Effects of *Aspergillus oryzae* Fermentation Extract on Performance of Lactating Cows in the Summer and Winter in Taiwan

Peter Wen-Shyg Chiou*, Chao-Ren Chen and Bi Yu
Department of Animal Science, National Chung-Hsing University, Taichung, Taiwan, ROC

ABSTRACT : The aims of this study is to evaluate the effect of *Aspergillus oryzae* Fermentation Extract (AFE) on the performance of lactating cows in summer (May to July) and winter (December to February). The experiment was a completely randomized design (CRD) and dietary treatments were 1) basal diet without AFE, 2) basal plus 3 g/d AFE into the basal total mixed ration (TMR), 3) basal plus 45.4 mg AFE/kg the ensiling corn silage and 4) AFE inclusion in silage and TMR. Twenty-eight cows from each trial were selected and randomly allocated into the four treatment groups, confined in individual pens, and fed *ad libitum* for 8 weeks in both seasons of feeding trials. Results showed that AFE inclusion in corn silage significantly improved DM intake by 4.4% and milk yield by 3.1% ($p<0.05$) during summer. In the winter season, AFE inclusion in the diet significantly improved milk yield by 10%. Direct addition of AFE to the TMR even further significantly improved milk yield over the addition through corn silage by 7.4% in winter ($p<0.05$). An additive effect of AFE inclusion into TMR and through corn silage was also demonstrated in the winter-feeding. AFE inclusion however, did not improve DM intake during the winter trial. In the summer trial, inclusion of AFE showed an adverse effect on the percentage of milk fat, but did not impact on the milk fat yield. Adding AFE through corn silage showed a trend towards alleviating the negative effects of milk fat from direct AFE inclusion in TMR. The similar trend occurred in the winter trial. The inclusion of AFE through corn silage significantly lowered the milk protein content over direct AFE addition, but did not significantly impacted the milk protein yield in summer. AFE supplementation during the winter season significantly increased milk protein content. Adding AFE to the corn silage significantly increased milk protein content over direct AFE addition in winter although inclusion of AFE significantly decreased total milk solid content in the summer ($p<0.005$). During the winter season, inclusion of AFE required less DM to produce a unit of milk. Inclusion of AFE into corn silage required less DM, energy and protein to produce a unit of milk. But inclusion of AFE did not alleviate heat stress on the lactating cows. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 3 : 382-389)***

Key Words : *Aspergillus Oryzae* Fermentation Extract, Lactating Cows, Seasons, Milk Yield, Milk Compositions, Blood Urea

INTRODUCTION

For many years, ruminant nutritionists have been interested in manipulating the rumen environment to improve production efficiency in ruminants. Consumers have expressed concern over pesticide residues and bacterial resistance to antibiotics used as feed additives in animal production. Therefore, the animal feed industry has increased interest in evaluating the effects of probiotics or direct-fed microbial (DFM) on animal performance and dairy production in particular. Nonbacterial DFM added into ruminant diets generally consists of *Aspergillus oryzae* products and/or *Saccharomyces cerevisiae* culture.

The *Aspergillus oryzae* fermentation product (AFE) has a long history of use as a human food. It is a naturally safe, nonbacterial source food product. Since AFE is not a metabolic product like antibiotics with a specific quantifiable molecular structure, it does not have a well-defined traceable mode of action. The influence of fungal culture on ruminal fermentation and microbial population via proteolytic, cellulolytic bacteria counts (Yoon and Sterns, 1996) and lactic acid fermenting bacteria (Beharka and Nagaraja, 1998) have been demonstrated. However, the AFE response was difficult to identify its effects in a feeding trial and it led researchers to suspect the effectiveness of AFE as a feed additive for ruminants. From nine feeding trials with AFE inclusion, Newbold (1990) concluded that the mean improvement in milk yield was +4.3% ranging from -9.0% to +12% depending on the quality of AFE, feed composition, sources of ingredients and environmental conditions. Recent study on AFE have still shown inconsistent results; Yu et al. (1997) showed a trend to increase milk protein and SNF content by the AFE inclusion. Bertrand and Grimes (1997) however, obtained no positive response on fiber digestion or milk production by the AFE inclusion.

Higginbotham et al. (1993, 1994) showed that AFE could alleviate heat stress. Huber et al. (1985) also indicated a decrease in the rectal temperature from the AFE inclusion while Denigan et al. (1992) did not obtain a decrease in body temperature in cows fed an AFE inclusion diet. Wallentine et al. (1986) on the other hand, showed an inconsistent result in decrease in body temperature from AFE inclusion. Huber et al. (1985) indicated that inclusion of AFE to the normal ratio of concentrate resulted in a significant decrease in body temperature over cows fed a high forage diet. In addition to the lactation stage and dietary composition, these inconsistent responses are most

* Address reprint request to Peter Wen-Shyg Chiou. Tel: +886-4-22870613, Fax: +886-4-22860265, E-mail: wschiou@dragon.nchu.edu.tw
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likely attributed to management and environment conditions. Our previous *in situ* degradation of fedstuffs study has also shown an inconsistent result on byproducts feed by AFE inclusion (Chiou et al., 2000). From a *in vitro* ensiling study, the AFE inclusion in ensiling improved silage quality through an increase in the Flieg score calculated from lactic acid and butyric acids (Chiou et al., 2001). This study is therefore aimed at evaluating the effects of AFE through direct or silage inclusion on the lactation performance of dairy cows in Taiwan at the cool or warm seasons.

MATERIALS AND MOTHODS

Feeding trials were conducted in two different seasons, warm summer from May to July and cool winter from December to February, to evaluate the effect of direct feeding of *Aspergillus oryzae* Fermentation Extract (AFE) or through corn silage on lactating performance. The experiment was a complete randomized design and dietary treatments were 1) basal diet without AFE, 2) basal plus 3 g/d AFE into the basal total mixed ration (TMR), 3) basal plus 45.4 mg AFE/kg the ensiling corn silage and 4) AFE inclusion in silage and TMR. The basal diet was formulated according to the NRC (1989) nutrient requirements and is presented in table 1. The chemical composition of the silage was described in Chiou et al. (2001). Biozyme Ltd., USA, provided the *Aspergillus oryzae* fermentation extract with the trade name “Amaferm” for direct added into the basal diet in a dose of 3 g/d and “Regular GX” for added into the ensiling corn silage in 45.4 mg/kg.

Table 1. The basal diet formulation, on dry matter basis in g/kg

Ingredients	Summer diet	Winter diet
Alfalfa hay	80.0	225.0
Corn silage	320.0	225.0
Oat hay	100.0	-
Soybean, full-fat	100.0	35.5
Soybean meal, 44%	40.0	106.1
Yellow corn, dent	170.0	277.1
Wheat bran	130.0	78.6
Protect fat	-	19.0
Sodium bicarbonate	15.0	15.0
Limestone	10.0	5.9
Dicalcium phosphate	-	7.8
Common salt	3.0	3.0
Premix*	2.0	2.0
Molasses	30.0	-
Total	1000.0	1000.0
Calculated analysis		
Crude protein	160.0	170.0
NE_L, Mcal/kg	1.65	1.68
Analyzed value		
Dry matter	536.1	564.4
Crude protein	155.6	167.8
ADF	197.2	193.0
NDF	383.1	365.0

*Premix contains (each kg of diet): Vitamin A, 10,000 IU; Vitamin E, 70 IU; Vitamin D_3, 1,600 IU; Fe, 50 mg; Zn, 40 mg; Mn, 40 mg; Co. 0.1 mg; Cu, 10 mg and Se, 0.1 mg.

The diets were fed as total mixed rations (TMR) with the ratio of concentrate to roughage in the TMR at 50 to 50 in the summer trial and 55 to 45 in the winter trial, respectively, on dry matter basis in accordant with the dietary pattern of local dairy farmers. The concentrates were premixed and mixed with corn silage and alfalfa hay in both the summer and winter trials. The moisture content of the roughage was measured weekly for adjustment of the as-fed ration composition. With the exceptions of season, diet and cows, all experimental procedures were similar in the two feeding trials.

Twenty-eight cows, within three months post-partum with a mean body weight of 550 kg, producing more than 25 kg of milk in summer or 35 kg in the winter trial, respectively, were selected and allocated to the four treatment groups. The cows were confined to individual pens for most of the time during the experimental period. They were released for exercise twice daily from 4:00 to 10:00 AM and 1:00 to 5:00 PM. The experimental cattle were dewormed once every two weeks during the experimental period.

After one week of adaptation, the cows started an eight-week feeding trial. These cows were fed individually, *ad libitum* with 2-3 kg orts in two meals per day. Water was provided individually with an automatic bowl type drinker. The animals were milked twice a day at 4:30 AM and 4:00 PM.

During the feeding period, feed consumption and milk yield were recorded daily. Milk samples were taken once every week for analysis of milk constituents and urea nitrogen. The cows body weights were measured at the beginning, in the middle and at the end of the feeding trial. Feed samples were taken weekly for proximate analysis and analysis of organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF). In the middle and end of the trial, blood samples were taken for analysis of urea concentration.

Analysis of the feed samples was done according to the methods of the Association of Official Analytical Chemists (AOAC, 1984). The NDF and ADF were analyzed according to the methods of Van Soest et al. (1991) using an automatic fiber analyzer (Fibertec System M. Tecator AB). The milk composition of fat, protein, lactose and total solids were analyzed using a milk scanner (Foss Electric Co., Milko Scan 255 A/B types) according to the infra-red method of the AOAC (1984). Analysis of blood and milk urea nitrogen was done by the automatic blood chemical analyzer (Gilford system 103) according to the method of

Cross and Jenny (1976).

Analysis of the variance was calculated with the general linear model procedure of the Statistical Analysis System Institute Inc. (1985). Least square means and orthogonal contrast was used to compare the difference in the treatment means.

RESULTS AND DISCUSSION

Feeding trials were conducted both in summer and winter, respectively. The summer trial was conducted in a warm and humid environment. The winter trial was conducted in the cool and rainy season. The summer trial began from May to July where the mean high ambient temperature was 37.3°C and the mean low temperature 26.3°C with a mean relative humidity of high 81% and low 38.8%. The winter trial began from December to February with a mean high ambient temperature of 18.6°C and a low of 13.5°C with a relative humidity of 85.7% high and 67.7% low. These two weather environments greatly impacted DM intakes, with 46% more consumed in the winter than in the summer trial. This greatly influenced the nutrient utilization and lactation performance. The effects of AFE inclusion on the lactation performance, either directly or through corn silage is presented in table 2. The protein and energy utilization is presented in table 3 and the live-weight changes and concentration of urea nitrogen in the blood and milk is shown in table 4.

Dry matter intake

Effects of AFE inclusion in the diet either directly or through corn silage, did not significantly influence the DM intake of lactating cows during the winter season (p>0.05). On the other hand, inclusion of AFE significantly influenced the DM intake of lactating cows during the summer season. During the summer season, cows fed the AFE diet significantly improved DM intake (A vs. BCD) (p<0.005) as compared to the basal diet without AFE. The way of AFE inclusion (B vs. C) also significantly influenced the DM intake of the cows (p<0.005), inclusion of AFE through silage significantly improved DM intake over inclusion through TMR (p<0.005). Double inclusion of AFE through both silage and TMR showed a significant improvement of DM intake as compared to either in the silage or directly into TMR (BC vs. D) (p<0.005), indicating an additive effect of AFE on DM intake during the summer season. This implicated that inclusion of AFE through corn silage improved DM intake by 5.1% during the warm season. This increase in the DM intake by the inclusion of fungal probiotics in the diet may be attributed to the intake-driven characteristics of the probiotics as suggested by Wallace et al. (1990). Some researchers have suggested that AFE inclusion in the ruminant diet stimulated growth and metabolism of the lactic acid metabolizing bacteria, i.e., *Megasphaera elsderii* and *Selenomonas ruminatium* and this increase in the bacteria growth depressed lactic acid concentration in ruminal fermentation and stabilized ruminal pH (Waldrip and Martin, 1993; Beharka and Nagaraja, 1998). AFE provided nutrients to the fermentation that might also stimulate rapid growth and multiplication of ruminal bacteria, hence the improved rate of fiber degradation (Harris and Lobo, 1988; Yoon and Stern, 1996). This increase in the fiber digestion will decrease gut fill and enhance DM intake. From the *in situ* studies, our data also showed an increase in the ADF degradation in a number of feedstuff with AFE inclusion, i.e., alfalfa hay, corn silage, bermuda straw, napiergrass and peanut vine (Chiou et al., 2000). Sievert and Shaver (1993) also demonstrated an increase in DM intake in the high non-fibrous carbohydrate diet from the inclusion of AFE. It appears that adding AFE to the diet, either through corn silage or direct inclusion in TMR, will alleviate the adverse effects of heat stress on DM intake in lactating cows. Adding AFE through corn silage obtained a better result than directly adding AFE into TMR.

Milk yield

In the winter season, the effects of AFE inclusion, either added into TMR directly or through corn silage, significantly increased milk yield and the 4% FCM of the lactating cows (p<0.05). Direct inclusion of AFE into TMR significantly improved milk yield over the inclusion into corn silage group (36.8 vs. 34.3 kg/day) (p<0.005) in the winter. This indicated that direct inclusion of AFE into TMR produced a 7.4% improvement in milk yield over inclusion through corn silage during the winter, the season without heat stress. Adding AFE through corn silage however, still improved milk yield as compared to the control without AFE inclusion (p<0.05). Data also showed an additive effect of AFE inclusion, both directly and through corn silage on milk yield (BC vs. D) (p<0.05). Gomez-Alarcon et al. (1990) showed an improvement in milk yield from daily inclusion of 3 g AFE in the diet.

The results of the summer trial showed that the effects of AFE inclusion in the diet significantly produced more milk over those diets without AFE inclusion (A vs. BCD) (p<0.05). AFE inclusion through corn silage showed a trend towards improved milk yield over AFE added directly into TMR in the summer, although it did not reach significant level (p>0.05). However, AFE inclusion in the dairy diet through corn silage significantly improved 4% FCM over AFE added directly into TMR (p<0.01) in the summer trial.

Wallentine et al. (1986) and Gomez-Alarcon et al. (1988) both suggested that the diet composition and the ratio of concentrate to roughage in the diet influenced the effectiveness of AFE on milk yield. After reviewing a

Table 2. Effects of *Aspergillus oryzae* inclusion in the diet on lactating performance of dairy cows

Treatment	Basal diet	AFE in TMR	AFE in silage	AFE in silage & TMR	SEM	Orthogonal contrast		
	A	B	C	D		A vs. BCD	BC vs. D	B vs. C
Summer trial (May to July)								
DMI and milk yield (kg/day)								
DM intake	16.00	16.14	16.81	17.16	0.39	***	***	***
Milk yield	24.61	25.06	25.70	25.52	0.76	*	NS	NS
4% FCM	24.20	23.36	24.88	24.29	1.03	NS	NS	**
Milk composition (%)								
Total solids	12.67	12.28	12.40	12.48	0.23	***	NS	NS
Milk fat	3.88	3.59	3.74	3.74	0.14	***	NS	*
Milk protein	3.30	3.33	3.22	3.28	0.08	NS	NS	*
Milk components yield (kg/day)								
Total solids	3.12	3.06	3.21	3.16	0.12	NS	NS	*
Milk fat	0.95	0.89	0.96	0.95	0.05	NS	NS	**
Milk protein	0.80	0.83	0.82	0.83	0.03	*	NS	NS
Winter trial (December to February)								
DMI and milk yield (kg/day)								
DM intake	23.36	23.31	22.86	23.07	0.40	NS	NS	NS
Milk yield	32.67	36.80	34.27	36.92	0.60	***	*	***
4% FCM	30.17	32.53	32.32	32.83	0.58	***	NS	NS
Milk composition (%)								
Total solids	11.86	11.74	12.20	11.65	0.09	NS	***	***
Milk fat	3.50	3.36	3.63	3.39	0.08	NS	NS	***
Milk protein	2.97	3.00	3.09	2.96	0.02	*	***	***
Milk components yield (kg/day)								
Total solids	3.85	4.27	4.15	4.24	0.07	***	NS	‡
Milk fat	1.14	1.19	1.23	1.23	0.03	***	NS	NS
Milk protein	0.96	1.09	1.05	1.08	0.02	***	NS	**

*,**,*** Mean the orthogonal comparison are significantly different at 0.05, 0.01 and 0.005, respectively.
‡ Mean the orthogonal comparison is significantly different at 0.1.

number of feeding trials, Newbold (1990) found that AFE inclusion improved milk yield by +4.3% with a range from -9% to +12%, implicating that the factors of feedstuff, available nutrients and animal requirements influenced the effectiveness of AFE. The ratio of concentrate to roughage was 50 to 50 and 55 to 45 in the summer and winter trial, respectively, and also contained a higher NFC (52%) in our TMR in both seasons. Our result therefore may not agree with the result of Huber et al. (1985) on the effectiveness of AFE in a high roughage diet.

Much research reported that the effects of AFE on ruminant performance might be associated with the increase in DM intake and the rate of ruminal fiber digestion (Harries and Lobo, 1988; Yoon and Nagaraja, 1998). The milk yield increase from AFE addition to the diet in both the summer and winter seasons did not agree with Harris et al. (1983) that adding *Aspergillus oryzae* enzyme product did not influence the milk yield. Data from our laboratory mini-silo silage study showed that the pH decreased and NDF and ADF degradation significantly increased from the inclusion of AFE in corn silage. The lactic acid, DM and crude protein content were also significantly higher ($p<0.05$) in the silage containing AFE. The Flieg score of the silage was also higher from AFE inclusion over the control silage at the end of the ensilage in our previous trial (Chiou et al., 2000). It appears that inclusion of AFE either directly or through silage will improve milk yield during the early lactation period. Inclusion of AFE directly into TMR in winter or adding AFE through corn silage during the summer season will result in a better lactating performance.

Milk composition

Effects of AFE inclusion in the diet significantly reduced the percent of milk fat during the summer season (A vs. BCD) ($p<0.05$). The effect of AFE inclusion on milk fat decrease was significantly more severe in the direct AFE inclusion in the TMR over the addition to the corn silage in summer ($p<0.05$).

Table 3. Effects of *Aspergillus oryzae* inclusion in the diet on protein and energy utilization of lactating dairy cows

Treatment	Basal diet	AFE in TMR	AFE in silage	AFE in silage & TMR	SEM	Orthogonal contrast		
	A	B	C	D		A vs. BCD	BC vs. D	B vs. C
Summer trial (May to July), kg/kg								
DM intake/milk yield	0.68	0.65	0.67	0.66	0.03	NS	NS	NS
DM intake/4% FCM	0.70	0.70	0.70	0.68	0.03	NS	NS	NS
NE intake/4% FCM	1.16	1.15	1.16	1.13	0.06	NS	NS	NS
CP intake/4% FCM	0.11	0.11	0.11	0.11	0.005	NS	NS	NS
CP intake/milk protein	3.19	3.15	3.36	3.34	0.14	NS	NS	**
Winter trial (December to February), kg/kg								
DM intake/milk yield	0.71	0.66	0.69	0.64	0.02	**	*	NS
DM intake/4% FCM	0.77	0.74	0.73	0.70	0.02	***	**	NS
NE intake/4% FCM	1.29	1.24	1.23	1.17	0.033	***	**	NS
CP intake/4% FCM	0.131	0.126	0.125	0.118	0.003	***	**	NS
CP intake/milk protein	4.06	3.85	3.76	3.63	0.09	***	*	NS

*,**,*** Mean the orthogonal comparison are significantly different at 0.05, 0.01 and 0.005, respectively.
Above data was calculated on feed intake and milk production without making an adjustment for the live-weight changes during the feeding trial.

Table 4. Effects of *Aspergillus oryzae* inclusion in the diet on live-weight changes and urea nitrogen in blood and milk of lactating cows

Treatment	Basal diet	AFE in TMR	AFE in silage	AFE in silage and TMR	SEM	Orthogonal contrast		
	A	B	C	D		A vs. BCD	BC vs. D	B vs. C
Summer trial (May to July), kg/kg								
Initial Wt, kg	592.14	549.58	509.33	565.75	31.00			
Wt change, kg/day	-0.19	0.60	0.11	0.03	0.19	*	NS	*
Live-weight changes in percentage, %								
4th/0 week	100.04	105.00	101.73	98.63	1.93	NS	*	NS
8th/4th wk	97.97	100.78	99.26	101.50	0.93	*	NS	NS
8th/0th wk	97.98	105.78	100.95	100.02	1.75	*	NS	NS
Milk urea N, mg/dL	6.85	7.34	7.77	7.63	0.44	***	NS	‡
Serum urea N, mg/dL								
4th week	10.69	11.68	12.45	12.48	0.73	‡	NS	NS
8th week	10.36	11.60	12.28	12.42	0.90	‡	NS	NS
Rectal Temp. °C								
4th week	40.04	40.36	40.17	40.03	0.11	NS	NS	NS
8th week	40.04	40.18	39.85	39.98	0.09	NS	NS	*
Winter trial (December to February)								
Initial Wt, kg	577.03	611.50	582.14	619.64	19.34			
Wt change, kg/day	0.21	0.22	0.53	0.22	0.16	NS	NS	NS
Live-weight changes in percentage, %								
4th/0 week	100.84	100.57	102.07	100.83	1.26	NS	NS	NS
8th/4th wk	101.05	101.17	102.43	101.29	1.11	NS	NS	NS
8th/0th wk	101.86	101.71	104.45	101.91	1.27	NS	NS	NS

*,**,*** Mean the orthogonal comparison are significantly different at 0.05, 0.01 and 0.005, respectively.
‡: Mean the orthogonal comparison is significantly different at 0.1.

The effects of AFE inclusion on the milk fat in winter on the other hand, was different. AFE inclusion did not significantly influence the milk fat percentage in winter. AFE inclusion in the corn silage however, significantly improved milk fat over the direct inclusion (B vs. C) ($p<0.05$).

The AFE inclusion however significantly influenced the milk fat yield in winter, but did not influence it in the summer season. AFE inclusion in corn silage in fact significantly increased milk fat yield over the direct addition of AFE in TMR in summer ($p<0.01$). AFE inclusion in the diet generally can stabilize ruminal pH, promote the growth of cellulose digesting bacteria, and hence increase the milk fat content. Sievert and Shaver (1993) showed that AFE inclusion improved milk fat in the high non-fibrous-carbohydrates (NFC) diet (42% NFC), but not in the low NFC diet (35%). Gomez-Alarcon et al. (1990) however, showed no improvement in milk fat from AFE inclusion in early and mid lactation cows on alfalfa hay. Harris et al. (1983) suggested a trend toward increased milk fat from AFE inclusion in corn silage. This agreed to our results in the summer trial.

The effects of AFE inclusion in the lactation diet was not significant on the increase in milk protein content ($p>0.05$), but significantly increased milk protein yield during the summer trial. In the winter trial AFE inclusion significantly increased the milk protein content ($p<0.05$) and yield ($p<0.005$). Direct inclusion of AFE into TMR significantly increased milk protein content as compared with adding AFE in the corn silage ($p<0.05$), but did not significantly influence the milk protein yield in the summer trial. During the winter trial however, inclusion of AFE in corn silage significantly increased the milk protein content ($p<0.005$) over direct inclusion into the TMR ($p<0.005$) and this result in contrary to the summer trial where the milk protein content decrease. The effects of double AFE inclusion, both through corn silage and directly into TMR, was significantly lowered milk protein content than either corn silage addition or TMR ($p<0.005$) in the winter trial.

The AFE inclusion in the diet also significantly influenced the percent of total milk solids content ($p<0.05$) in both the summer and winter trials. In the summer, AFE inclusion significantly lowered the total milk solids content ($p<0.005$); the AFE inclusion into TMR significantly decreased milk total solids yield over the AFE addition in silage ($p<0.05$). In winter, the AFE corn silage inclusion diet significantly increased the total milk solids content over the directly added AFE into TMR group ($p<0.005$). The double AFE inclusion group was significant lowest on the total solids content ($p<0.005$). These results indicated that directly adding AFE in the diet significantly decreased total milk solids. The depression effect of directly adding AFE on milk total solids was significantly alleviated through AFE inclusion into corn silage ($p<0.005$). Research data on the effects of AFE on the milk protein and total solids did not agree with each other in the literature. Denigan et al. (1992) fed alfalfa hay as forage to early and mid lactating cows and found no AFE affect upon the milk composition. With alfalfa hay and corn silage as a forage source, Bertrand and Grimes (1997) also showed similar results. Higginbotham et al. (1993) showed an increase in milk protein and total milk solids from the AFE inclusion in the mid-lactating cows fed corn silage and alfalfa hay as a source of forage. It appears that there are so many factors i.e., diet composition, method of AFE application, management, etc. that compound the effects of AFE on the milk composition, resulting inconsistent results.

Protein and energy efficiency

Table 3 presents the effects of AFE in the diet on protein and energy utilization in lactating dairy cows. The effects of AFE on protein and energy efficiency were not significant during the summer trial, except for protein intake per unit of milk protein. AFE inclusion in corn silage required significantly more protein intake to produce a unit of milk protein as compared to adding AFE directly into TMR ($p<0.05$) in the summer. AFE inclusion however, significantly gained live-weight ($p<0.05$) while the basal dietary group lost body weight (table 4). This live-weight increase with no adverse effect on the efficiency of DMI per unit of milk yield indicates an improvement in energy efficiency due to the AFE inclusion in the summer.

During the winter, inclusion of AFE utilized protein was significantly more efficient over the basal dietary group ($p<0.01$). Double inclusion of AFE through both corn silage and TMR showed an additive effect on nutrient utilization over addition to corn silage or TMR ($p<0.05$). Adding AFE through corn silage did not show any beneficial affect over the direct inclusion into TMR on the nutrient utilization in winter. It appears that AFE inclusion in the diet improve energy efficiency in summer and winter, and AFE either through corn silage or direct addition to TMR improved nutrient utilization in lactating cows during winter.

Serum urea nitrogen

Table 4 presents the effects of AFE inclusion on live-weight changes and urea nitrogen concentration in the blood and milk, and the rectal temperature. AFE inclusion in the corn silage significantly increased milk urea N over the group of basal diet ($p<0.005$). AFE inclusion in the diet also showed a trend toward increase in serum urea N over the control ($p<0.1$) in the 4th and the 8th weeks of feeding. The high soluble protein from AFE inclusion may contribute this increase in the milk and serum urea level. Our mini-silo trial showed that adding AFE to corn silage increased soluble protein (protein fraction B_1) and NPN (protein fraction A) in silage as compared to those without AFE inclusion (Chiou et al., 2000). These high soluble protein fractions may provide an excess amount of ruminal ammonia, which exceeds bacterial ammonia removal capability, resulting in high blood urea N concentration. Both Compos et al. (1990) and Wiedmeier et al. (1987)

showed an increase in deamination and protein digestibility in rumen from AFE inclusion in the diet. It appears that inclusion of AFE in the diet may increase blood and milk urea concentrations.

Rectal temperature

The effect of AFE inclusion on the rectal temperature of lactating cows was not significant at the 4th week, but was significant at the 8th weeks of feeding in the summer trial ($p<0.05$). It however showed a trend toward higher rectal temperatures in the AFE direct inclusion into TMR groups over the corn silage inclusion group ($p<0.1$) at the 4th week of feeding. AFE inclusion did not significantly influence rectal temperature as compared to the basal dietary group, but AFE inclusion through corn silage significantly lowered rectal temperature over direct AFE inclusion at the 8th weeks of feeding. Our result agreed with Bertrand and Grimes (1997) and Denigan et al. (1992) that AFE inclusion in the diet did not alleviate the high rectal temperature of lactating cows due to heat stress. Some research however showed a significant decrease in rectal temperature under heat stress from the AFE inclusion in the diet (Gomez-Alarcon et al., 1990; Higginbotham et al., 1993; Higginbotham et al., 1994). The increase in rectal temperature likely to be attributed to the increase in DM intake, fiber digestion and milk yield from increased fermentation heat in the rumen and an accelerated rate of metabolism in the body. Our data also showed a similar trend toward improvement in DM intake and milk yield from AFE inclusion in the summer. The data did not show a beneficial effect from AFE inclusion in the diet on the rectal temperature in the lactating cows. However, our data showed a significant alleviation of heat stress in lactating cows from the addition of AFE to corn silage over the direct inclusion into TMR at the end of the feeding trial.

It appears that adding AFE into the corn silage alleviated the adverse effects of heat stress on the DM intake in the lactating cows, but did not alleviate the high rectal temperature due to the heat stress. AFE inclusion, either directly or through silage, will improve milk yield during the early lactation period. AFE inclusion either directly into TMR in winter or through corn silage during the summer seasons will result in a better lactating performance. Inclusion of AFE in the diet may increase milk and blood urea concentration due to the increase in soluble protein. The increase in the rectal temperature may be attributed to the increase in DM intake, fiber digestion and milk yield from increased fermentation heat in the rumen and an accelerated rate of metabolism in the body.

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