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Effect of feeding *Aspergillus oryzae* extract on milk production and rumen parameters

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Abstract

The objective of this study was to examine the effects of an *Aspergillus oryzae* extract on milk production and composition and rumen metabolites in a commercial dairy herd. Early lactation Holstein cows, 282, in their second or greater lactation were used in a 60-day trial from August to October 2001. Cows were assigned to one of two treatments in a switchback design with two 30-day treatment periods. Treatments were control (no additive) or 5 g/day of *A. oryzae* extract. Both groups were fed the same total mixed ration (TMR). *A. oryzae* extract was blended with the TMR and fed once daily. Feeding a culture of *A. oryzae* had no effect on yields of milk and 3.5% fat-corrected milk. Milk components were also unaffected by *A. oryzae* extract, but cows fed with the control diet had higher concentration and yield of milk true protein. Rumen pH, rumen concentrations of volatile fatty acids, and NH₃-N, as well as blood urea N, were similar for both treatment groups. **Feeding 5 g/day of *A. oryzae* extract to early lactation multiparous Holstein cows had no effect on lactation performance and rumen parameters.**

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1. Introduction

There is continued interest in the use of nonbacterial direct-fed microbials (DFM) as feed additives for lactating cows. The use of a DFM such as *Aspergillus oryzae* (Amaferm[®], Biozyme, St. Joseph, MO) has been shown to increase DM digestibility of high

concentrate diets through enhanced fiber digestion (Van Horn et al., 1984; Weidmeier et al., 1987; Gomez-Alarcon et al., 1990). The increased number of cellulolytic bacteria present in the rumen fluid and the more stable rumen environment when cows are fed *A. oryzae* might account for the increased fiber digestibility. Moreover, higher milk or fat-corrected milk yields and milk protein, and solids-not-fat percentages resulted from feeding Amaferm to lactating cows (Gomez-Alarcon et al., 1991; Kellems et al., 1990; Higginbotham et al., 1993).

Previous studies on feeding Amaferm to lactating cows used 3 g/day of an *A. oryzae* culture. When Yu

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et al. (1997) fed lactating dairy cows 3 g/day of a culture of *A. oryzae* in diets containing either steam-flaked or steam-rolled corn, they observed an increase in milk solids nonfat content and a tendency for higher milk protein content in cows fed *A. oryzae*. Denigan et al. (1992) evaluated the top dressing of a culture of *A. oryzae* to dairy cows at levels of 0, 1.5, 3, and 6 g/day in two lactation trials, and they observed that none of the levels of *A. oryzae* culture fed affected the performance of lactating cows. This is the only study that evaluated doses higher than 3 g/day of a culture of *A. oryzae* in the diet of high-producing lactating dairy cows.

The supplementation of a culture of *A. oryzae* incorporated into the total mixed ration (TMR) at a high dose rate has not been investigated with large number of high-producing cows fed high concentrate diets. The objective of this study was to determine the effects of feeding 5 g/day of a culture of *A. oryzae* on yields of milk and milk components and on rumen parameters of early lactation multiparous Holstein cows.

2. Materials and methods

2.1. Cows and management

The study was conducted on a 1500-cow commercial dairy farm located in the central valley of California. Multiparous Holstein cows ($n=282$) at 86 DIM (S.E.M. = ± 3.1) with an average lactation number of 2.90 (S.E.M. = ± 0.07) were randomly assigned to one of two treatments in a switchback design. The study was conducted from August to October 2001 and it lasted 60 days, with two 30-day treatment periods. Measurements were taken during the last week of the 30-day treatment period.

At the beginning of the study, cows were randomly assigned to be housed in one of the two study pens, and milk yield and DIM were similar for the two groups. The pens were identical in size, location, design, located just across each other, and housed the same number of animals. Both pens were located in the same free-stall barn and they had access to an open dirt exercise yard. Treatments were control (no additive) or 5 g/cow/day of a culture of *A. oryzae* incorporated into the TMR during the afternoon feeding. Cows were milked three times daily.

2.2. Experimental diets and feeding

A TMR containing on a DM basis 14.6% alfalfa hay, 7.2% alfalfa silage, 16.8% corn silage, 56.8% concentrate mix, and 4.6% mineral supplement was fed to all cows twice daily. The nutrient content of the diet offered (DM basis) was 1.68 Mcal NE¹/kg, 17.8% CP, 6.2% crude fat, 37.0% NFC, 30.6% NDF, 21.1% ADF, 37.3% UIP, and 8.4% ash. Cows fed *A. oryzae* extract received a daily premix containing 5 g of Amaferm mixed with the TMR once daily, in the afternoon feeding. Cows were fed for ad libitum intake with an expected 3–5% of the daily amount offered to be left as refusal the next day.

2.3. Milk sampling

Yields of milk were recorded by measuring milk weights from two consecutive milkings for 3 days during the last week of each period. At the same time, individual milk samples were collected from each milking, composited within each day, and analyzed for the concentration of fat, true protein, lactose, solids-not-fat (SNF), and SCC (Foss 303 Milk-O-Scan[®]; Foss Foods, Eden Prairie, MN) at the DHIA Laboratory in Fresno, CA.

2.4. Rumen and blood metabolites determination

In the last week of each period, rumen contents were sampled by rumenocentesis using 16-gauge, 14-cm needle, 2–3 h after the morning feeding from 20 cows selected randomly, which represented 10 cows/treatment in each period. Upon collection, a 5-ml aliquot of rumen fluid was acidified with 1 ml of 25% metaphosphoric acid and allowed to stand for 30 min. Rumen fluid was then centrifuged at $2000 \times g$ for 10 min. Supernatant was collected and frozen for later analysis of NH₃-N (Noel and Hambleton, 1975), and for the analysis of VFA by GLC (Baker, 1966). At the same time of rumen sampling, blood was sampled by puncture of the coccygeal vein or artery using Vacutainer serum tubes (Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed on ice and transported to the laboratory within 6 h of collection. Blood was centrifuged at $2000 \times g$, and serum was frozen at -5°C until analysis of urea N (Marsh et al., 1957).

2.5. Experimental design and statistical analysis

The study was conducted following a switchback design using two pens in the same free-stall barn. Within each of the 30-day period, each pen was assigned to one of the two treatment diets, with treatments being switched at the end of the first 30-day period.

Within each period, data collected during the last week of treatment were averaged into a single mean and then analyzed by ANOVA using the GLM procedure of SAS (2001) with observed mean, treatment, period, and interaction between treatment and period. Treatment differences with $P \leq 0.05$ were considered significant, and $0.05 < P \leq 0.15$ were considered a trend.

3. Results

Yields of milk were not different between the two treatment groups, and they averaged 47.6 and 46.8 kg/

Table 1
Effect of feeding yeast culture on lactation performance of dairy cows

	Treatment ^a		S.E.M.	Effect ^b		
	Control	AO		TRT	Period	TRT × period
	(P <)					
Milk yield, kg/day	47.6	46.8	0.45	0.17	0.01	0.01
3.5% FCM, kg/day	44.0	44.1	0.35	0.88	0.01	0.84
Milk fat %	3.16	3.19	0.03	0.41	0.01	0.001
Yield, kg/day	1.50	1.48	0.02	0.52	0.01	0.94
Milk true protein %	3.14	3.06	0.01	0.001	0.08	0.001
Yield, kg/day	1.50	1.43	0.03	0.001	0.001	0.41
Lactose, %	4.71	4.72	0.01	0.62	0.001	0.94
SNF, %	8.77	8.73	0.02	0.24	0.08	0.01
SCC, × 10 ³ /ml	254.6	256.6				
Linear SCC	3.29	3.11	0.10	0.21	0.78	0.01

^a AO = *Aspergillus oryzae* extract.

^b TRT = treatment; TRT × period = interaction between TRT and period.

Table 2

Effect of feeding yeast culture on rumen and blood parameters of dairy cows

	Treatment ^a		S.E.M.	Effect ^b		
	Control	AO		TRT	Period	TRT × period
	(P <)					
VFA, ^c mmol						
Total	126.9	124.4	7.10	0.81	0.86	0.92
Acetic	77.6	76.4	4.09	0.84	0.95	0.69
Propionic	32.6	31.9	2.09	0.82	0.89	0.46
Butyric	13.4	12.8	0.88	0.66	0.26	0.45
Isovaleric	1.54	1.45	0.10	0.49	0.08	0.64
Valeric	1.90	1.90	0.16	0.99	0.68	0.79
Acetic/propionic	2.43	2.43	0.06	0.99	0.60	0.01
Blood urea-N, mg/dl	20.6	20.6	0.86	0.97	0.18	0.01
Rumen pH	5.85	6.02	0.10	0.21	0.001	0.77
Rumen NH ₃ -N, mg/dl	16.38	15.85	1.13	0.74	0.01	0.01

^a AO = *Aspergillus oryzae* extract.

^b TRT = treatment; TRT × period = interaction between TRT and period.

^c VFA = volatile fatty acids.

day for controls and *A. oryzae* extract fed cows. Similarly, daily production of 3.5% fat-corrected milk did not differ between the treatment groups and they averaged 44.1 kg/day (Table 1).

Compared to control cows, feeding 5 g/day of a culture of *A. oryzae* had no effect on content and yield of milk fat. Similarly, lactose and SNF content of milk were similar between the two treatment groups. However, cows fed *A. oryzae* extract produced milk with a lower true protein content, which resulted in a lower milk protein yield. Interaction between treatment and period for yields of milk and concentrations in milk of fat, true protein, SNF, and linear SCC indicates that responses of cows to treatments varied with period. It is not clear to us why responses to treatments differed with period, mainly considering that a switchback design was utilized to eliminate any possible pen grouping effect.

Blood urea N, rumen pH and rumen concentration of VFA and NH₃-N were unaffected by treatment (Table 2).

4. Discussion

4.1. Milk production and composition

Gomez-Alarcon et al. (1991) reported that cows in early lactation consuming high concentrate diets had increased DMI and milk yields when fed *A. oryzae* extract. In addition, Kellems et al. (1990) observed the greatest effect of feeding *A. oryzae* extract during the early stages of the lactation cycle. Although cows in the current study were in early lactation and consuming a high concentrate diet, only milk protein content and yield were affected, and no other changes in lactation performance occurred when a culture of *A. oryzae* was added to the diet. Results from the current study confirm a previous report by Denigan et al. (1992) that failed to show increases in milk yields when *A. oryzae* extract was fed at levels higher than 3 g/day.

No difference was observed between treatments for any of the milk constituents except for milk true protein being higher for cows receiving the control diet. Previous reports (Denigan et al., 1992; Huber et al., 1994; Yu et al., 1997) have shown little or no differences in responses in milk components when feeding *A. oryzae* extract including milk protein. Higginbotham et al. (1993) reported higher milk protein and SNF percentages in cows fed *A. oryzae*. Williams and Newbold (1990) proposed that the addition of *A. oryzae* increases energy supply to the lactating cow by stabilizing the ruminal environment and increasing fiber digestion, thus increasing the energy available from the diet for productive purposes. These effects can potentially improve DM intakes. Huber et al. (1994) summarized 17 comparisons in which *A. oryzae* extract was fed to lactating dairy cows, and they concluded that DM intake was similar between controls and cows receiving *A. oryzae*. Differences in feed components among trials may contribute to the differences in response to *A. oryzae* feeding.

4.2. Rumen and blood metabolites

The current study failed to detect differences in serum urea N, rumen VFA, or rumen $\text{NH}_3\text{-N}$ concentrations. Higginbotham et al. (1993) found that *A. oryzae* significantly reduced blood urea N concentra-

tions. Previous reports (Fondevila et al., 1990; Newbold et al., 1990) on fungal cultures have shown that yeast cultures, but not *A. oryzae* products, reduced the concentrations of rumen $\text{NH}_3\text{-N}$. It was suggested that the improved rumen fermentation might be responsible for the lower concentrations of $\text{NH}_3\text{-N}$ in the rumen fluid as a consequence of improved nitrogen utilization by rumen microorganisms. Gomez-Alarcon et al. (1990) found that *A. oryzae* supplementation increased rumen and total tract digestibility of fiber fractions, but rumen VFA and $\text{NH}_3\text{-N}$ concentrations were not affected. Because *A. oryzae* has proteolytic activity (Arambel et al., 1987), rumen $\text{NH}_3\text{-N}$ concentrations might increase with the use of *A. oryzae* when digestion of carbohydrates is not improved and asynchrony of energy and N availability in the rumen occurs.

Rumen pH was not affected by feeding a culture of *A. oryzae*. Stabilization of rumen pH is reported to be an important property of direct fed microbials, and that is usually an argument to justify improvements in fiber digestion. Frumholtz et al. (1989) using the rumen simulation technique (Rusitec) observed a reduction in the post-feeding pH drop with *A. oryzae* supplementation. Rumen pH is controlled by the concentrations of the commonly found VFA, in association with the presence of lactate. Since no differences in total VFA concentrations in the rumen fluid were observed for cows fed *A. oryzae* compared to cows fed the control diet in the present study, changes in rumen pH would not be expected. Martin (1990) proposed that changes in rumen pH when direct feed microbials were fed might be related to lactate availability in the rumen. Because no changes in rumen pH were observed for cows in the current study, it is unlikely that lactate concentrations in the rumen fluid differed between cows fed *A. oryzae* or the control diet.

5. Conclusion

Addition of an *A. oryzae* extract to a dairy diet high in concentrate ingredients did not have significant effects on production of milk or milk components with the exception of decreased milk true protein percentage and yield. Similarly, feeding *A. oryzae* extract at 5 g/cow/day did not affect rumen parameters

and serum concentrations of urea N. Our findings do not support the benefits of feeding *A. oryzae* extract in the diet of lactating dairy cows.

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References

- Arambel, M.J., Wiedmeier, R.D., Walters, J.L., 1987. Influence of donor animal adaptation to added yeast culture and/or *Aspergillus oryzae* fermentation extract on in vitro rumen fermentation. *Nutr. Rep. Int.* 35, 433–436.
- Baker, R.A., 1966. Volatile fatty acids in aqueous solution by gas liquid chromatography. *J. Gas Chromatogr.* 4, 418–419.
- Denigan, M.E., Huber, J.T., Alhadhrami, G., Al-Dehneh, A., 1992. Influence of feeding varying levels of Amaferm® on performance of lactating dairy cows. *J. Dairy Sci.* 75, 1616–1621.
- Fondevila, M., Newbold, C.J., Hotten, P.M., Ørskov, E.R., 1990. A note on the effect of *Aspergillus oryzae* fermentation extract on the rumen fermentation of sheep given straw. *Anim. Prod.* 51, 422–425.
- Frumholtz, P.P., Newbold, C.J., Wallace, R.J., 1989. Influence of *Aspergillus oryzae* fermentation extract on the fermentation of a basal ration in the rumen simulation technique (Rusitec). *J. Agric. Sci. Camb.* 113, 169–172.
- Gomez-Alarcon, R.A., Dudas, D., Huber, J.T., 1990. Influence of *Aspergillus oryzae* on rumen and total tract digestion of dietary components. *J. Dairy Sci.* 73, 703–710.
- Gomez-Alarcon, R.A., Huber, J.T., Higginbotham, G.E., Wiersma, F., Ammon, D., Taylor, B., 1991. Influence of feeding an *Aspergillus oryzae* culture on the milk yields, eating patterns, and body temperatures of lactating cows. *J. Anim. Sci.* 69, 1733–1740.
- Higginbotham, G.E., Bath, D.L., Butler, L.J., 1993. Effect of feeding an *Aspergillus oryzae* extract on milk production and related responses in a commercial dairy herd. *J. Dairy Sci.* 76, 1484–1489.
- Huber, J.T., Higginbotham, G., Gomez-Alarcon, A., Taylor, R.B., Chen, K.H., Chan, S.C., Wu, Z., 1994. Heat stress interactions with protein, supplemental fat, and fungal cultures. *J. Dairy Sci.* 77, 2080–2090.
- Kellems, R.O., Lagerstedt, A., Wallentine, M.V., 1990. Effect of feeding *Aspergillus oryzae* fermentation extract or *Aspergillus oryzae* plus yeast culture plus mineral and vitamin supplement on performance of Holstein cows during a complete lactation. *J. Dairy Sci.* 73, 2922–2928.
- Marsh, W.B., Fingerhut, B., Kirch, E., 1957. Determination of urea nitrogen with diacetyl method and an automatic dialyzing apparatus. *Am. J. Clin. Pathol.* 28, 681–688.
- Martin, S.A., 1990. Nutrient transport by ruminal bacteria: potential for manipulating the rumen fermentation? Service Through Science Biozyme® Technical Symposium, March 14, Fresno, CA.
- Newbold, C.J., Williams, P.E.V., McKain, N., Walker, A., Wallace, R.J., 1990. The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. *Prod. Nutr. Soc.* 49, 47A (Abstract).
- Noel, R., Hambleton, L.G., 1975. Collaborative study of a semi-automated method for the determination of crude protein in animal feeds. Presented at the 89th Annual meeting of the AOAC, Washington, DC.
- SAS, 2001. SAS/STAT® User's Guide (Release 8.2). SAS Inst., Cary, NC.
- Van Horn, H.H., Harris, B., Taylor, M.J., Bachman, K.C., Wilcox, C.J., 1984. By-product feeds for lactating dairy cows: effects of cottonseed hulls, corrugated paper, peanut hulls, sugarcane bagasse, and whole cottonseed with additives of fat, sodium bicarbonate, and *Aspergillus oryzae* product on milk production. *J. Dairy Sci.* 67, 2922–2938.
- Weidmeier, R.D., Arambel, M.J., Walters, J.L., 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. *J. Dairy Sci.* 70, 2063–2068.
- Williams, P.E.V., Newbold, C.J., 1990. Rumen probios: the effects of novel microorganisms on rumen fermentation and ruminant productivity. In: Haresign, W., Cole, D.J.A. (Eds.), *Recent Advances in Animal Nutrition*. Butterworths, London, England, p. 211.
- Yu, P., Huber, J.T., Theurer, C.B., Chen, K.H., Nussio, L.G., Wu, Z., 1997. Effect of steam-flaked or steam-rolled corn with or without *Aspergillus oryzae* in the diet on performance of dairy cows fed during hot weather. *J. Dairy Sci.* 80, 3293–3297.