Effects of Aspergillus oryzae Fermentation Extract on In Situ Degradation of Feedstuffs

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ABSTRACT: The aim of this study was to evaluate the effect of Aspergillus oryzae fermentation extract (AFE) on in situ degradation of the various concentrates, forages and by-products in Taiwan. The in situ trial was conducted to determine the effect of AFE on the rate of ruminal degradation of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of the various local available feedstuffs commonly used for dairy cattle. Two ruminal fistulated cows were arranged into a two by two switchback trial. Two dietary treatments were control without AFE inclusion diet and diet with 3 g of AFE (Amaferm) added daily into the total mixed ration (TMR). Results showed that effect of AFE inclusion on the ruminal degradability of concentrates vary; soybean meal is the most responsive feedstuff, corn is the next, whereas full-fat soybean did not respond the AFE inclusion at all. The inclusion of AFE significantly depressed most of the nutrient degradation of the concentrates of soybean meal in the first 12-hour in situ incubation. The effect declined in the next 12 hours. Rapeseed meal showed a different trend of response; addition of AFE improved its NDF degradation. The conclusions of AFE significantly improved ADF degradation of roughage after 24 or 48 hours of incubation. However, corn silage and peanut vines showed a different trend. Effects of AFE inclusion on the by-products degradation were inconsistent. Most of nutrients in rice distillers grain and some in bean and corn meal did show increased degradation by the AFE inclusion. (Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1076-1083)

Key Words: Aspergillus oryzae Fermentation Extract, In Situ Degradation, Forages, By-Products, ADF, NDF

INTRODUCTION

The Aspergillus oryzae fermentation extract (AFE) has long been used as a human food. It is a natural safe nonbacterial food product. Since AFE is not a metabolic product, such as antibiotics with a specific quantifiable molecular action, a well defined traceable mode of action is difficult to identify in feeding trials. This leads researchers to suspect the effectiveness of AFE as a feed additive for ruminants. After reviewing nine feeding trials involving AFE inclusion, Newbold (1990) concluded that the mean improvement in milk yield was 4.3% and ranged from 91.0% to 112% depending upon the quality of the AFE, feed composition, ingredient sources and environmental conditions.

Wallace et al. (1990) suggested that the inclusion of probiotics from fungus in ruminant diets increased DM intake using the intake-driven characteristic of the probiotics. Some researchers proposed a theory that AFE inclusion in the ruminant diet would stimulate the growth and metabolism of lactic acid metabolizing bacteria, i.e., Megasphaera elsderii and Selenomonas ruminantium. This therefore stabilizes ruminal pH by depressing the concentration of lactic acid produced from ruminal fermentation (Waldrip and Martin, 1993; Nisbet and Martin, 1991, 1993). AFE may also provide nutrients that stimulate rapid growth and multiplication of ruminal bacteria, and hence improve the rate of fiber degradation (Harris and Lobo, 1998; Williams et al., 1991). This increase in fiber digestion will decrease gut fill and enhance DM intake. The aim of this study was to evaluate the effect of Aspergillus oryzae fermentation extract on in situ degradation of the various forages and by-products in Taiwan. The in situ trial was conducted to determine the effect of AFE on the rate of ruminal degradation of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of the various local available feedstuffs commonly used with dairy cattle.

MATERIALS AND METHODS

The experiment was designed in a 2×2 switchback arrangement with two ruminal fistulated dry dairy cows which were fed two different diets. The treatments were a control diet without the inclusion of Aspergillus oryzae fermentation extract (AFE) in the basal diet and a treatment diet with 3 g/day of AFE per animal added into the basal diet according to the manufacturer's recommendation. BioZyme Enterprises, Inc., St. Jose, Mo. supplied the AFE (Amaferm).

Two Holstein dairy cows with a 600-kg live-weight were fistulated in the rumen, and placed into a 50 m² pen with a concrete floor and holding stanchion inside. The cows were fed 15 kg total
mixed ration Bermuda hay with a 1 to 1 ratio of concentrate on a dry basis. Table 1 presents the diet formulation and chemical analysis of the basal diet. After a 10-day adaptation, the cows were fed every four hours with equal portions of feed every day. The feeding hours were 0400, 0800, 1200, 1600, 2000 and 2400 during the experimental period.

Table 1. Basal diet formulation for the in situ ruminal degradation study, g/kg

| Ingredients       | Basal diet |
|-------------------|------------|
| Bermuda hay       | 500.0      |
| Soybean meal, 44% | 122.5      |
| Corn, dent        | 249.0      |
| Wheat bran        | 100.0      |
| Limestone         | 10.0       |
| Dicalcium phosphate | 15.0    |
| Common salt       | 2.5        |
| Premix*           | 1.0        |
| Total             | 1000.0     |

*Premix components (each kg contain): vitamin A, 10,000,000 IU; vitamin E, 70,000; vitamin D, 1,600,000 IU; Fe, 50 g; Zn, 40 g; Co, 0.1 g; Cu, 10 g; I, 0.5 g; Se, 0.1 g.

The 21 of the most commonly used feed ingredients in dairy cattle were collected and oven dried in a 60°C forced-driven oven for 48 hours. Samples were then ground through a 2 mm mesh screen and stored at -18°C for in situ incubation and chemical analysis.

The procedure for ruminal incubation in this trial followed the method of Oskov and McDonald (1979) modified by Chiou et al. (1995). A feedstuff sample of approximately 8 g was packed and sealed into a 10 x 20 cm polyester bag (Arkorn Co. Ltd., Spencerport, N.Y., USA). The pore size of the polyester bag was 53 ± 10 μm. Each sample of 42 replicates for roughage and 30 replicates for other feedstuff with three replications per cow and an additional 6 replicates for 0 hour were prepared into individual nylon bags for the assay. Before being placed into the rumen for incubation, all sample bags were heat sealed, and placed into a 39°C water bath for a 10 min. presoak. Three replicate sample for each incubation time were placed into the two fistulated cows. Every feed sample of the different replicates, with exception of the 0 h samples, were placed into three ruminal incubation periods, 12, 24, and 48 h for roughage, and two incubation periods 12, 24 h, for the other feedstuffs. The three 0 h incubation sample bags were directly rinsed with water only. The bags were strung onto an iron ring of 450 g. The string ring was then connected to the fistula outside the rumen by a nylon cord. The sample bags were placed into the rumen for different periods and were removed from the rumen simultaneously. After incubation, samples were put on ice to stop ruminal fermentation and then washed with water three times. Samples were then dried in a 60°C force-driven oven for 48 h. Dried samples of the feedstuffs and the incubated samples were determined by proximate analysis according to AOAC (1984). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also analyzed according to Van Soest et al. (1991).

Analysis of variance was calculated with the general linear model procedure of the Statistical Analysis Systems Institute Inc. (1985). Duncan’s new multiple range test was used to compare the treatment means (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Table 2 presents the chemical composition of ingredients that are commonly used in the dairy cattle industry in Taiwan. The feedstuffs include seven concentrates, eight forages and six by-products. Tables 3, 5 and 7 present the effects of AFE supplementation on in situ dry matter and organic matter degradation for the concentrates, forages and by-products, respectively. Table 4, 6 and 8 present the effects of in situ acid detergent fiber and neutral detergent fiber degradation of the concentrates, forages, and by-products, respectively.

The effects of AFE inclusion on the in situ nutrient degradation in rumen varied according the specific characteristics of the feedstuff. Among the concentrates, inclusion of AFE significantly depressed the first 12 hours of degradation in DM, OM, ADF, and NDF in soybean meal (p<0.05); it however, did not significantly influence the degradability of DM, OM, ADF, and NDF in soybean meal in 24 hours incubation. It showed a significant interaction by AFE inclusion and incubation period on the DM, OM and ADF degradation of soybean meal (p<0.05). Inclusion of AFE showed a trend of depressing DM and OM degradation of corn in 24 hours incubation as compared to the control diet. This may be attributed to the starch granular structure of corn. This can also
Table 2. The chemical composition of common feedstuffs in Taiwan, %

| Ingredients                  | IFI number | DM   | OM   | CP    | ADF   | NDF   |
|------------------------------|------------|------|------|-------|-------|-------|
| Concentrates                 |            |      |      |       |       |       |
| Corn, dent                   | 4-02-935   | 89.98| 90.57| 10.31 | 3.40  | 13.16 |
| Corn, flake                  | 4-02-864   | 90.40| 89.24| 9.88  | 4.43  | 10.80 |
| Wheat                        | 4-05-211   | 89.83| 87.19| 17.90 | 4.49  | 15.06 |
| Soybean meal, 44%            | 5-04-612   | 90.85| 93.38| 47.12 | 8.02  | 14.18 |
| Soybean, full-fat            | 5-04-608   | 93.30| 91.21| 43.15 | 8.76  | 11.31 |
| Cottonseed                   | 5-01-614   | 92.82| 94.77| 25.00 | 32.13 | 37.36 |
| Rapeseed meal                | 5-08-135   | 90.76| 79.41| 44.94 | 18.13 | 24.58 |
| Forages                      |            |      |      |       |       |       |
| Alfalfa hay                  | 1-00-050   | 90.00| 82.24| 24.36 | 33.38 | 39.80 |
| Corn silage                  | 3-02-912   | 21.99| 90.32| 8.08  | 28.84 | 51.49 |
| Bermuda hay                  | 1-00-713   | 91.65| 84.43| 11.34 | 26.85 | 60.04 |
| Bermuda straw                | 1-00-210   | 91.20| 83.19| 7.10  | 31.03 | 67.43 |
| Oat hay                      | 1-03-     | 90.46| 87.46| 9.01  | 44.01 | 63.25 |
| Pangola hay                  | 2-03-493   | 92.65| 84.46| 8.79  | 35.94 | 64.73 |
| Nupiergrass                  | 1-08-462   | 14.00| 85.55| 10.86 | 37.59 | 62.50 |
| Peanut vine                  | 1-03-619   | 89.93| 88.29| 13.70 | 33.40 | 39.08 |
| By-products                  |            |      |      |       |       |       |
| Wheat middling               | 4-05-205   | 89.93| 86.85| 19.87 | 8.04  | 27.92 |
| Brewers grain, wet           | 5-02-142   | 23.00| 90.80| 28.53 | 22.78 | 53.41 |
| Sorghum distiller's grain, wet| 5-04-374  | 28.68| 89.08| 22.34 | 25.32 | 42.76 |
| Rice distillers' grain, dried| 5-         | 95.09| 94.10| 25.08 | 30.48 | 48.77 |
| Soy pomace, dried            | 5-         | 90.36| 87.99| 25.79 | 27.40 | 36.18 |
| Beancurd pomace, wet         | 4-         | 13.06| 90.66| 19.90 | 21.21 | 35.83 |

Table 3. Effect of *Aspergillus oryzae* fermentation extract supplementation on in situ dry matter and organic matter degradation of concentrates, %

| Ingredients                  | Dry matter | Organic matter |
|------------------------------|------------|----------------|
|                              | Control    | AFE           | SEM | P-value | Control    | AFE | SEM | P-value |
| Corn yellow dent             |            |               |     |         |            |     |     |         |
| 12 h incubation              | 73.20      | 73.33         | 2.76| 0.9746  | 71.75      | 71.89| 2.81| 0.9975  |
| 24 h incubation              | 93.91<sup>a</sup> | 92.14<sup>d</sup> | 0.58| 0.0827  | 93.54<sup>a</sup> | 91.75<sup>d</sup> | 0.60| 0.0885  |
| Corn, flake                  |            |               |     |         |            |     |     |         |
| 12 h incubation              | 67.08      | 64.04         | 3.43| 0.5587  | 63.18      | 60.57| 3.75| 0.6435  |
| 24 h incubation              | 87.58      | 87.00         | 3.82| 0.9183  | 86.94      | 86.01| 4.40| 0.8868  |
| Wheat                        |            |               |     |         |            |     |     |         |
| 12 h incubation              | 91.70      | 92.25         | 0.48| 0.4540  | 90.93      | 91.54| 0.53| 0.4514  |
| 24 h incubation              | 93.99      | 94.27         | 0.15| 0.2450  | 93.44      | 93.79| 0.15| 0.1624  |
| Soybean meal, 44%            |            |               |     |         |            |     |     |         |
| 12 h incubation              | 91.65<sup>b</sup> | 81.95<sup>b</sup> | 1.96| 0.0175  | 91.42<sup>b</sup> | 81.05<sup>b</sup> | 2.12| 0.0180  |
| 24 h incubation              | 96.51      | 97.66         | 0.76| 0.3345  | 96.42      | 97.60| 0.78| 0.3339  |
| Soybean, full-fat            |            |               |     |         |            |     |     |         |
| 12 h incubation              | 82.96      | 77.49         | 3.88| 0.3646  | 81.25      | 75.93| 4.17| 0.4079  |
| 24 h incubation              | 87.78      | 91.98         | 6.63| 0.6732  | 86.41      | 91.34| 7.34| 0.6547  |
| Cottonseed                   |            |               |     |         |            |     |     |         |
| 12 h incubation              | 52.34      | 52.47         | 1.94| 0.9640  | 43.75      | 43.72| 1.68| 0.9911  |
| 24 h incubation              | 58.27      | 54.97         | 1.57| 0.1963  | 49.98      | 46.63| 1.53| 0.1835  |
| Rapeseed meal                |            |               |     |         |            |     |     |         |
| 12 h incubation              | 59.05      | 61.21         | 0.77| 0.1052  | 61.00      | 55.52| 5.78| 0.5326  |
| 24 h incubation              | 72.13      | 71.61         | 0.60| 0.5673  | 75.73      | 81.84| 4.59| 0.3898  |

<sup>a,b</sup> Means in the same row followed by different letters are significantly different (p<0.05).
<sup>c,d</sup> Means in the same row followed by different letters are significantly different (p<0.10).
Table 4. Effect of *Aspergillus oryzae* fermentation extract supplementation on in situ acid detergent fiber and neutral detergent fiber degradation of concentrates, %

|                        | Acid detergent fiber | Neutral detergent fiber |
|------------------------|----------------------|-------------------------|
|                        | Control | AFE | SEM | P-value | Control | AFE | SEM | P-value |
| **Corn, yellow dent**  |         |     |     |         |         |     |     |         |
| 12 h incubation        | 50.92   | 34.08 | 11.05 | 0.3305  | 44.49   | 50.04 | 6.40  | 0.5670  |
| 24 h incubation        | 68.46   | 66.04 | 6.84  | 0.8037  | 71.43   | 68.19 | 3.83  | 0.5754  |
| **Corn, flake**        |         |     |     |         |         |     |     |         |
| 12 h incubation        | 55.52   | 44.26 | 2.77  | 0.0349  | 18.45   | 6.88  | 11.13 | 0.4954  |
| 24 h incubation        | 73.91   | 23.47 | 27.35 | 0.2490  | 61.62   | 41.88 | 10.65 | 0.2471  |
| **Wheat**              |         |     |     |         |         |     |     |         |
| 12 h incubation        | 46.27   | 52.67 | 2.40  | 0.1171  | 66.79   | 64.35 | 2.37  | 0.4992  |
| 24 h incubation        | 53.36   | 56.60 | 2.97  | 0.4768  | 67.76   | 69.47 | 0.68  | 0.1370  |
| **Soybean meal, 44%**  |         |     |     |         |         |     |     |         |
| 12 h incubation        | 66.56   | 42.50 | 4.39  | 0.0117  | 70.61   | 55.18 | 2.93  | 0.0136  |
| 24 h incubation        | 82.07   | 88.76 | 5.63  | 0.4385  | 84.18   | 90.10 | 3.63  | 0.3099  |
| **Soybean, full-fat**  |         |     |     |         |         |     |     |         |
| 12 h incubation        | 46.30   | 44.84 | 9.43  | 0.9168  | 37.18   | 25.37 | 14.64 | 0.5930  |
| 24 h incubation        | 31.76   | 85.72 | 37.83 | 0.3595  | 34.71   | 85.73 | 33.16 | 0.3263  |
| **Cottonseed**         |         |     |     |         |         |     |     |         |
| 12 h incubation        | 5.06    | 0.03  | 3.93  | 0.4066  | -5.12   | -2.75 | 1.98  | 0.4368  |
| 24 h incubation        | 10.18   | 3.53  | 2.71  | 0.1437  | 5.83    | -1.24 | 4.35  | 0.3027  |
| **Rapeseed meal**      |         |     |     |         |         |     |     |         |
| 12 h incubation        | 19.68   | 24.69 | 5.18  | 0.5244  | 27.57   | 41.87 | 2.37  | 0.0079  |
| 24 h incubation        | 39.61   | 37.09 | 1.44  | 0.2705  | 49.97   | 53.97 | 2.63  | 0.4067  |

a,b Means in the same row followed by different letters are significantly different (p<0.05).

It appears that the effects of AFE inclusion on the ruminal degradability of concentrates vary; soybean meal is the most responsive feedstuff, corn is the next, whereas full-fat soybean did not respond to AFE inclusion at all. The inclusion of AFE significantly depressed degradation of most nutrients in the concentrates, especially soybean meal during the first 12 hour *in situ* incubation; this effect declined during 12 to 24 hour period. Rapeseed meal showed a different trend of response; addition of AFE improved its NDF degradation.

Response to AFE inclusion in the diet on nutrient degradation in the forages was most marked in corn silage, napier grass and peanut vine; Bermuda straw showed a modest response, and Bermuda hay the least response. Inclusion of AFE influenced ruminal degradation of most nutrients in corn silage; it depressed degradation of DM, OM, ADF, and NDF in both 12 and 24 h incubation. However, it increased degradation of ADF and NDF in the 48 h incubation. Napier grass showed a similar response in most of the nutrients except NDF. Most of the depressing effect of AFE inclusion on nutrient degradation became evident by 24 h incubation, with the exception of ADF degradation which showed a trend to increase with the inclusion. Inclusion of AFE showed an increased response of DM, OM, ADF, and NDF degradation in peanut-vine. Inclusion of AFE significantly increased DM and OM degradation (p<0.05) in peanut-vine during the first 12 hours, and showed only a trend towards increase during the second 12 hours (p<0.10) of ruminal incubation. The ADF and NDF however, showed significantly increased degradation by the AFE inclusion with the 24 hours incubation (p<0.05). Bermuda straw, on the other hand, did not significantly respond in increased OM, ADF, and NDF degradation until 24 hours incubation.

It appears that inclusion of AFE in the diet promoted ADF degradation in forage, but it normally
Table 5. Effect of *Aspergillus oryzae* fermentation extract supplementation on *in situ* dry matter and organic matter degradation of forages the in Taiwan, %

|                | Dry matter | Organic matter |
|----------------|------------|----------------|
|                | Control    | AFE            | SEM | P-value | Control | AFE     | SEM | P-value |
| Alfalfa hay    |            |                |     |         |         |         |     |         |
| 12 h incubation| 64.35      | 62.87          | 1.54| 0.5252  | 55.00   | 54.00   | 2.03| 0.6541  |
| 24 h incubation| 74.52      | 75.77          | 1.25| 0.5116  | 68.13   | 69.91   | 1.58| 0.4633  |
| 48 h incubation| 80.02      | 79.74          | 0.37| 0.6088  | 74.93   | 74.69   | 0.45| 0.7218  |
| Corn silage    |            |                |     |         |         |         |     |         |
| 12 h incubation| 53.31      | 47.23          | 1.01| 0.0079  | 52.82   | 46.53   | 1.04| 0.0080  |
| 24 h incubation| 65.37      | 59.78          | 1.36| 0.0336  | 63.67   | 59.45   | 0.77| 0.0118  |
| 48 h incubation| 72.40      | 72.74          | 0.24| 0.3697  | 72.26   | 72.99   | 0.33| 0.1762  |
| Bermuda hay    |            |                |     |         |         |         |     |         |
| 12 h incubation| 48.30      | 52.90          | 4.69| 0.5192  | 42.33   | 47.42   | 5.15| 0.5162  |
| 24 h incubation| 62.64      | 60.57          | 4.55| 0.7604  | 48.37   | 56.63   | 7.56| 0.4749  |
| 48 h incubation| 70.02      | 70.45          | 1.29| 0.8211  | 66.80   | 67.55   | 1.38| 0.7193  |
| Bermuda straw  |            |                |     |         |         |         |     |         |
| 12 h incubation| 41.37      | 41.50          | 0.82| 0.9319  | 33.25   | 34.01   | 1.13| 0.6507  |
| 24 h incubation| 52.31      | 53.37          | 2.11| 0.7374  | 46.44   | 52.75   | 1.38| 0.0231  |
| 48 h incubation| 64.45      | 63.56          | 1.04| 0.5731  | 60.44   | 59.38   | 1.14| 0.5393  |
| Oat hay        |            |                |     |         |         |         |     |         |
| 12 h incubation| 43.48      | 42.87          | 2.13| 0.8476  | 39.17   | 38.44   | 2.34| 0.8331  |
| 24 h incubation| 52.87      | 53.17          | 2.69| 0.9390  | 49.32   | 50.42   | 3.07| 0.8097  |
| 48 h incubation| 62.13      | 62.62          | 1.57| 0.8364  | 60.02   | 60.18   | 1.78| 0.9519  |
| Pangola hay    |            |                |     |         |         |         |     |         |
| 12 h incubation| 48.78      | 46.34          | 2.19| 0.4661  | 41.69   | 38.30   | 2.85| 0.4385  |
| 24 h incubation| 55.23      | 59.07          | 2.71| 0.3620  | 49.38   | 53.01   | 3.44| 0.4887  |
| 48 h incubation| 66.49      | 67.49          | 1.31| 0.6136  | 62.27   | 63.27   | 1.66| 0.6889  |
| Napier grass   |            |                |     |         |         |         |     |         |
| 12 h incubation| 35.59      | 39.13          | 2.30| 0.3264  | 31.43   | 35.10   | 2.50| 0.3474  |
| 24 h incubation| 60.89      | 45.75          | 4.32| 0.0884  | 59.41   | 42.46   | 4.89| 0.0879  |
| 48 h incubation| 55.35      | 57.94          | 2.88| 0.5542  | 52.52   | 55.89   | 3.10| 0.4987  |
| Peanut vine    |            |                |     |         |         |         |     |         |
| 12 h incubation| 76.04      | 82.44          | 0.48| 0.0002  | 74.14   | 81.06   | 0.51| 0.0002  |
| 24 h incubation| 80.99      | 83.20          | 0.74| 0.0882  | 79.37   | 81.81   | 0.81| 0.0872  |
| 48 h incubation| 82.12      | 85.77          | 1.21| 0.2675  | 80.66   | 84.62   | 2.22| 0.2641  |

*Means in the same row followed by different letters are significantly different (p<0.05).*

took a longer incubation period to respond. The response of AFE inclusion in nutrient degradation in corn silage and peanut-vine was different. This may attributed to the different ratio of carbohydrate fractions in the feedstuff. Corn silage contains all starch in 37.2% of non-structural CHO and 44.6% structural CHO with 35.0% slow degradable CHO (B2) and 9.6% unavailable CHO (C) (Sniffer et al., 1992). Peanut-vine contains 39.9% non-structural CHO with 38.1% sugar (A) and 1.8% starch, and 35.3% structural CHO with 19.4% slow degradable CHO and 16.0% unavailable CHO (Chiou, unpublished data). Most of the non-structural CHO in peanut-vine is soluble CHO and starch in corn silage, while both feedstuffs contain about same amount of non-structural CHO. These fast degradable carbohydrates in peanut-vine may have enhanced rumen fermentation, which resulted in faster response of AFE in nutrient degradation as compared to the intermediate degradable carbohydrate in corn silage.

The most responsive by-products on *in situ* nutrient degradation in rumen is rice distillers grains; wheat
Table 6. Effect of *Aspergillus oryzae* fermentation extract supplementation on *in situ* acid detergent fiber and neutral detergent fiber degradation of forages in Taiwan, %

|               | Control | AFE | SEM | P-value | Control | AFE | SEM | P-value |
|---------------|---------|-----|-----|---------|---------|-----|-----|---------|
| **Alfalfa hay** |         |     |     |         |         |     |     |         |
| 12 h incubation | 31.83   | 28.63 | 3.41 | 0.5367  | 26.31   | 24.62 | 2.48 | 0.6514  |
| 24 h incubation | 37.78<sup>a</sup> | 56.53<sup>c</sup> | 6.30 | 0.0895  | 44.69   | 47.62 | 2.44 | 0.4349  |
| 48 h incubation | 55.73   | 54.46 | 1.58 | 0.5943  | 56.16   | 59.19 | 1.77 | 0.2803  |
| **Corn silage** |         |     |     |         |         |     |     |         |
| 12 h incubation | 26.32<sup>a</sup> | 16.80<sup>b</sup> | 1.82 | 0.0141  | 30.78<sup>c</sup> | 20.88<sup>b</sup> | 1.90 | 0.0142  |
| 24 h incubation | 42.51<sup>a</sup> | 33.71<sup>b</sup> | 1.27 | 0.0045  | 46.33<sup>c</sup> | 39.94<sup>c</sup> | 1.26 | 0.0158  |
| 48 h incubation | 56.32<sup>a</sup> | 59.22<sup>c</sup> | 0.89 | 0.0689  | 59.21<sup>b</sup> | 61.23<sup>c</sup> | 0.60 | 0.0637  |
| **Bermuda hay** |         |     |     |         |         |     |     |         |
| 12 h incubation | 32.12   | 35.63 | 6.49 | 0.7176  | 35.16   | 40.56 | 5.65 | 0.5292  |
| 24 h incubation | 49.49   | 47.90 | 5.42 | 0.8434  | 53.62   | 51.40 | 5.92 | 0.8015  |
| 48 h incubation | 54.14   | 63.95 | 3.98 | 0.1415  | 62.94   | 65.52 | 1.76 | 0.348B  |
| **Bermuda straw** |         |     |     |         |         |     |     |         |
| 12 h incubation | 26.03   | 27.47 | 0.99 | 0.3501  | 31.89   | 20.24 | 1.62 | 0.9502  |
| 24 h incubation | 41.17<sup>b</sup> | 49.68<sup>a</sup> | 1.98 | 0.0287  | 45.41<sup>b</sup> | 51.97<sup>c</sup> | 1.42 | 0.0220  |
| 48 h incubation | 57.03   | 56.53 | 2.02 | 0.8688  | 59.97   | 59.51 | 1.61 | 0.8493  |
| **Oat hay** |         |     |     |         |         |     |     |         |
| 12 h incubation | 32.48   | 32.68 | 2.82 | 0.9607  | 27.70   | 27.34 | 2.56 | 0.8670  |
| 24 h incubation | 47.25   | 48.35 | 2.98 | 0.8034  | 40.93   | 41.01 | 3.66 | 0.9874  |
| 48 h incubation | 56.18   | 59.84 | 1.81 | 0.2115  | 52.32   | 54.24 | 1.98 | 0.522B  |
| **Pangola hay** |         |     |     |         |         |     |     |         |
| 12 h incubation | 28.12   | 19.53 | 5.59 | 0.3263  | 33.50   | 31.15 | 6.25 | 0.6512  |
| 24 h incubation | 34.01   | 40.50 | 4.82 | 0.3982  | 43.26   | 48.46 | 4.39 | 0.4407  |
| 48 h incubation | 49.65   | 55.65 | 3.11 | 0.2307  | 58.44   | 59.74 | 1.87 | 0.6426  |
| **Napier grass** |         |     |     |         |         |     |     |         |
| 12 h incubation | 15.46   | 23.26 | 2.17 | 0.0517  | 20.56   | 25.77 | 2.98 | 0.2706  |
| 24 h incubation | 48.07   | 26.32 | 7.35 | 0.1311  | 52.02<sup>c</sup> | 35.25<sup>d</sup> | 4.69 | 0.0851  |
| 48 h incubation | 42.53   | 45.63 | 4.28 | 0.6700  | 45.50   | 49.08 | 3.69 | 0.4693  |
| **Peanut vine** |         |     |     |         |         |     |     |         |
| 12 h incubation | 51.78<sup>b</sup> | 66.88<sup>c</sup> | 1.21 | 0.0003  | 51.11<sup>b</sup> | 65.77<sup>c</sup> | 1.68 | 0.0016  |
| 24 h incubation | 61.24<sup>b</sup> | 66.15<sup>c</sup> | 1.25 | 0.0394  | 60.05<sup>b</sup> | 65.76<sup>c</sup> | 1.30 | 0.0268  |
| 48 h incubation | 63.62   | 70.67 | 4.17 | 0.2849  | 62.56   | 70.84 | 4.60 | 0.3917  |

<sup>a</sup>,<sup>b</sup>,<sup>c</sup> Means in the same row followed by different letters are significantly different (p<0.05).

<sup>AB</sup> Means in the same row followed by different letters are significantly different (p<0.10).

During the incubation, the AFE significantly increased the DM and NDF of rice distillers grain in 12 hours and 24 hours incubation; OM degradation increased in 12 hours incubation and ADF in 24 hours incubation. The DM and OM degradation were increased by the AFE inclusion in 12 hours incubation (p<0.05). The response of AFE inclusion on wheat middlings and sorghum distillers grain was inconsistent.

In general, effects of AFE inclusion on by-products degradability were inconsistent. Most of nutrients in rice distillers grain and some in beancurd pomace did show increased degradation by the AFE inclusion.

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Table 7. Effect of *Aspergillus oryzae* fermentation extract supplementation on *in situ* dry matter and organic matter degradation of by-products in Taiwan, %

|                     | Dry matter       | Organic matter   |
|---------------------|------------------|------------------|
|                     | Control | AFE   | SEM    | P-value | Control | AFE   | SEM    | P-value |
| Wheat middlings     |         |       |        |         |         |       |        |         |
| 12 h incubation     | 83.59   | 81.81 | 1.28   | 0.3704  | 82.01   | 79.74 | 1.28   | 0.2664  |
| 24 h incubation     | 87.42   | 87.25 | 0.56   | 0.8408  | 86.16   | 86.15 | 0.57   | 0.9865  |
| Brewers grains, wet |         |       |        |         |         |       |        |         |
| 12 h incubation     | 57.03   | 53.75 | 1.87   | 0.2681  | 54.55   | 53.52 | 3.12   | 0.8256  |
| 24 h incubation     | 65.87   | 67.02 | 1.44   | 0.5987  | 63.68   | 65.52 | 1.51   | 0.4277  |
| Sorghum distillers grain, wet | |       |        |         |         |       |        |         |
| 12 h incubation     | 63.49   | 68.96 | 4.25   | 0.4052  | 57.25   | 65.90 | 6.87   | 0.4148  |
| 24 h incubation     | 78.78   | 76.90 | 2.42   | 0.6072  | 76.86   | 75.14 | 2.52   | 0.6504  |
| Rice distillers grain, dried |         |       |        |         |         |       |        |         |
| 12 h incubation     | 38.23^a | 45.35^a | 0.96 | 0.0033  | 35.18^b | 41.10^b | 0.85 | 0.0044  |
| 24 h incubation     | 49.23^b | 60.89^b | 2.22 | 0.0139  | 47.72   | 68.90 | 8.50   | 0.1385  |
| Soy pomace, dried |         |       |        |         |         |       |        |         |
| 12 h incubation     | 79.01   | 77.34 | 0.22   | 0.6220  | 77.73   | 75.92 | 2.30   | 0.6020  |
| 24 h incubation     | 87.62   | 85.33 | 1.46   | 0.3184  | 86.68   | 84.34 | 1.56   | 0.3182  |
| Bean curd pomace, wet |         |       |        |         |         |       |        |         |
| 12 h incubation     | 49.70^b | 51.12^a | 0.35 | 0.0359  | 46.76^b | 48.35^a | 0.41 | 0.0396  |
| 24 h incubation     | 87.59   | 85.76 | 3.13   | 0.6975  | 86.92   | 85.64 | 3.33   | 0.7063  |

^ab^ Means in the same row followed by different letters are significantly different (p<0.05).

^cd^ Means in the same row followed by different letters are significantly different (p<0.10).

Table 8. Effect of *Aspergillus oryzae* fermentation extract supplementation on *in situ* and acid detergent fiber and neutral detergent degradation of by-products in Taiwan, %

|                     | Acid detergent fiber | Neutral detergent fiber |
|---------------------|----------------------|------------------------|
|                     | Control | AFE   | SEM    | P-value | Control | AFE   | SEM    | P-value |
| Wheat middlings     |         |       |        |         |         |       |        |         |
| 12 h incubation     | 38.56^a | 30.56^b | 2.02 | 0.0429  | 58.12^c | 48.31 | 3.42   | 0.0985  |
| 24 h incubation     | 45.60   | 47.43 | 2.76   | 0.6586  | 63.85   | 66.40 | 1.05   | 0.1458  |
| Brewer’s grains, wet |         |       |        |         |         |       |        |         |
| 12 h incubation     | 32.42   | 29.67 | 2.01   | 0.3778  | 37.44   | 35.55 | 2.32   | 0.5879  |
| 24 h incubation     | 43.50^c | 29.86^c | 4.04 | 0.0627  | 48.67   | 51.20 | 1.82   | 0.3688  |
| Sorghum distillers grain, wet | |       |        |         |         |       |        |         |
| 12 h incubation     | 29.91   | 44.23 | 10.55  | 0.3809  | 42.10   | 39.58 | 13.08  | 0.8969  |
| 24 h incubation     | 55.20   | 53.80 | 3.07   | 0.7600  | 68.30   | 65.22 | 3.26   | 0.5323  |
| Rice distillers grain, dried |         |       |        |         |         |       |        |         |
| 12 h incubation     | 27.11   | 34.53 | 2.67   | 0.1016  | 31.33^b | 38.13^a | 1.76 | 0.0408  |
| 24 h incubation     | 33.75^b | 50.80^a | 2.95 | 0.0094  | 41.04^b | 53.95 | 2.67   | 0.0189  |
| Soy pomace, dried   |         |       |        |         |         |       |        |         |
| 12 h incubation     | 65.46   | 64.42 | 4.77   | 0.8840  | 62.33   | 55.19 | 5.82   | 0.4250  |
| 24 h incubation     | 79.74   | 77.65 | 2.10   | 0.5134  | 73.74   | 69.78 | 3.44   | 0.4520  |
| Bean curd pomace, wet |         |       |        |         |         |       |        |         |
| 12 h incubation     | 21.91   | 15.84 | 3.31   | 0.2506  | 29.95   | 28.83 | 3.78   | 0.8423  |
| 24 h incubation     | 65.78   | 72.82 | 6.34   | 0.4681  | 75.65   | 75.29 | 5.58   | 0.9649  |

^ab^ Means in the same row followed by different letters are significantly different (p<0.05).

^cd^ Means in the same row followed by different letters are significantly different (p<0.10).

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