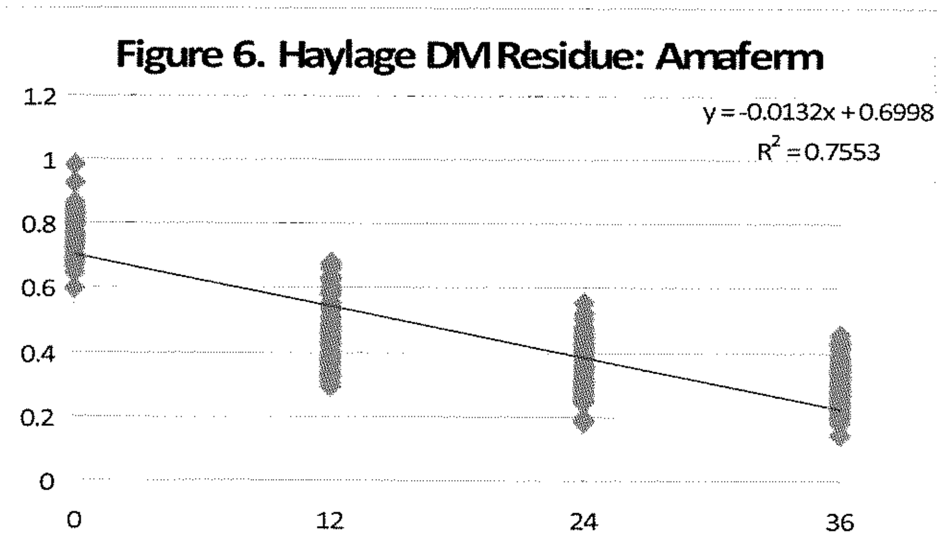
Figure 6. Haylage DM Residue: Amaferm
y = -0.0132x + 0.6998
R² = 0.7553
1.2
1
0.8
0.6
0.4
0.2
0
0
12
24
36

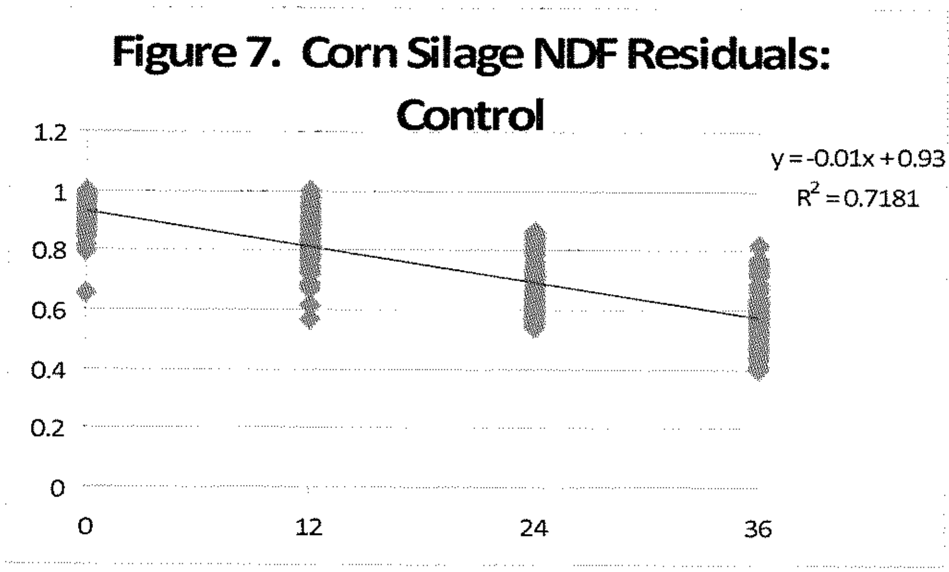

Figure 7. Corn Silage NDF Residuals: Control

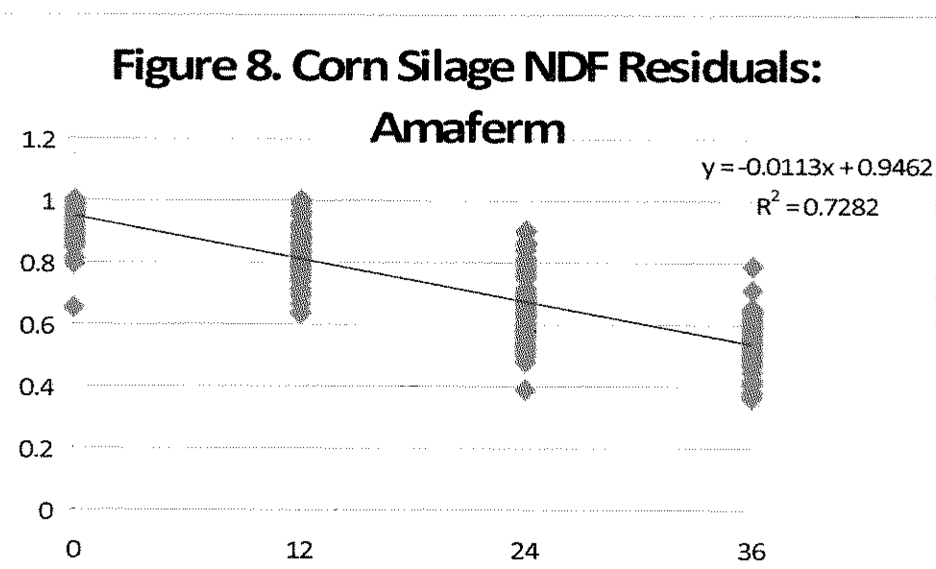

Figure 8. Corn Silage NDF Residuals: Amaferm

Figure 9. Hay NDF Residuals: Control

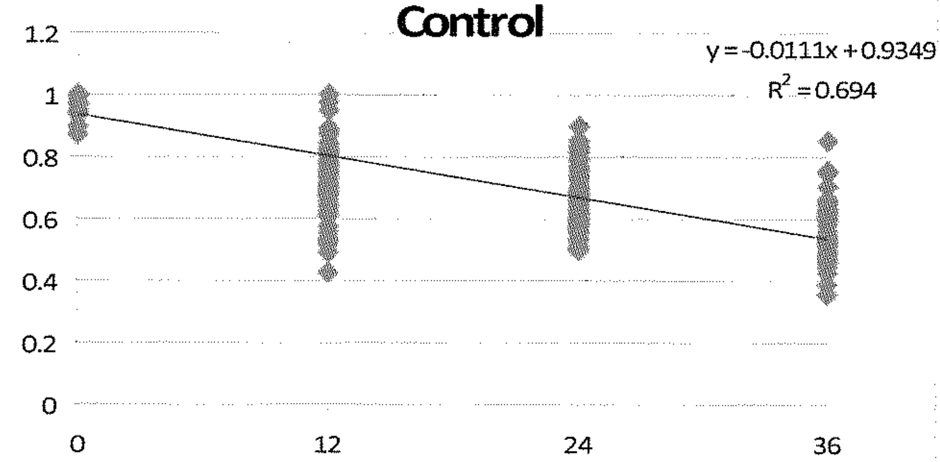

Figure 10. Hay NDF Residuals: Amaferm

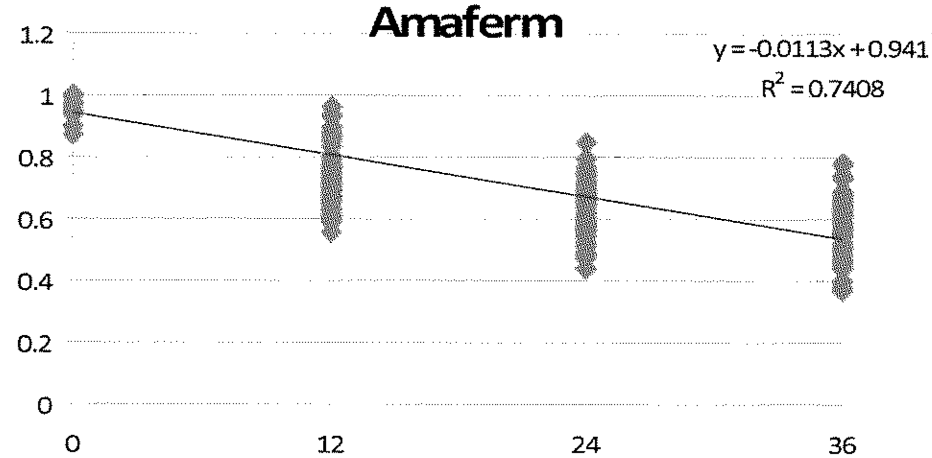

Figure 11. Haylage NDF Residual: Control

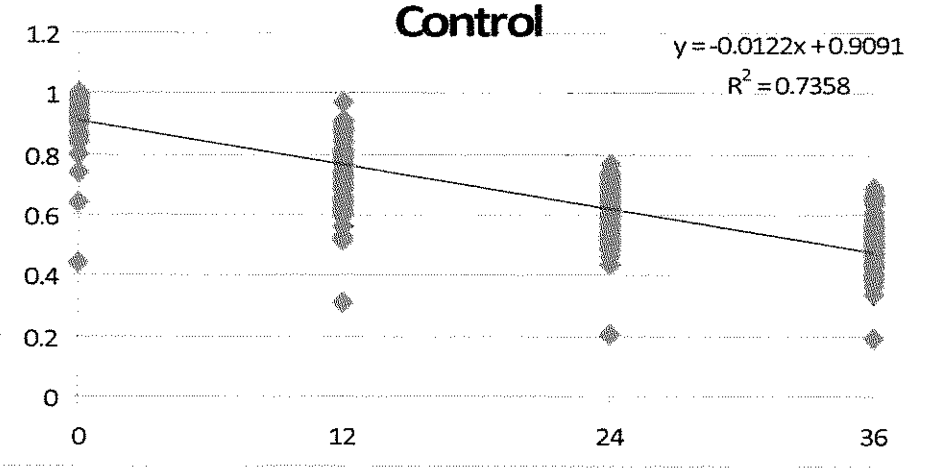

Figure 12. Haylage NDF Residuals: Amaferm

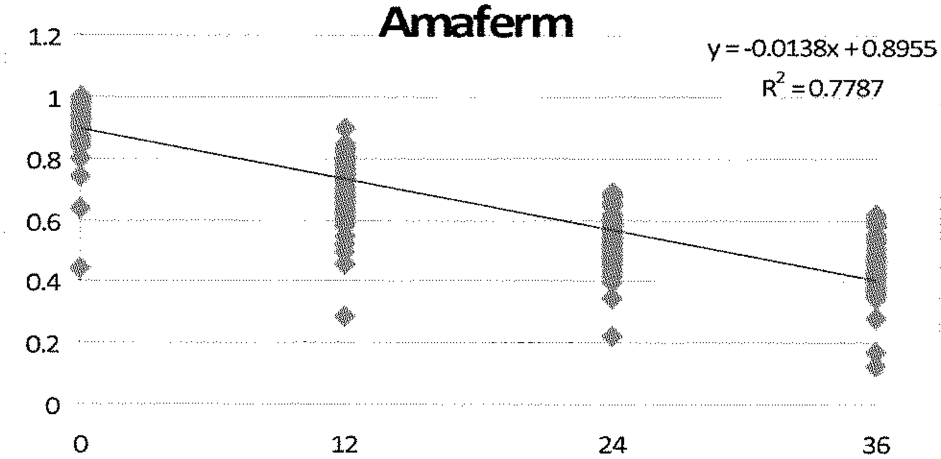

Figure 13. Corn Silage NDF Comparison 0-36 Hour

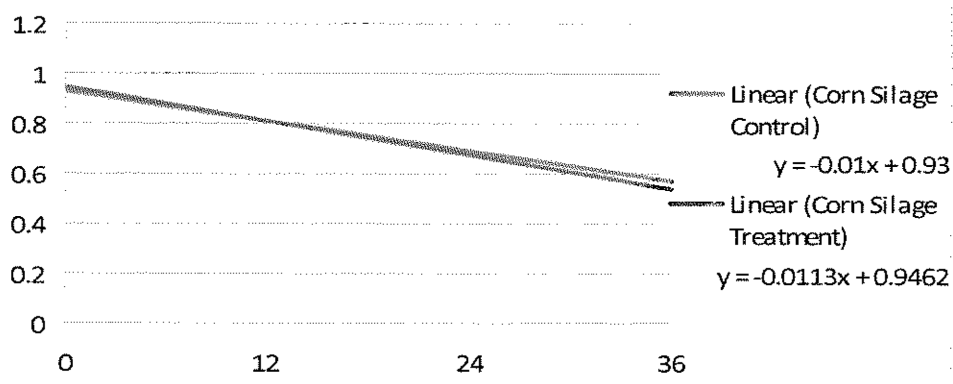

Figure 14. Hay NDF Comparison 0-36 Hour

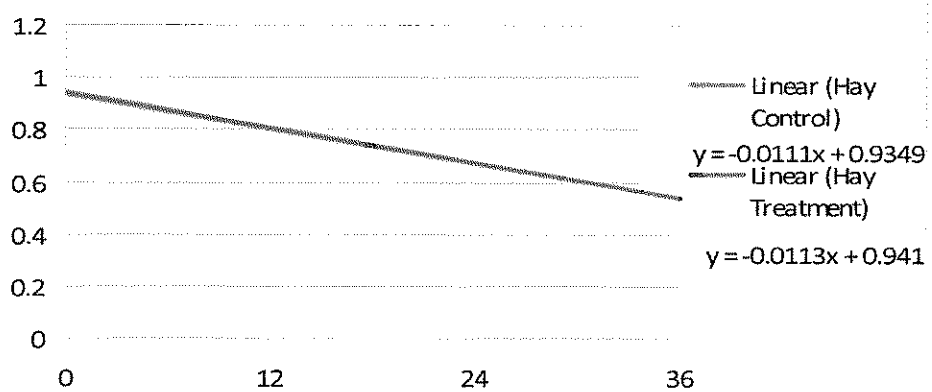

Figure 15. Haylage NDF Comparison 0-36 Hour