Roughage Energy and Degradability Estimation with *Aspergillus oryzae* Inclusion Using Daisy® *In vitro* Fermentation

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ABSTRACT : The aim of this study was to predict the energy value and dynamic degradation of roughage in Taiwan using the Daisy® *in vitro* fermentation method to provide information on one of the very important nutrients for ration formulation. The second objective was to study the effects of *Aspergillus oryzae* (AFE) inclusion on nutrient utilization. Three ruminal fistulated dry dairy cows were used for rumen fluid and fifteen conventional forages used in dairy cattle were collected around this island. The degradability of these feedstuffs with and without AFE (Amaferm®) treatment was measured using the Daisy® *in vitro* method. The roughage energy values, including TDN and NEL, were calculated according to Robinson (2000). Results from the 30 h *in vitro* neutral detergent fiber (NDF) degradability and predicted energy evaluations showed that alfalfa (among the forages) contained the highest degradability and energy values, Bermuda straw having the lowest. Peanut vines and corn silage contained higher energy values and the lowest value found in Pangola and Napier grasses among the locally produced forages. Pangola and Napier grasses had lower values than most imported forages except Bermuda straw. Among the by-products, wheat middling contained the highest NDF degradability, while rice bran contained the richest energy value due to its high oil content. From the dynamic dry matter (DM), organic matter (OM), acid detergent fiber (ADF) and neutral detergent fiber (NDF) degradation, corn silage contained the highest effective degradation among the local forages; wheat middling (among the by-products) degraded the fastest in DM, OM, ADF and NDF and showed the highest effective degradability. AFE inclusion was inconsistent among the forages. Alfalfa hay showed significantly increased 30 h NDF degradability and energy values, Pangola hay, Napier grass and brewer's grains showed decreased degradability and energy values. AFE inclusion increased the DM, OM and NDF degradation rate in most forage, but only increased the DM degradation rate in sorghum distiller's grains, the OM degradation rate in bean curd pomace and the NDF and ADF degradation rates in soy pomace (among the by-products).
(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 1 : 53-62)

Key Words : Daisy®, *In vitro* Fermentation, Degradability, *Aspergillus oryzae*, Energy Value, Forage

INTRODUCTION

Forage energy content is a specific nutrient used as an index in evaluating the economic value of forages. Metabolizable or net energy is one of the most critical nutrients and is most difficult to measure because it is not a chemical constituent and cannot be obtained through chemical analyses. Great variations in the energy content of forage due to various factors including cultivation, growth condition, climate, fertilizations, harvesting and processing make energy value estimations more difficult. By-product feedstuffs also contain variable energy content according to the raw material source and processing procedures. The conventional dairy cattle roughage used in Taiwan includes imported forage (alfalfa hay, Timothy hay, oat hay and Bermuda hay and straw) and locally produced forage (corn silage, Pangola grass, Napier grass and peanut vines) and by-products (wheat middling, bean curd pomace, soy pomace, brewer's grains, sorghum distiller's grains and rice distiller's grains). The roughage energy values are taken from the NRC table values or estimated from the proximate composition. Dairy farmers in this country formulate their total mixed rations (TMR) based on unreliable energy values. Precise estimation of the most critical nutrients-(energy content in conventional forage) is very important in improving dairy farming efficiency here in Taiwan.

Aspergillus oryzae has been used as human food for centuries and is as safe as most food from nature. Gomez-Alarcon et al. (1990) concluded from three lactation trials that AFE improved total tract DM, ADF and neutral detergent fiber (NDF) digestibility. They also indicated that *Aspergillus* fermentation extract (AFE) improved total tract digestibility and rate of digestion in alfalfa hay. This digestive rate increase in alfalfa hay may be attributed to the increase in DM intake and production performance. Using an *in situ* nylon bag trial, Fondevila et al. (1990) demonstrated an increased rate in wheat straw degradation with AFE inclusion. Using the same *in situ* method, Chiou et al. (2000) examined the effects of AFE inclusion on 21 conventional feedstuffs and found an increase in ADF degradation after 24 and 48 h rumen incubation.

The conventional *in vitro* Tilley and Terry (1963) two-stage method has also gained worldwide acceptance for nutrient evaluation for ruminant feedstuff due to its simplicity and precise DM degradation estimation. This method is still regarded as time consuming and samples must be individually measured. The improved Daisy® *in*

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Received November 29, 2002; Accepted September 16, 2003

Table 1. Basal diet formulation for the *in vitro* trial, %

Ingredients	Basal diet
Bermuda hay	50.00
Soybean meal	12.25
Corn	24.90
Wheat bran	10.00
Limestone	1.00
Dicalcium phosphate	1.50
Salt	0.25
Premix[1]	0.10
Total	100.00
Calculated value	
CP (%)	15.40
NEL (Mcal/kg)	1.40
ADF (%)	45.30
NDF (%)	24.50
Ca (%)	0.90
P (%)	0.70

[1] Each kilogram contain: Vit. A, 10,000 KIU; Vit. D_3, 1,600 KIU; Vit E, 70 KIU; Fe, 50 mg; Zn, 40 ma; Mn, 40 ma; I, 0.5 mg; Co, 0.1 mg; Cu, 10 mg; Se, 0.1 mg.

vitro incubator and fiber analyzer method saves labor and time. Holden (1999) and Mabjeesh et al. (2000) conducted a comparison trial using the conventional *in vitro* Tilley and Terry (1963) two-stage fermentation and Daisy® *in vitro* incubation methods and found no significant differences in analytical values between the two methods. The multi-sample incubation method within same incubator did not create a significant interaction that influenced the degradation result. This new method requires no filtration processing after incubation in addition to the continued agitation in the incubator to mix the substrates with the rumen fluid. The aim of this study was to predict the energy values and dynamic degradation of forage in Taiwan using the Daisy® *in vitro* fermentation method. The second objective was to study the effects of *Aspergillus oryzae* (AFE) inclusion on the nutrient utilization on various roughages.

MATERIALS AND METHODS

The traditional feedstuffs, commonly used forage and byproduct were collected from various locations in Taiwan. Samples of approximately 5 kg (in dry weight) were dried in a 60°C air-driven oven for 24-72 h, depending on the moisture content. Samples were ground through a 1 mm mesh screen and stored at -18°C for further analysis. All feedstuff samples were placed into two incubation treatments; the control and treatment with Amaferm® (BioZyme) inclusion 0.4 g/L incubation solution as described by Martin and Nisbet (1990).

Three ruminal fistulated dry 700 kg live-weight Holstein cows were selected and fed total mixed ration (TMR) with roughage at a 1:1 concentrate ratio, as presented in Table 1. After 8 days adaptation, the cows were fed 8 kg (as fed) of evenly divided TMR amounts twice (8:00 am and 8:00 pm) daily. Water was accessible to the cows all of the time. Rumen fluid was taken from the fistula 2 h. After feeding, filtrated through 4 layers of cheesecloth and mixed with McDougall's buffer at a 1:4 ratio as incubation solution. 0.4 g Amaferm® was added to each litter of incubation solution in the treatment group.

The Daisy® *in vitro* incubation procedure was performed according to the ANKOM technology bulletin. The Daisy® *in vitro* incubator was pre-set at 39°C with a rolling speed of 49 sec. per round. CO_2 was pumped into the incubator for 30 sec. before the sample was inserted. Approximately 0.5 g of feedstuff sample was placed into the Daisy® nylon bag (ANKOM Co. Ltd., Spencerport, NY), sealed and placed into the incubator jar. Four jars with 24 sample nylon bags in each jar were evenly distributed within each digestion jar divider and placed into the incubator. By-product feedstuffs were incubated at 0, 6, 12, 24 and 30 h. Forage was incubated an additional 48 h. in addition to the 6 incubation periods. After incubation, sample bags were completely water rinsed, and dried in a 60°C air-driven oven for 48 h and stored at -18°C for further analysis.

The incubated nylon bag forage samples were analyzed for DM and OM according to AOAC (1984) and NDF and ADF according to the method of Van Soest et al. (1991) using an ANKOM fiber analyzer. The feedstuff energy content was calculated using the following prediction equations based on the proximate composition and 30 h. *In vitro* degradation according to Robinson (2000):

$$TDN(1\times M)=((CP-ADICP)\times(FT/5)\times0.98)+((CP-ADICP)\times(1-(FT/5))\times0.8)+((EE-1)\times0.98\times2.25)+(NDF\times dNDF+(0.98\times(100-ASH-EE-NDF-CP)))$$

$$NEL(1\times M)=(TDN(1\times M)\times0.0266)-0.12$$

$$Discount=((0.033+(0.132\times NDF(\%\ of\ DM)))-(0.033\times NEL(1\times M, Mcal/kg)))+(NFC(\%of\ DM)\times0.05)$$

$$NEL(1\times3M)=NEL(1\times M)-(NEL(1\times M)\times(Discount\times2/100))$$

Where CP=crude protein (%DM), ADICP=acid detergent insoluble CP (%DM), FT=feed type (silage=1, wet by-products=2, others=3), EE=ether extract (% of DM), NDF=ash-free NDF assayed with sodium sulfate and amylase (% of DM), dNDF=*in vitro* or in sacco NDF digestibility at 30 h (% of DM), ASH=ash (% of DM), NEL=energy value at 1×M intake, NFC=non-fiber carbohydrate calculated as: 100-ASH-EE-NDF-CP.

Table 2. The *in vitro* NDF digestibility and energy value of forage in Taiwan with or without supplementation of *Aspergillus oryzae* fermentation extract

	DNDF[1] (%)	$TDN_{(1\times M)}$(%)	$DE_{(1\times M)}$ (Mcal/kg)	$ME_{(1\times M)}$ (Mcal/kg)	$NEL_{(1\times M)}$ (Mcal/kg)	$NEL_{(3\times M)}$ (Mcal/kg)
Alfalfa hay						
-AO*	41.57^{b}	64.88^{b}	3.08^{b}	2.66^{b}	1.61^{b}	1.34^{b}
+AO	46.10^{a}	67.18^{a}	3.18^{a}	2.76^{a}	1.67^{a}	1.39^{a}
SEM	1.272	0.643	0.028	0.029	0.017	0.014
Peanut vine						
-AO	39.84	62.18	2.87	2.45	1.53	1.29
+AO	38.38	61.52	2.84	2.42	1.52	1.27
SEM	0.721	0.326	0.014	0.015	0.009	0.007
Timothy hay						
-AO	36.12	49.18	2.26	1.84	1.19	0.95
+AO	38.21	50.56	2.33	1.90	1.22	0.98
SEM	1.550	1.030	0.045	0.046	0.027	0.022
Oats hay						
-AO	29.36	42.45	1.94	1.51	1.01	0.80
+AO	27.92	41.42	1.89	1.46	0.98	0.78
SEM	0.657	0.466	0.021	0.021	0.012	0.010
Bermuda hay						
-AO	33.50	49.57	2.28	1.85	1.20	0.95
+AO	31.23	47.89	2.21	1.78	1.15	0.91
SEM	1.086	0.804	0.035	0.036	0.021	0.017
Bermuda straw						
-AO	23.77	41.33	1.91	1.48	0.98	0.78
+AO	23.32	41.03	1.90	1.47	0.97	0.78
SEM	0.578	0.389	0.017	0.017	0.010	0.008
Pangola hay						
-AO	38.68^{a}	44.30^{a}	2.08^{a}	1.65^{a}	1.06^{a}	0.82^{a}
+AO	33.82^{b}	40.48^{b}	1.91^{b}	1.48^{b}	0.96^{b}	0.74^{b}
SEM	0.997	0.783	0.035	0.035	0.021	0.016
Napier grass						
-AO	33.96^{a}	42.56^{a}	1.97^{a}	1.54^{a}	1.01^{a}	0.79^{a}
+AO	30.50^{b}	39.88^{b}	1.85^{b}	1.42^{b}	0.94^{b}	0.74^{b}
SEM	1.069	0.826	0.036	0.037	0.022	0.017
Corn silage						
-AO	35.87	57.05	2.26	2.20	1.40	1.14
+AO	36.21	57.25	2.63	2.21	1.40	1.14
SEM	0.988	0.588	0.026	0.026	0.016	0.013

[1] *In vitro* NDF digestibility at 30 h (% of NDF).

[a, b] Means within the same column without the same superscripts are significantly different ($p<0.05$).

* −AO: represents the *Aspergillus oryzae* inclusion. -AO: without the *Aspergillus oryzae* inclusion.

The dynamic degradation model was performed according to Ørskov and McDonald (1979) and calculated from the DM, OM, NDF and ADF disappearance rate at each time point using iterative least square procedures according to SAS (1985). The nutrient degradation rate is from the slope (b) of the degradation curve from the degradation model, $P=a+b(1-e^{-ct})$ where "P" is the actual degradation after time "t", "a" is the soluble portion (percentage) at incubation initiation, "b" is the potentially degradable portion in the rumen, and "c" is the degradation rate constant (%/h) of "b" or the slope of the degradation curve. The available nutrient or the effective degradability (ED) of the nutrient, $ED=a+b\times c/(c+k)$ was calculated at the rumen solid outflow rates of 2, 5 and 8%/h (k).

Analysis of variance was calculated using the general linear model procedure (GLM) of the Statistical Analysis Systems Institute Inc. (1985). Least square means were used to compare the differences between the treatment effects.

RESULTS AND DISCUSSION

Predict energy value from 30 h *in vitro* NDF degradability

NDF degradability can be regarded as NDF digestibility as no more NDF digestion in the GI tract beyond the rumen. The *in vitro* NDF degradability or digestibility and energy value for the respective forage and by-product feedstuff in Taiwan included those with or without AFE treatment and

Table 3. The *in vitro* NDF digestibility and energy value of by-products in Taiwan with or without supplementation of *Aspergillus oryzae* fermentation extract

	DNDF[1] (%)	$TDN_{(1\times M)}$ (%)	$DE_{(1\times M)}$ (Mcal/kg)	$ME_{(1\times M)}$ (Mcal/kg)	$NEL_{(1\times M)}$ (Mcal/kg)	$NEL_{(3\times M)}$ (Mcal/kg)
Wheat middling						
-AO*	66.32	82.41	3.89	3.48	2.07	1.82
+AO	65.51	82.18	3.88	3.47	2.07	1.81
SEM	0.867	0.242	0.011	0.011	0.006	0.006
Soy pomace						
-AO	45.91	72.31	3.46	3.05	1.80	1.56
+AO	47.56	72.90	3.49	3.07	1.82	1.57
SEM	0.781	0.283	0.012	0.013	0.008	0.007
Beancurd pomace						
-AO	34.39	73.41	3.50	3.08	1.83	1.55
+AO	35.67	73.94	3.52	3.10	1.85	1.56
SEM	0.937	0.384	0.017	0.017	0.010	0.009
Rice distiller grain						
-AO	34.93	88.13	4.13	3.72	2.22	1.88
+AO	34.84	88.08	4.13	3.72	2.22	1.88
SEM	0.447	0.239	0.011	0.011	0.006	0.005
Brewers' grain						
-AO	41.29 [a]	63.97 [a]	3.14 [a]	2.72 [a]	1.58 [a]	1.28 [a]
+AO	39.52 [b]	62.82 [b]	3.09 [b]	2.67 [b]	1.55 [b]	1.25 [b]
SEM	0.459	0.297	0.013	0.013	0.008	0.006
Sorghum distiller grain						
-AO	59.00	81.51	3.80	3.39	2.05	1.75
+AO	58.55	81.36	3.80	3.38	2.04	1.75
SEM	0.488	0.163	0.007	0.007	0.004	0.004

[1] *in vitro* NDF digestibility at 30 h (% of NDF).
[a, b] Means within the same column without the same superscripts are significantly different ($p<0.05$).
[*] +AO; represents the *Aspergillus oryzae* inclusion, -AO; without the *Aspergillus oryzae* inclusion.

presented in Table 2 and 3. In general, NDF degradability was higher in legumes than in grasses and alfalfa was higher than peanut vines among the legumes. The alfalfa hay (CP 18.9%) sampled in this study was supposedly harvested at pre- to mid-blossom and graded to 124 to 140% relative nutritional value (Van Soest, 1994) according to the proximate composition. Peanut vines (CP 11.2%), on the other hand, are tropical legumes and are by-products after harvesting losing the leaves during the post-harvesting process.

Corn silage contains nonstructural carbohydrate (NFC) from its grain content, hence it has the richest in energy value although in the NDF degradability was not the highest among the forage. Corn silage contained lower NEL_{3xM} value (3×M) in this study compared to the value obtained by Robinson (2000) (1.14 vs. 1.45 Mcal/kg DM) relatively highest NDF with lower degradability than the corn silage used in this trial. This is probably attributed to the different corn strains with different cultivation methods, climate, fertilization and harvesting. The NDF degradability and energy value was greatest in Timothy hay among the imported forages. The imported Timothy hay still contained lower CP (7.5%) and energy compared to the average quality hay, reflecting the fact that the imported hay was post-head harvested with a relative feed value lower than 85% (Van Soest, 1994). Next to Timothy, Bermuda hay (among the imported forages) contained better NDF degradability and predicted energy value. Although oat is temperate C3 type forage, the oat hay used in this study contained relatively lower CP (5.2%) with higher NDF. This revealed late harvesting in low forage quality. The energy content of oat hay in this trial was 27.7% lower than the value obtained by Robinson (2000). Bermuda is tropical C-4 type forage and Bermuda straw is the by-product after seed harvesting. Bermuda hay therefore contained the poorest NDF degradability and estimated energy value among the imported forages. Bermuda straw is therefore used only to fill dry cows during the winter roughage shortage season.

Except for corn silage with rich energy, locally produced grasses including Pangola and Napier grasses contained low energy value. These two forages are tropical C4 type forages cultivated under tropical high temperatures in a high humidity climate. This grass grows at different maturity levels within the same plant, with rapid maturation during the hot summer (Van Soest, 1994). The quality of these grasses deteriorates fast without harvesting at the right time.

AFE inclusion significantly increased ($p<0.05$) NDF degradability in alfalfa, but decreased ($p<0.05$) in Pangola and Napier grasses. These results agreed with Chiou et al. (2000) in their *in situ* nylon bag measurements. AFE inclusion did not show significantly increased NDF degradability in peanut vines or a decrease in corn silage in this study. Our values were also lower than those described in Chiou et al. (2000). The lower values obtained from the *in vitro* compared to *in situ* methods may be attributed to the methodology differences described by Dewhurst et al. (1995). The higher values derived from *in situ* incubation may be attributed to two reasons. In addition to the small feed particles that passed through the nylon bag pores in the *in situ* incubation (regarded as an instantly soluble or degradable fraction), parts of these particles were, in fact, not degraded in the rumen. The three nylon bag texture dimensions used in ANKOM incubation make it difficult for the feed particles to pass through the pores in the *in vitro* incubation. The increased NDF degradation which enhances the energy content in alfalfa hay might be attributed to the improvement in lactating performance by AFE inclusion obtained from our previous lactation trial (Chiou et al., 2002).

The TDN values for most grasses calculated from 30 h. *in vitro* NDF digestibility, in addition to the proximate composition in this study, were lower than the NRC tabulated values predicted from the proximate analysis, except for the alfalfa hay values (64.9 vs. 63.2%) and peanut vines (62.2 vs. 58.8%). Weiss (1998) pointed out a 7 to 8% over-estimate for TDN and NEL using the current prediction equation for proximate composition (NRC, 2001) with an over-estimation for low digestible and an under-estimation for high digestible forages. The disadvantage of the current energy value predictions from the proximate composition is using unreliable lignin content values in estimating NDF digestibility, due to large error in lignin analysis (Weiss, 1993). Therefore, either the *in vitro* or *in situ* method is more reliable than the proximate composition using the lignin content to evaluate NDF digestibility.

The predicted forage $NEL_{(3\times M)}$ "discount value" at production levels was subtracted twice from the $NEL_{(1\times M)}$ at maintenance level in this study according to the equations from Robinson (2000). Eight percent was subtracted for the decrease in digestibility, or 10% for forage TDN content below 60% in the NRC (2001) estimation (19.68 vs. 10.0%). The discount value in this study was calculated from the NDF and NFC content of the feedstuff and was greater than the value subtracted from the maintenance level in the NRC calculation. This resulted in lower predicted $NEL_{(3\times M)}$ values in this study than those from the NRC (2001). The lower $NEL_{(3\times M)}$ values reflect a more precise NEL prediction at production levels as described by Weiss (1998).

Rice distiller's grains contained lower NDF degradability, with the highest energy value among the by-product feedstuffs. The lower NDF degradability in dried rice distiller's grains might be attributed to 1) increased CHO fraction C (non-degradable and non-available fraction) during distillation in the winery process due to heat damage and 2) high lipid content in dried rice distiller's grains (31% of DM). Although the predicted NEL value in dried rice distiller's grain was high, the inclusion level in lactating cows was limited to 10% for safety reasons due to the high fat content, as described by Huang et al. (1999). Wheat middling and sorghum distiller's grains contained richer TDN among the by-product feedstuffs. Wheat middling contained slightly higher TDN than sorghum distiller's grains (82.4 vs. 81.5%). The NDF degradability was close, while wheat middling contained higher NDF digestibility and lower CP content than sorghum distiller's grains (19.9 vs. 21.3). The TDN contents in the two by-product feedstuffs were quite close, but slightly lower in wheat middling compared to sorghum distiller's grains (74.3 vs. 76.5%), according to the NRC (2001) calculation from the proximate composition.

Two factors have long been regarded as the determinants for forage energy content, lipid content (high energy density) and structural carbohydrate digestibility. This leads to under-estimating the energy value in high NDF digestible forage and over estimating it in low NDF digestible forage, using the proximate composition for the energy prediction. Therefore, NDF digestibility determination is required in precise forage TDN content predictions.

The estimated $NEL_{(3\times M)}$ in brewer's grains in this study was considerably lower than that obtained by Robinson (2000) (1.28 vs. 1.83 Mcal/kg). This is probably due to the different brewery process because of the higher NDF content with lower NDF digestibility observed in the locally produced by-products used in this study. Bean-curd pomace contained richer energy than soy pomace, although both by-products were produced from the same raw material-soybeans. The different processing and high acid detergent insoluble nitrogen (ADIN) probably caused the lower energy content in soy pomace. Bean curd pomace is an excellent source of roughage for dairy cattle because of the dietary inclusion improved lactation performance (Chiou et al., 1998).

When energy values in locally produced by-products feedstuff are compared to the NRC (2001) values, TDN values were larger in this study than NRC when the by-product NDF degradability was relatively high, as shown in wheat middling (82.4 vs. 74.3) and sorghum distiller's grains (81.5 vs. 76.5). Conversely, the TDN values were lower in this study than the NRC values when the by-product NDF degradability was low, i.e., soy pomace (72.3 vs. 75.1%), bean-curd pomace (73.4 vs. 84.1%), brewer's grains (64.0 vs. 74.9%) and rice distiller's grains (88.1 vs. 98.9%). The heat damage via Maillard reaction during the

Table 4. The DM and OM degradability parameters and effective degradability of forage in Taiwan with or without supplementation of *Aspergillus oryzae* fermentation extract

	DM parameter			EDDM (%h)			OM parameter			EDOM (%h)		
	a	b	c	2	5	8	a	b	c	2	5	8
Alfalfa hay												
-AO*	27.21	54.32	3.43	61.52	49.31	43.51	26.86	52.40	4.34	62.73	51.21	45.29
+AO	27.40	48.29	4.11	59.88	49.19	43.79	26.87	48.05	5.04	61.27	50.99	45.44
Peanut vine												
-AO	8.53	55.76	4.96	48.27	36.30	29.87	7.73	57.64	5.80	50.59	38.68	31.96
+AO	7.74	55.53	5.37	48.20	36.50	30.04	6.97	57.19	6.23	50.26	38.70	32.01
Timothy hay												
-AO	23.66	46.55	2.85	51.01	40.56	35.89	22.28	46.36	3.57	51.99	41.59	36.58
+AO	23.53	56.74	2.01	51.97	39.80	34.92	22.48	53.48	2.63	52.86	40.91	35.71
Oats hay												
-AO	21.87	27.80	6.58	43.10	37.58	34.33	21.17	29.88	7.38	44.68	38.98	35.51
+AO	21.37	27.28	7.08	42.64	37.36	34.18	20.75	29.35	7.77	44.09	38.61	35.21
Bermuda hay												
-AO	17.12	39.71	3.57	42.57	33.66	29.37	18.52	39.26	4.08	44.87	36.16	31.78
+AO	17.47	32.61	4.49	40.03	32.90	29.19	18.38	33.16	5.31	42.47	35.46	31.61
Bermuda straw												
-AO	10.81	33.45	3.57	32.25	24.74	21.23	11.61	33.76	4.56	35.08	27.71	23.87
+AO	10.22	35.09	3.39	32.29	24.40	20.66	11.41	36.40	3.93	35.53	27.43	23.40
Pangola hay												
-AO	7.51	40.17	5.38	36.79	28.33	23.66	8.27	43.03	5.83	40.31	31.43	26.41
+AO	7.36	36.52	6.97	35.74	28.63	24.36	7.51	39.74	7.74	39.09	31.65	27.05
Napier grass												
-AO	6.45	48.54	5.34	41.76	31.52	25.88	6.99	50.63	6.05	45.04	34.71	28.79
+AO	7.99	48.06	4.92	42.16	31.83	26.29	8.28	50.35	5.74	45.62	35.19	29.31
Corn silage												
-AO	21.97	39.43	4.04	48.34	39.59	35.20	22.04	39.82	4.85	50.23	41.65	37.07
+AO	20.66	33.29	5.86	45.48	38.62	34.74	17.30	36.62	6.64	45.44	38.19	33.91

[1] Dry matter degradability described by the exponential equation $p=a+b(1-e^{-ct})$ for incubation time of 0, 6, 12, 24, 30 and 48 h with 3 replicate in each time point. a= fraction of soluble dry matter; b=fraction of potentially degradable dry matter; c=rate of degradation for fraction b. [2] EDDM calculate on k=2, 5 and 8 (%/h) solid outflow rate. * +AO; represents the *Aspergillus oryzae* inclusion, -AO; without the *Aspergillus oryzae* inclusion.

distillation process may influence the nutrient availability, hence the energy content in by-products like distiller's grains. In fact, damaged protein was not highly correlated to the ADIN. ADIN partially available to post-ruminal digestion in ruminants as indicated by many researches (Nakamura et al., 1994; Rogers et al., 1986; Weiss et al., 1989) using an *in vitro* post ruminal digestion assay. Chiou (2002) also confirmed a 40 to 78% ADIN post-ruminal digestion using the mobile nylon bag method on the rumen and duodenum in fistulated cows. The actual available energy, or NEL, in distiller's grains might be much greater than that estimated from TDN.

Except for the significantly decreased ($p<0.05$) NDF degradability, hence decreased 1.8% $NEL_{(3xM)}$ value in brewer's grains, AFE inclusion did not show any significant influence on NDF digestion in the other by-product feedstuffs. AFE inclusion also did not promote NDF digestibility in dry rice distiller's grains from our previous *in situ* trial (Chiou et al., 2000). The effectiveness of AFE inclusion apparently depends on many factors other than the feedstuff characteristics.

Forage DM and OM degradation

Table 4 presents forage DM and OM degradability parameters and effective degradability with or without AFE treatment. The DM and OM degradation rate was fastest in oat hay, followed by Pangola and Napier grasses, while Timothy was the slowest. Alfalfa hay was the largest in the DM and OM soluble portion and degradability effect, followed by Timothy hay. The least degradability was found in Bermuda straw among the imported forage. Corn silage was the largest in the DM and OM soluble portion and degradability among the locally produced forage. The DM degradation rate observed in this trial agreed with the values obtained by Mandebvu et al. (1998) (4.04 vs. 3.99).

AFE inclusion significantly increased the DM and OM degradation rate in alfalfa hay, oat hay, Pangola hay, peanut vines, Bermuda straw and corn silage with an average of a 19.3% (7.1 to 31.1%) and 18.1% (5.0 to 27.0%) rate increase in DM and OM degradation, respectively. The most prominent rate increase was in corn silage, and the least in oat hay. Fondevila et al. (1990) observed an increase in the viable bacteria counts and rate of DM degradation in straw using AFE inclusion. Gomez-Alarcon et al. (1990)

Table 5. The ADF and NDF degradability parameters and effective degradability of forage in Taiwan with or without supplementation of *Aspergillus oryzae* fermentation extract

	ADF parameter[1]			EDADF (%h)[2]			NDF parameter			EDNDF(%h)		
	a	b	c	2	5	8	a	b	c	2	5	8
Alfalfa hay												
-AO*	14.51	44.30	4.13	44.36	34.55	29.59	15.55	41.71	4.19	43.78	34.57	29.89
+AO	13.73	39.25	5.08	41.89	33.51	28.97	13.39	40.59	5.72	43.46	35.05	30.31
Peanut vine												
-AO	4.85	54.38	3.02	37.56	25.33	19.75	7.83	52.97	2.80	38.73	26.84	21.56
+AO	6.36	54.55	2.75	37.94	25.72	20.31	6.56	46.89	3.68	36.94	26.44	21.33
Timothy hay												
-AO	4.71	50.16	3.62	37.02	25.77	20.34	6.03	52.12	3.43	38.95	27.24	21.67
+AO	5.16	55.39	2.51	35.99	23.67	18.39	8.84	57.57	2.35	39.94	27.25	21.91
Oats hay												
-AO	22.51	17.19	4.95	34.75	31.06	29.08	21.18	19.27	4.59	34.60	30.40	28.21
+AO	23.86	14.76	4.54	34.11	30.88	29.20	19.72	18.94	5.25	33.44	29.42	27.22
Bermuda hay												
-AO	17.81	43.05	1.85	38.50	29.44	25.90	16.21	57.45	1.70	42.61	30.79	26.28
+AO	18.02	42.4	1.67	37.31	28.64	25.34	16.25	44.46	2.20	39.54	29.84	25.84
Bermuda straw												
-AO	7.29	36.67	1.96	25.44	17.62	14.51	5.89	32.21	4.06	27.47	20.32	16.73
+AO	6.55	42.26	1.57	25.23	16.75	13.58	5.40	34.25	3.79	27.82	20.17	16.41
Pangola hay												
-AO	5.30	51.40	2.30	32.79	21.49	16.78	5.10	46.88	3.59	35.21	24.69	19.62
+AO	5.59	44.41	2.70	31.10	21.16	16.80	5.27	40.48	4.59	33.46	24.64	20.03
Napier grass												
-AO	3.63	57.08	4.07	41.90	29.24	22.88	4.07	57.86	3.38	40.31	27.33	21.20
+AO	5.08	60.31	3.52	43.54	30.00	23.51	5.55	68.37	2.37	42.63	27.54	21.18
Corn silage												
-AO	20.49	38.03	2.12	40.06	31.81	28.46	21.50	39.12	2.64	43.76	35.02	31.21
+AO	19.03	32.97	2.96	38.71	31.29	27.93	20.20	34.41	3.78	42.70	35.01	31.24

[1] Acid detergent fiber degradability described by the exponential equation $p=a+b(1-e^{-ct})$ for incubation time of 0, 6, 12, 24, 30 and 48 h with 3 replicate in each time point. a=fraction of soluble dry matter; b=fraction of potentially degradable acid detergent fiber; c=rate of degradation for fraction b. [2] EDADF calculate on k=2, 5 and 8 (%/h) solid outflow rate. * +AO; represents the *Aspergillus oryzae* inclusion, –AO; without the *Aspergillus oryzae* inclusion.

demonstrated an improvement in lactation performance using dietary AFE inclusion. This was probably due to the increase in the DM degradation rate in one of the ration ingredients - alfalfa hay. The effect of AFE inclusion on the DM and OM degradation increase rate in alfalfa hay and corn silage in this study was also confirmed from our previous *in vivo* total tract digestion trial. The in vivo trial showed that dietary AFE inclusion increased the apparent DM and OM digestibility when alfalfa hay and corn silage were used as the roughage source (Chiou, unpublished data). The AFE inclusion positive responses were reflected in the lactation performance and proven in our previous lactation trial (Chiou et al., 2002). Therefore, AFE inclusion increased the rate of DM and OM degradation; however, it did not increase DM and OM degradability.

Forage NDF and ADF degradation

Table 5 presents forage ADF and NDF degradability parameters and effective degradability with or without AFE treatment. The NDF and ADF degradation rate was fastest and degradability was highest in both alfalfa hay and oat hay, with the least degradability found in Bermuda straw. Among the legumes, alfalfa hay was better in ADF and NDF degradation than peanut vines, both in rate and effective degradability, because peanut vine is a by-product of peanut harvesting. The vines contain high lignified-fiber and high nitrate salts. This may lead to a decrease in the NDF degradation rate. The NDF degradation rate observed in this study was lower than that obtained by Bruno-Soars et al. (2000) from their *in situ* trial (2.80 vs. 3.60%/h).

AFE inclusion increased the NDF degradation rate in alfalfa hay, oat hay, Pangola hay, peanut vines, Bermuda straw and corn silage. AFE inclusion also increased the ADF degradation rate in alfalfa hay, Pangola hay and corn silage and was most prominent in corn silage. Because AFE contained cellulolytic enzymes (Saddler, 1986) and also promoted viable cellulolytic bacteria counts (Weidmeier et al., 1987; Frumholtz et al., 1989), it promoted fiber digestion. Beharka and Nagartaja (1993) indicated that AFE promotes bacteria activity in rumen fermentation and therefore increased NDF and ADF degradability. AFE inclusion in this study was positive to the NDF and ADF rate without a positive response on the effective degradability in some forage.

Table 6. The DM and OM degradability parameters and effective degradability of by-products in Taiwan with or without supplementation of *Aspergillus oryzae* fermentation extract

	DM parameter			EDDM (%h)			OM parameter			EDOM(%h)		
	a	b	c	2	5	8	a	b	c	2	5	8
Wheat middling												
-AO*	24.70	57.91	14.31	75.51	67.62	61.84	25.68	57.41	14.24	76.02	68.17	62.44
+AO	23.28	61.04	11.96	75.58	66.32	59.86	24.20	60.41	12.05	76.01	66.89	60.51
Soy pomace												
-AO	39.45	23.32	6.23	57.10	52.39	49.66	37.76	23.60	8.54	56.88	52.65	49.95
+AO	37.61	27.29	5.66	57.77	52.10	48.92	35.58	27.00	8.26	57.32	52.40	49.30
Beancurd pomace												
-AO	3.31	73.98	2.94	47.34	30.70	23.19	3.93	70.12	3.58	48.92	33.19	25.61
+AO	4.08	72.51	3.05	47.87	31.55	24.09	5.21	71.92	3.79	52.29	36.22	28.33
Rice distiller grain												
-AO	4.39	33.98	2.76	24.09	16.48	13.11	5.18	28.94	4.18	24.75	18.36	15.11
+AO	7.26	29.64	3.61	26.33	19.69	16.48	5.34	32.93	3.45	26.19	18.78	15.26
Brewers' grain												
-AO	6.88	32.39	5.09	30.13	23.22	19.47	7.25	29.32	7.48	30.38	24.82	21.42
+AO	8.68	39.47	3.07	32.58	23.70	19.63	8.60	29.58	6.20	30.97	24.97	21.52
Sorghum distiller grain												
-AO	27.87	36.11	10.62	58.26	52.42	48.47	28.46	36.60	11.35	59.58	53.87	49.93
+AO	28.69	35.69	9.69	58.27	52.23	48.24	29.09	35.84	10.74	59.30	53.55	49.63

[1] Dry matter degradability described by the exponential equation $p=a-b(1-e^{-ct})$ for incubation time of 0, 6, 12, 24 and 30 h with 3 replicate in each time point. a=fraction of soluble dry matter; b=fraction of potentially degradable dry matter; c=rate of degradation for fraction b. [2] EDDM calculate on k=2, 5 and 8 (%/h) solid outflow rate. * +AO: represents the *Aspergillus oryzae* inclusion, -AO without the *Aspergillus oryzae* inclusion.

Table 7. The ADF and NDF degradability parameters and effective degradability of by-products in Taiwan with or without supplementation of *Aspergillus oryzae* fermentation extract

	ADF parameter[1]			EDADF (%h)[2]			NDF parameter			EDNDF (%h)		
	a	b	c	2	5	8	a	b	c	2	5	8
Wheat middling												
-AO*	9.28	45.45	12.02	48.25	41.38	36.57	20.26	46.74	15.00	61.50	55.32	50.74
+AO	11.99	49.34	7.27	50.68	41.22	35.48	19.59	49.30	11.64	61.66	54.08	48.81
Soy pomace												
-AO	40.49	27.76	2.03	54.47	48.51	46.11	42.00	13.13	6.35	51.99	49.35	47.81
+AO	36.36	23.43	5.30	53.37	48.42	45.70	37.36	18.03	9.34	52.21	49.10	47.07
Beancurd pomace												
-AO	3.38	27.54	5.03	23.09	17.19	14.01	5.54	43.52	3.45	33.09	23.31	18.65
+AO	3.53	28.55	4.39	23.14	16.88	13.65	4.59	42.01	4.02	32.64	23.31	18.64
Rice distiller grain												
-AO	4.89	39.25	5.21	33.25	24.92	20.37	4.52	43.17	7.29	38.40	30.13	25.10
+AO	5.58	37.46	4.50	31.51	23.32	19.07	4.50	45.31	6.26	38.84	29.69	24.39
Brewers' grain												
-AO	7.30	50.52	1.60	29.75	19.55	15.72	5.62	51.58	2.32	33.32	21.97	17.22
+AO	8.04	52.42	1.86	33.30	22.25	17.93	5.84	53.77	2.11	33.44	21.80	17.06
Sorghum distiller grain												
-AO	19.45	24.37	9.29	39.50	35.29	32.54	14.50	43.73	8.31	49.57	41.80	36.78
+AO	13.65	23.02	9.16	32.54	28.54	25.94	14.51	43.07	8.36	49.27	41.46	36.52

[1] Acid detergent fiber degradability described by the exponential equation $p=a+b(1-e^{-ct})$ for incubation time of 0, 6, 12, 24 and 30 h with 3 replicate in each time point. a=fraction of soluble dry matter; b=fraction of potentially degradable acid detergent fiber; c=rate of degradation for fraction b.
[2] EDADF calculate on k=2, 5 and 8 (%/h) solid outflow rate. * −AO: represents the *Aspergillus oryzae* inclusion, -AO; without the *Aspergillus oryzae* inclusion.

By-product DM and OM degradation

Table 6 presents the DM and OM degradability parameters and effective degradability for by-product feedstuffs with or without AFE treatment. Wheat middling exhibited a faster degradation rate and higher effective DM and OM degradability among the by-product feedstuffs, probably due to the high NFC content in wheat middling. Sorghum distiller's grains contained the most rapidly degradable and effective DM and OM degradability among the winery by-products, and was almost twice as fast as the other distillation by-products. Rice distiller's grain contained the lowest DM and OM degradation. This was probably due to the different raw material grain source used in distillation. Because sorghum contains starch granules that are harder to break down in the GI tract or during the distillation process, hence much richer partially hydrolyzed starch granules are contained in this by-product than other grains. The DM and OM degradation rate and effective degradability between soy pomace and bean curd pomace were different. The former was two times greater than the later, although the two by-products come from the same raw material, soybeans. This again reflected the process effect on DM and OM degradation.

AFE inclusion increased both rice distiller's grains and bean curd pomace in the DM degradation rate. Bean curd pomace was increased, while rice distiller's grain was depressed in OM degradability. The AFE inclusion on the DM degradation rate was most prominent on rice distiller's grain, reaching 23.6%. Effective degradability was also promoted in rice distiller's grains by AFE inclusion.

By-product NDF and ADF degradation

Table 7 presents the ADF and NDF degradability parameters and effective degradability for by-products with or without AFE treatment. Wheat middling contained the fastest rate of NDF and ADF degradation and the highest effective degradability among the by-products. Soy pomace exhibited NDF and ADF degradation three times faster than bean curd pomace from the same raw material by-product. Sorghum distiller's grains contained the fastest and greatest, while brewer's grains the slowest and least in NDF and ADF degradation rate and degradability among distillation by-products.

AFE inclusion in by-product feedstuff greatly increased (2.6 times) soy pomace and slightly increased brewer's grains ADF degradation rate in addition to promoting the NDF degradation rate in soy pomace, bean curd pomace and sorghum distiller's grains with soy pomace the most prominent with a 47% rate increase. AFE inclusion, however, decreased NDF and ADF degradation in wheat middling, both in rate and degradability. Bertrand and Grimes (1997) indicated that dietary inclusion of unprotected lipid depressed the cellulolytic bacteria on fiber degradation. The promotion effect of dietary AFE inclusion on cellulolytic bacteria has not yet compensated for the negative effect from dietary lipid inclusion. The AFE inclusion result on fat rich rice distiller's grains for NDF and ADF degradation reflected this phenomenon.

CONCLUSION

The Daisy® *in vitro* fermentation method can provide important nutrient information in predicting the energy value and dynamic degradation of forage in ration formulation for dairy cattle. AFE inclusion promoted NEL in alfalfa and the DM, OM and NDF degradation rate for most forage.

ACKNOWLEDEGEMENT

The authors wish to thank the National Science Council of Taiwan, ROC, for the financial support of this project (NSC86-2811-B-005-081).

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