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J Anim Sci 1991. 69:1733-1740.

INFLUENCE OF FEEDING *ASPERGILLUS ORYZAE* FERMENTATION EXTRACT ON THE MILK YIELDS, EATING PATTERNS, AND BODY TEMPERATURES OF LACTATING COWS[1]

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ABSTRACT

Trials were conducted to evaluate effects of a fermentation extract of *Aspergillus oryzae* (AO) on milk production and composition, diet digestibility, and rectal temperature changes in lactating dairy cows. Treatments were incorporated as a top dressing at the morning feeding and consisted of control (90 g/d of ground sorghum) or AO (3 g of culture + 87 g of ground sorghum daily). Twenty-four mid-lactation Holstein cows were paired for production in Lactation Trial 1 (LT-1). In Lactation Trial 2 (LT-2), 46 cows (20 primiparous and 26 multiparous) in early lactation were used. Trials lasted 12 wk. In LT-1, AO supplementation increased milk yields only at 2 ($P < .05$) and 8 wk ($P < .10$) of treatment. Rectal temperatures were lower ($P < .05$) for cows fed AO for 4 of 10 readings made during summer. Supplementation of AO culture in LT-2 (early lactation cows) increased milk production and feed efficiency ($P < .05$). Inner ear temperatures tended to be lower ($P < .11$) for cows fed AO. Digestion trials, conducted at the end of lactation trials, used Cr_2O_3 as an indigestible marker. In Digestion Trial 1, digestibilities were not significantly ($P > .10$) affected by AO supplementation. However, in Digestion Trial 2, AO increased ($P < .05$) digestibilities of DM, OM, CP, NDF, and ADF. Length and number of meals were not affected ($P > .10$) by feeding AO. In summary, milk yields, efficiency of milk production, and nutrient digestibilities were higher for early lactation cows fed a high-concentrate diet supplemented with 3 g of AO/d. Mid-lactation cows fed a lower-energy diet were less responsive to AO than early lactation cows, though similar trends were shown.

Key Words: *Aspergillus oryzae*, Dairy cows, Digestibility, Heat Stress, Body Temperature

J. Anim. Sci. 1991. 69:1733–1740

Introduction

Supplementation of *Aspergillus oryzae* (AO) increased DM digestibility of high-concentrate diets through enhanced fiber digestion (Van Horn et al., 1984; Weidmeier et al., 1987; Gomez-Alarcon et al., 1990). Moreover, higher fat-corrected milk yields resulted from feeding AO to lactating dairy cows (Harris et al., 1983; Marcus et al., 1986; Kellems et al., 1987).

The objective of this study was to further evaluate effects of AO on milk production and

[1]Arizona Agric. Exp. Sta. J. Article Number 7204. Partially supported by Biozyme Enterprises, Inc., St. Joseph, MO.

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Received June 18, 1990.

Accepted November 2, 1990.

milk composition, body temperatures, diet digestibility and eating patterns when 3 g of AO/d were added to diets of lactating cows at different stages of lactation.

Materials and Methods

Lactation Trial 1. Twenty-four Holstein cows in mid-lactation (averaging 145 d in milk) were used in an 84-d trial from July to September 1985. During a 10-d pretreatment period all cows were placed in experimental pens and fed a basal diet (Table 1) formulated to meet nutrient requirements (NRC, 1978).

Experimental pens housed 12 cows each in an open lot (600 m^2) and provided 48 m^2 of concrete flooring at feeders. Two shade areas in each pen measured 72 m^2, one was over feeders and the other in the middle of pens. Feeding was ad libitum through electronically controlled Calan gates[6] to monitor individual DMI. Cows were offered total mixed rations twice daily at 0600 and 1800 in sufficient quantity to ensure approximately 5% orts. Orts were recorded and amount of feed offered was adjusted daily. Water and trace mineralized salt blocks[7] were available at all times. Cows were milked twice daily at 0500 and 1700, and milk yields were recorded every milking.

Representative samples of milk from consecutive evening and morning milkings were collected weekly from each cow, composited, and analyzed by infrared procedures at the Arizona DHIA laboratory (Phoenix, AZ) for fat, protein, lactose, and total solids.

Cows were paired on the basis of pretreatment milk production and number of lactations and randomly assigned to two treatments, control (90 g ground sorghum grain/d) and AO (3 g AO[8]/d + 87 g ground sorghum grain/d). The experimental design was a continuous trial of 12 wk duration using pretreatment milk production for covariate adjustment of treatment means. Supplements were incorporated as top dressing to the morning feeding and seemed to be totally consumed by 1 h postfeeding. The C and AO cows were placed in adjacent pens to monitor group water consumption. The diet consisted of 50% forage and 50% concentrate (Table 1). Other measurements included weekly rectal temperatures, weekly respiration rates counted by using a wrist watch to measure lapsed time while breaths were counted, and BW (twice at the beginning and end of the trial and once every 14 d). Diets were sampled twice weekly and samples were composited every 2 wk for analyses of DM, OM, CP (AOAC, 1980) and NDF and ADF (Van Soest and Robertson, 1986). Estimates for net energy for lactation in diets were calculated from ingredients according to NRC (1989). Means for composited feed samples are reported in Table 1.

Statistical analyses were conducted following the BMDP (1985) procedures for the following model: $Y_{ijk} = \mu + T_i + Cov_j + E_{ijk}$. This model accounted for a treatment effect

TABLE 1. INGREDIENT AND NUTRIENT COMPOSITION OF DIETS USED IN LACTATION AND DIGESTION TRIALS 1 AND 2

Item	Lactation and digestion Trial 1	Lactation and digestion Trial 2
Ingredients, % of DM		
Alfalfa hay	50	25
Alfalfa cubes	—	10
Flaked barley	20	—
Shelled corn	10	—
Commercial concentrate[a]	—	46
Commercial protein mix[b]	20	—
Whole cottonseed	—	15
Cottonseed hulls	—	4
Nutrients		
DM, %	88.8	93.9
OM, % of DM	91.8	94.5
CP, % of DM	16.9	18.0
NDF, % of DM	37.9	43.1
ADF, % of DM	23.3	29.4
NE_L, Mcal/kg[c]	1.50	1.66

[a]Ingredients, percentage: rolled corn, 44; malt pellets, 25; almond hulls, 10; alfalfa pellet, 6; wheat mids, 5; cottonseed meal, 2.5; molasses, 2.5; dicalcium phosphate, 1.7; urea, 1.5; salt, .9; calcium carbonate, .6. Trace minerals, mg/kg: Mn, 54, Zn, 100, Fe, 84, Cu, 12, I, 1, Co, .4, Se, .5. Vitamin A, IU/kg, 10,000; vitamin D-3, IU/kg, 4,400; vitamin E, mg/kg, 4.

[b]Ingredients: cottonseed meal; ground milo; dehydrated alfalfa; wheat bran; urea; biophos (a mixture of monocalcium and dicalcium phosphates containing 17% Ca and 21% P.); trace minerals; vitamins A, D, and E (formula not provided).

[c]Net energy for lactation estimated from ingredient composition according to NRC (1989).

[6]American Calan Inc., Norwood, NH.

[7]Provided by Eagle Milling Co., Tucson, AZ.

[8]Trade name Amaferm, which is a fermentation extract of *Aspergillus oryzae* furnished by Biozyme Enterprises, St. Joseph, MO, produced under Patent No. 3,043,748, U.S. Patent Office, Washington, DC.

(T_i), a covariate effect (Cov_j), and random error (E_{ijk}). The covariate used in Lactation Trial 1 (LT-1) and Lactation Trial 2 (LT-2) was mean daily milk production observed during pretreatment.

Lactation Trial 2. Forty-six Holstein cows (20 primiparous and 26 multiparous) that calved between January and June 1987 were assigned to treatments 3 to 5 wk postpartum. Management was similar to that in Trial 1, except the diet consisted of 40% forage and 60% concentrate (Table 1) and cows were housed in pens in a random manner so group water intakes were not measured. At the end of 14 d of pretreatment, cows were paired by production level and lactation number and randomly assigned to the control or AO treatments. The experimental design was a continuous lactation trial of 12 wk duration, and pretreatment milk production was used for covariate adjustment of means.

Milk production, DMI, milk composition, feed composition, BW, and rectal temperatures were determined as in Trial 1. Statistical analyses were conducted with BMDP (1985) procedures for the following model: $Y_{ijkl} = \mu + T_i + L_j + TL_{ij} + Cov_k + E_{ijkl}$. This model accounted for the effects of the treatment (T_i), lactation number (L_j), treatment × lactation interaction (TL_{ij}), covariate (Cov_k), and random error (E_{ijkl}). Weekly measurements were also analyzed by the above models for their respective trials.

Digestion Trial 1. Twenty-two of the Holstein cows from LT-1 were used in Digestion Trial 1 (DT-1) to determine the effect of AO on nutrient digestibilities. Treatments, diets, and feeding management were as described for LT-1, except feeds and weighbacks were sampled daily. Throughout the 14-d digestion trial, diets were top-dressed with 24 g of Cr_2O_3/d in two 12-g doses. It was assumed that all the Cr_2O_3 was consumed because placement was directly on top of the feed at time of feeding, followed by a small amount of mixing of Cr_2O_3 with the top layer of feed. Fecal grab samples were collected twice daily at approximately 0600 and 1800 during the last 7 d of the trial and stored at –5°C until they were composited for the complete 7 d of collection. Samples of feeds, orts, and feces were dried at 55°C and ground through a Wiley mill (2-mm screen).

Chemical analyses included Cr_2O_3 by atomic absorption spectrometry using a multi-element hallow cathode lamp at 357.9 nm after redigestion with periodic acid, percentage of DM, OM, and CP by AOAC (1984), and NDF and ADF according to Van Soest and Robertson (1986).

Data were analyzed as a randomized block design using the procedures of Steel and Torrie (1980) because there was no logical covariate for these data. Blocks were based on cow pairs as assigned in LT-1. The statistical model was as follows: $Y_{ijk} = \mu + T_i + B_j + E_{ijk}$ and accounted for treatment effect (T_i), a block effect (B_j) and random error (E_{ijk}).

Digestion Trial 2. Thirty-four of the 46 cows from LT-2 were used in Digestion Trial 2 (DT-2). They were handled in three separate groups of 12, 10, and 12 each. Diet was the same as in LT-2.

A single dose of Cr_2O_3 (20 g/d) was given to each cow in a gelatin capsule at 0800 for 10 d. Fecal grab samples were collected once daily at 1000 for the last 5 d of the trial. Feed, orts, and fecal samples were collected and analyzed as in DT-1.

Data were analyzed as randomized blocks with blocks based on pairs as assigned in LT-2 in a 2 × 3 factorial arrangement of treatments (Steel and Torrie, 1980); factors were two levels of AO (0 and 3 g/d), and three groups of cows placed on treatment at different times.

Temperature Monitoring. Six cows receiving AO and their paired controls from LT-2 were selected randomly and fitted with tympanic temperature probes for continuous monitoring of body temperatures. Probes remained in the cows' ears for 10 d.

The temperature transmitters[9] were placed deep into the ear canal of cows and fixed in place with adhesive foam. Pulse interval modulation was used for maximizing transmission life. Data were collected by a receiver and sent to a computer terminal with calibration curves for each transmitter. Data were then summarized for each cow for 24 h and results were analyzed statistically (Steel and Torrie, 1980), similarly to the model used for DT-1.

Eating Patterns. The eating patterns of six cows receiving AO and their paired controls during LT-2 were evaluated by placing electronic interrupters on Calan gates. The cows were randomly selected and had been adapted

[9]Model TTE-IG, Stuart Enterprises, Grass Valley, CA.

TABLE 2. EFFECT OF *ASPERGILLUS ORYZAE* FERMENTATION EXTRACT ON LACTATION PERFORMANCE OF HOLSTEIN COWS[a]

Item	Trial 1[b]			Trial 2		
	C	AO	SEM	C	AO	SEM
	kg/d					
Pretreatment milk	27.2	27.8	—	37.1	37.9	—
Treatment milk	22.3	23.2	.70	37.3[c]	39.8[d]	1.10
Fat-corrected milk (3.5%)	20.5	21.4	.79	34.1[e]	35.9[f]	1.06
DMI	19.1	19.9	1.75	25.1	25.6	.71
Water intake	108.6	118.9	—	—	—	—
Kg milk/kg feed	1.19	1.18	.05	1.49[c]	1.57[d]	.03

[a]Covariate adjusted means: 12 wk duration, 12 cows/treatment in Trial 1, 23 cows/treatment in Trial 2.

[b]C = control; AO = *Aspergillus oryzae* fermentation extract (3 g/d).

[c,d]Means not sharing a common superscript are different ($P < .05$).

[e,f]Means not sharing a common superscript are different ($P < .10$).

to experimental treatments. The interrupters were connected to a receiver that recorded the time when a gate was open or closed. When gates were open for < 1 min, it was not considered a meal. When gates were closed for > 3 min, it was considered the end of a meal. Wiring of gates did not interfere with the gate function or animal behavior. The experimental period was 23 d. Statistical analyses were made by standard procedures (Steel and Torrie, 1980) using the model described for DT-1.

Results and Discussion

Cow performances in LT-1 and LT-2 are summarized in Table 2. Pretreatment milk yields were determined by analysis of variance procedures (Steel and Torrie, 1980) and found to be similar ($P > .10$) for control and AO groups. During treatment, cows fed AO in LT-1 produced more milk than controls at 2 ($P < .05$, 24.6 vs 22.7 kg/d) and 8 wk ($P < .10$, 21.7 vs 20.2 kg/d). Moreover, 3.5% fat-corrected milk yields, DMI, and feed efficiency were not affected ($P > .10$) by the treatment. Because water consumption was determined only for treatment groups, it could not be statistically evaluated, but means were about 10% higher for cows fed AO culture than for controls. This may have been due to a numerical increase in DMI.

In LT-2 no interaction ($P > .10$) between lactation number and treatment was detected for any item studied, so data for primiparous and multiparous cows within treatments were combined. Groups were again shown to be similar ($P > .10$) in milk production during pretreatment. However, during treatment, cows fed AO produced 7% more milk than controls ($P < .05$). Feed efficiency (kg milk/kg feed DM) also was higher for cows fed AO ($P < .10$). Higher milk yields in response to feeding

TABLE 3. EFFECT OF *ASPERGILLUS ORYZAE* FERMENTATION EXTRACT ON NUTRIENT DIGESTIBILITIES OF HOLSTEIN COWS

Item	Digestion Trial 1[a]			Digestion Trial 2		
	C	AO	SEM	C	AO	SEM
DMI, % of BW	2.95	2.99	.15	3.95[b]	4.20[c]	.08
Digestibility, %						
DM	77.6	79.4	1.52	64.0[b]	71.9[c]	2.10
OM	78.5	79.9	1.21	65.3[b]	72.9[c]	2.00
CP	81.2	82.9	1.36	70.5[b]	77.6[c]	1.90
NDF	68.4	70.5	2.34	50.7[b]	57.1[c]	2.10
ADF	62.4	63.7	3.12	40.3[b]	48.6[c]	2.80

[a]C, control; AO, *Aspergillus oryzae* fermentation extract.

[b,c]Means not sharing a common superscript are different ($P < .05$).

TABLE 4. EFFECT OF *ASPERGILLUS ORYZAE* FERMENTATION EXTRACT ON MILK COMPOSITION AND BW GAINS OF HOLSTEIN COWS[a]

Item	Trial 1[b]			Trial 2		
	C	AO	SEM	C	AO	SEM
Butterfat, %	2.97	2.93	.15	3.07	2.91	.10
Protein, %	3.15	3.20	.16	3.02	2.99	.03
Lactose, %	4.78	5.01	.10	4.99	5.03	.05
BW change, kg/d	.40	.44	.03	.24	.19	.02

[a]None of the differences was significant ($P > .10$).

[b]C, control; AO, *Aspergillus oryzae* fermentation extract.

AO have been reported elsewhere (Marcus et al., 1986; Walletine et al., 1986; Kellems et al., 1987), but in those trials feed intakes of individual cows were not determined.

Early lactation cows (LT-2) responded more to AO supplementation than those in mid-lactation (LT-1). This might have been related to the more highly concentrated diet fed cows in LT-2 vs LT-1 (Table 1). Williams and Newbold (1990) reported a response in milk yields to a *Saccharomyces cerevisiae* supplement in cows fed high vs low concentrate. Utah workers (Wallentine et al., 1986) demonstrated that high-producing cows in early lactation responded more to AO than lower producers in mid-lactation fed similar diets. Effects of fungal cultures at various production and dietary energy concentrations need clarification. Cows in LT-1 consumed about 3% of their BW as DM, in contrast to about 4% in LT-2 (Table 3). Waldo and Jorgensen (1981) calculated that variations in feed intake accounted for 50 to 75% of the differences in productivity of lactating cows.

Cows fed AO in LT-2 reached maximal DMI about 4 wk after treatment commenced, but control cows required 6 to 8 wk (data not shown). The faster increase in DMI may be explained by a greater stabilization in ruminal fluid pH (Frumholtz et al., 1989) or more rapid ruminal lactate uptake (Nisbet and Martin, 1989) in AO-treated vs untreated fermentations. Intakes were higher ($P < .05$) for cows fed AO than for controls in wk 4 and 5, respectively (26.4 vs 24.6 and 26.1 vs 24.4 kg/d; pooled SEM, .71). Thus, the AO cows required less time after parturition than controls to maximize intakes.

No differences ($P > .10$) were observed in any milk components due to treatment (Table 4). Increases in fat-corrected milk production by cows fed AO in other trials (Van Horn et al., 1984; Marcus et al., 1986; Wallentine et al., 1986; Kellems et al., 1987) resulted partially from an increased percentage of fat in milk.

In LT-1, weekly milk production (Figure 1) was consistently higher for the AO treatment, but the greatest differences in wk 2 ($P < .05$)

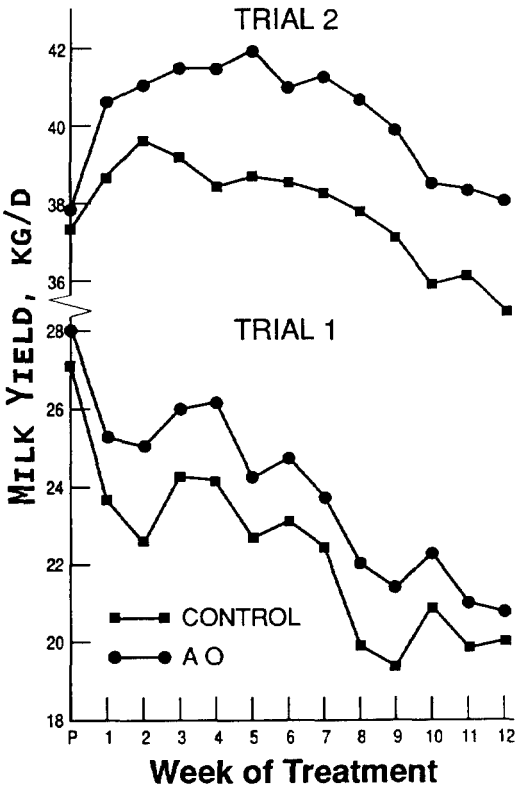

Figure 1. Weekly milk yields of cows fed diets containing 3 g/d of *Aspergillus oryzae*/d fermentation extract (AO) in two trials. Cows in Trial 1 commenced treatment an average of 145 postpartum and those in Trial 2 averaged 28 d. P = pretreatment. Pooled SEM:Trial 1, .7; Trial 2, 1.1.

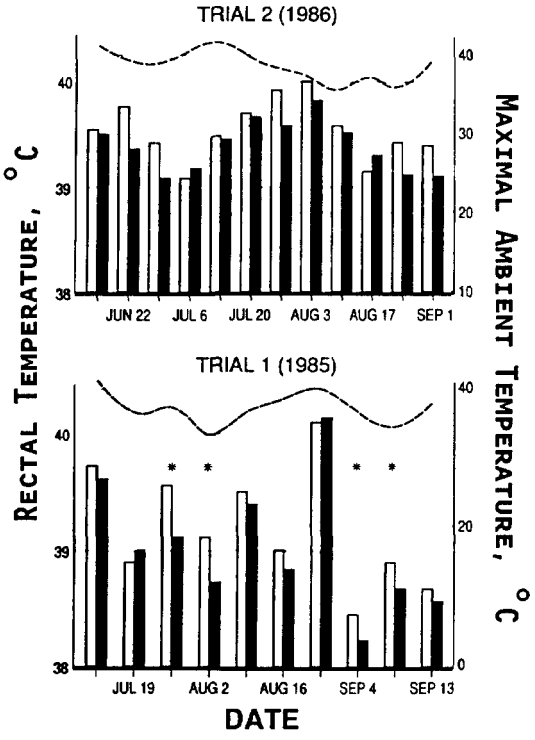

Figure 2. Rectal temperatures of cows fed 3 g/d of *Aspergillus oryzae* fermentation extract (■) or the control diet (□). Dashed line represents maximal ambient temperature; **P* < .05. Pooled SEM: Trial 1, .12; Trial 2, .15.

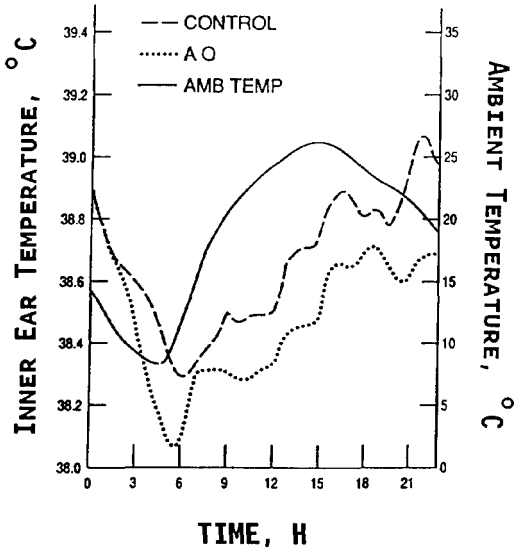

Figure 3. Effect of feeding *Aspergillus oryzae* fermentation extract (AO) on diurnal temperature patterns in the inner ear of lactating cows (mean of six cows/treatment for 7 d). *P* < .11. Pooled SEM, .17.

and 8 (*P* < .10) coincided with rises in ambient temperatures. Wallentine et al. (1986) reported that the largest production differences between controls and cows fed AO occurred when ambient temperatures were highest. Differences between treatments in weekly milk production for LT-2 (Figure 1) were of greater magnitude than in LT-1, and differences between treatments were significant (*P* < .10) for all weeks except 2, 10, and 11. In LT-1 and in the study of Wallentine et al. (1986), differences between treatments in weekly milk production diminished toward the end of the experimental periods. However, in LT-2, cows fed AO culture produced 6 to 7% more milk than controls throughout the entire treatment period.

Even though cows gained weight in both studies, treatments did not differ (*P* > .10) in BW gains (Table 4). Wallentine et al. (1986) reported that greater weight gains (270 vs 390 g/d) resulted from feeding AO compared with control diets.

Rectal temperatures and respiration rates (data not shown) averaged for all weeks of LT-1 were not different (*P* > .10) due to treatment. Means for AO and controls were 39.0 vs 39.2°C and 63 vs 67 breaths/min, respectively. A similar trend was shown for LT-2. When considering rectal temperatures in LT-1 for individual weeks, cows fed AO were lower (*P* < .05) for four of ten weekly determinations (Figure 2). Treatments did not differ (*P* > .10) for other weeks. In LT-2, which was conducted from January to September, rectal temperatures recorded before June 16 always averaged below 39°C, indicating thermal neutrality (Anderson, 1977).

Diurnal temperature curves determined in the inner ear were parallel for treatments. Cows fed AO averaged about .3°C less than cows fed C, except for early morning hours when ambient temperatures were lowest (Figure 3). This trial was conducted in late spring, and attempts to repeat the test during hotter weather failed due to malfunction of equipment. Reduced temperatures from feeding the AO culture were not always consistent in this study but have been observed previously (Huber and Higginbotham, 1985; Marcus et al., 1986). If the effect is real, it might have an etiology similar to that of the decrease in

temperature noted in cattle given *A. flavus* by Mertens (1977). Under thermal stress, thermoregulatory centers of the hypothalamus are more sensitive to drugs that cause hyper- and hypothermia than at moderate temperatures (Meyers, 1974). The 10% higher water consumption of treated cows in LT-1 might also have affected body temperatures, but we found no data in support of such a relationship. Further research is needed to define the role of fungal cultures in thermoregulation.

Digestion coefficients for all feed components in DT-1 did not differ ($P > .10$) between treatments (Table 3). Twice- instead of once-daily sampling of feces in DT-2 may have been preferable, as indicated by slightly higher SEM for DM, OM, and CP digestibilities in DT-2 than in DT-1, but resources prevented more frequent sampling because of the large number of cows per treatment. In DT-2, digestion coefficients for all components were lower than in DT-1 (Table 3) despite a higher proportion of concentrate in DT-2. This was probably caused by higher intakes by cows in DT-2 (4% of BW) than in DT-1 (3% of BW). Tyrrell and Moe (1975) suggested a decrease of 4% DM digestibility for every increment in maintenance intake.

In DT-2, feeding of AO culture resulted in increased ($P < .05$) digestibilities of DM, OM, CP, NDF, and ADF, despite higher intakes on the AO diet during the conduct of DT-2 compared with DT-1. These results are in agreement with other in vivo studies (Van Horn et al., 1984; Weidmeier et al., 1987; Gomez-Alarcon et al., 1990) and in vitro studies (Gomez-Alarcon, 1990).

Eating patterns showed no differences due to treatment in meals per day (9.0), total eating time (3.6 h), eating time per meal (24.4 min), or feed per meal (2.5 kg). Eating behavior was studied because cows fed AO achieved maximal intakes more rapidly after parturition and had less day-to-day variation in intakes than cows fed the control diet. However, no treatment differences ($P > .10$) in feed intake were observed during "eating pattern" observations, which may explain the lack of difference in eating behavior.

Implications

Results of this study suggest that *Aspergillus oryzae* fermentation extract positively affects milk production, but magnitude of response was greater in early than in mid-lactation. However, our data failed to separate diet and production level effects. The improved nutrient digestibilities observed from feeding *Aspergillus oryzae* fermentation extract to early lactation cows were associated with increased milk yields.

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