INFLUENCE OF DIRECT-FED MICROBIALS ON RUMINAL MICROBIAL FERMENTATION AND PERFORMANCE OF RUMINANTS : A REVIEW[1]

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Summary

Direct-fed microbials (DFM) have been used to enhance milk production in lactating cattle and to increase feed efficiency and body weight gain in growing ruminants. Primary microorganisms that have been used as DFM for ruminants are fungal cultures including *Aspergillus oryzae* and *Saccharomyces cerevisiae* and lactic acid bacteria such as *Lactobacillus* or *Streptococcus*. Attempts have been made to determine the basic mechanisms describing beneficial effects of DFM supplements. Various modes of action for DFM have been suggested including : stimulation of ruminal microbial growth, stabilization of ruminal pH, changes in ruminal microbial fermentation pattern, increases in digestibility of nutrients ingested, greater nutrient flow to the small intestine, greater nutrient retention and alleviation of stress, however, these responses have not been observed consistently. Variations in microbial supplements, dosage level, production level and age of the animal, diet and environmental condition or various combinations of the above may partially explain the inconsistencies in response. This review summarizes production responses that have been observed under various conditions with supplemental DFM and also corresponding modification of ruminal fermentation and other changes in the gastrointestinal tract of ruminant animals.

(**Key Words** : Direct-Fed Microbials, Mode of Action, Ruminal Fermentation, Production Response, Ruminant)

Introduction

Manipulation of the ruminal microbial ecosystem to maximize production efficiency by ruminants has been a challenge to ruminant nutritionists and rumen microbiologists. Various attempts have been made to optimize ruminal fermentation using methane inhibitors (halogenated methane analogues, sulphite, nitrate, etc.), propionate enhancers (monensin, lasalocid, salinomycin, avoparcin and other antibiotics), microbial growth factors (niacin, thiamin, and branched chain volatile fatty acids) and also by dietary modifications. Growing concern regarding the use of antibiotics and other growth stimulants in the animal feed industry has increased interest in evaluating the effects of microbial additives on animal performance.

Various terminology has been used to describe microorganisms supplemented in the ruminant diet. Probiotic, a word coined by Parker (1974), has been more clearly defined by Fuller (1989) as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". In the United States, the term probiotic has been used to refer to viable microbial cultures, enzyme preparations, culture extracts or various combinations of the above. Because of the confusion surrounding multiple definitions of the term probiotic, the US Food and Drug Administration (FDA) in 1989 required manufacturers to use the term direct-fed microbial (DFM) instead of probiotic (Miles and Bootwalla, 1991). The FDA defines DFM as "a source of live (viable) naturally-occurring microorganisms". The term DFM will be used throughout this review. Primary microorganisms that have been used as DFM for ruminants are fungal cultures including *Aspergillus oryzae* (AO) and *Saccharomyces cerevisiae* (SC) and lactic acid bacteria such as *Lactobacillus* or *Streptococcus*.

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Microbial cultures or culture extracts of various types have been used in three primary ways that affect ruminants. First, DFM has been used as an additive for silage or haylage or as a preservative for hay. Secondly, DFM have also recently been used to replace or reduce the use of antibiotics in stressed cattle. Finally, there is interest in using DFM to enhance milk production in dairy cows and to increase feed efficiency and body weight gain in beef cattle. Basic mechanisms to explain any beneficial effects of microbial supplements are not clearly understood. Therefore, the application of DFM is not based on well defined principles and optimal conditions for their use have not been determined. This review summarizes production responses observed with supplemental DFM and corresponding modification of ruminal fermentation and other changes in the gastrointestinal tract of ruminant animals. Other reviews on the use of DFM in animal production are available (Sandine, 1979; Sissons, 1989; Hutcheson, 1991; Martin and Nisbet, 1992; Dawson, 1993).

Fungal Cultures

1. History

Since Eckles and Williams (1925) published a report on the use of yeast as a supplementary feed for lactating cows, brewers yeast has been successfully used as a protein source in ruminant diets (Carter and Phillips, 1944; Steckley et al., 1979). Ethanol is one of the major fermentation products of SC which occasionally has led to ethanol toxicity when high levels of live yeast have been included in the diet. Toxicity can be avoided by using dead yeast (Ingledew and Jones, 1982; Bruning and Yokoyama, 1988). The application of low levels of yeast (< 1% of dietary DM) to dairy cow diets first received attention in the 1940's and 1950's. Renz (1954) reported that the inclusion of 50 g/d of an active yeast culture increased milk yield by 1.1 kg/d. Beeson and Perry (1952) reported a 6% increase in the daily gain of steers fed 8 g/d of active dried yeast. Ruf et al. (1953) suggested that yeast may beneficially alter ruminal fermentation, while Leatherwood et al. (1960) reported that an enzyme preparation prepared from *Aspergillus niger* enhanced *in vitro* cellulose digestion by ruminal microbes. However, no *in vivo* effect was observed for growth of steers or total tract DM, cellulose and CP digestion by sheep when the enzyme preparation was supplemented to the diet. Evaluation of fungal culture in many other studies reported little or no increase in production (Norton, 1945; Lassiter et al., 1958). Recently, the use of fungal cultures in ruminant diets to improve the health and productivity of animals has received renewed attention. Two types of fungal cultures, *Aspergillus oryzae* and *Saccharomyces cerevisiae*, have been examined. In most cases, products contain live cells plus growth medium. However, there are considerable differences in both the number of live cells and the nature of the growth medium among products. Products containing yeast and bacterial cultures are also available (Hoyos et al., 1987; Dawson et al., 1990).

2. Production responses

1) *Saccharomyces cerevisiae*

The official definition by AAFCO (1991) of yeast culture is "a dry product composed of yeast and the media on which it was grown, dried in such a manner as to preserve the fermenting capacity of the yeast. The media must be stated on the label". Production responses to *Saccharomyces cerevisiae* are summarized in tables 1 and 2.

Milk production and composition

Increases in response to yeast cultrue have been reported in lactating dairy cows (Hoyos et al., 1987; Harris and Webb, 1990; Williams et al., 1991) with an average increase in fat corrected milk yield of 2.5 kg/d. Improved milk production was also reported by Teh et al. (1987) in lactating goats fed a diet supplemented with 2.5 % yeast culture compared with those fed a control diet (3.25 vs. 2.66 kg/d). The magnitude of response was dependent on the stage of lactation. Wohlt et al. (1991) reported that cows fed supplemental yeast peaked earlier and had a higher average milk yield through wk 18 of lactation compared with control cows. Harris and Lobo (1988) demonstrated that cows receiving yeast culture produced higher FCM and milk fat percent and lower milk protein percent in early lactating cows (average 104 DIM), although no significant differences were found in mid lactation cows (average 170 DIM). In another study, Harris and Webb (1990) confirmed higher FCM yield and milk fat percent but also found a higher milk protein percent in early lactating cows (average 102 DIM) fed yeast culture.

Milk production responses to yeast culture may be dependent on the level of production. Hoyos et al. (1987) examined the effect of yeast culture in cows with different production levels. Treatment groups in both high and low production levels were fed supplements containing *Saccharomyces cerevisiae, Streptococcus faecium* and *Lactobacillus acidophilus*. Milk production increased in the yeast culture supplemented cows in the high

TABLE 1. EFFECTS OF *Saccharomyces cerevisiae* ON DRY MATTER INTAKE, MILK YIELD AND COMPOSITION IN LACTATING DAIRY COWS

Reference	Supplement[1]		DM intake[2]	Milk[2]				Diet[3]	DIM[4]	Comments on SC effects
	g/d	% of DM	kg/d	yield, kg/d	FCM, kg/d	protein, %	fat, %			
Hoyos et al., 1987	0(30)			30.9				High producer		Increase in milk yield (high producer) and milk fat percent.
	+(30)			32.8(+1.9)			(+19.4%)	High producer+SC		
	0(30)			22.3				Low producer		
	+(30)			22.3(0)			(+14.0%)	Low producer+SC		
Harris and Lobo, 1988	0(150)		20.9	30.4	26.6	3.17	3.20	cirb sukage/wet brewers grains /concentrate	170	
	110(150)		21.4(+.5)	30.7(+.3)	27.6(+1.0)	3.13(−.04)	3.33(+.13)			
Erdman and sharma, 1989		0(5)	19.6	26.3	23.2	3.44	3.42	40% corn silage:60% concentrate (corn/soybean meal)	154	A tendency for increased milk protein percent.
		1(5)	19.0(−.6)	25.6(−.7)	22.5(−.7)	3.50(+.06)	3.46(+.04)			
Arambel and Kent, 1990	0(10)		21.9	37.9	36.3	2.97	3.33	alfalfa hay/haylage/ corn silage/rolled barley/whole cottonseed/brewers dried grains/beet pulp/extruded soybean meal/beet molasses	65	
	90(10)		21.8(−.1)	36.5(−1.4)	35.5(−.8)	2.94(−0.3)	3.37(+.04)			
Harris and Webb, 1990	0(135)		26.8	30.9	29.9	3.10	3.27	Corn silage/corn meal/wet brewers grains/whole cottonseed/protein-mineral concentrate	102	Increase in FCM, milk fat and protein percent.
	+(134)		25.9(−.9)	31.7(+.8)	31.4(+1.5)	3.15(+.05)	3.41(+.14)			
Williams et al., 1991	0(4)		15.7	22.5	21.7	3.12	3.78	50C:50F straw	wk 1-12	Greatest improvement in intake and milk yield on 60C:40F hay diet.
	10(4)		16.5(+.8)	21.5(−1.0)	21.2(−.5)	3.22(+.10)	3.81(+.03)	50C:50F straw+SC		
	0(4)		18.1	23.4	21.1	3.57	3.44	50C:50F hay		
	10(4)		18.8(+.7)	23.3(−.1)	20.6(−.5)	3.36(−.21)	3.35(−.09)	50C:50F hay+SC		
	0(4)		17.8	23.3	19.4	3.31	3.19	60C:40F straw		
	10(4)		18.7(+.9)	23.5(+.2)	22.8(+3.4)	3.43(+.12)	3.66(+.47)	60C:40F straw+SC		
	0(4)		17.3	23.3	21.5	3.39	3.45	60C:40F hay		
	10(4)		19.6(+2.2)	27.4(+4.1)	24.5(+3.0)	3.54(+.15)	3.26(−.19)	60C:40F hay+SC		

TABLE 1. (CONTINUED)

Reference	Supplement[1]		DM intake[2]	Milk[2]				Diet[3]	DIM[4]	Comments on SC effects
	g/d	% of DM	kg/d	yield, kg/d	FCM, kg/d	protein, %	fat, %			
Wohlt et al. 1991	0(12)		19.2	26.0		3.23	3.96	corn silage/corn/ soybean meal/oats/ distillers grains/ molasses/wheat bran/wheat middings/beet pulp	wk 1-18	Earlier and higher peak milk yield.
	10(12)		18.5(−.7)	27.2(+1.2)		3.12(−.11)	3.91(−.05)			
Alikhani et al. 1992	0(6)		17.8	27.6		3.08	3.24	alfalfa silage		
	+(6)		18.7(+.9)	27.2(−.4)		3.07(−.01)	3.23(−.01)	alfalfa silage+SC		
	0(6)		24.4	30.3		3.12	3.00	alfalfa hay		
	+(6)		24.1(−.3)	30.7(+.4)		3.18(+.06)	2.83(−.17)	alfalfa hay+SC		
Erasmus et al. 1992	0(3)		21.8	18.9		3.41	3.19	65C:35F	56-94	Increase in DM intake.
	10(3)		23.2(+1.4)	20.1(+1.2)		3.38(−.03)	3.19(0)			
Harris et al. 1992	0(54)		22.0	25.3		3.12	3.43	corn silage/corn meal/soybean hulls/soybean meal/fish meal/urea	Early to mid lactation	
	57(54)		22.9(+.9)	26.5(+1.2)		3.03(−.09)	3.38(−.05)			
Smith et al., 1993	0(60)		26.0	22.0	21.9	3.22	3.53	50C:50F		Decrease in DM intake.
	10(48)		24.8(−1.2)	23.6(+1.6)	22.7(+.8)	3.03(−.19)	3.27(−.26)			
Swartz et al. 1994	0			31.8	32.8	3.15	3.69	Corn silage/legume silage/alfalfa hay/corn /oats/soybean meal	120	360 lactating Holstein cows from 7 farms
	5.3×10^{10} CFU/d			31.8(0)	32.4(−.4)	3.12(−.03)	3.67(−0.2)			
	5.1×10^{10} CFU/d			31.6(−.2)	32.4(−.4)	3.17(+.02)	3.76(+.07)			

[1] Numbers in parenthesis represent the number of animals.

[2] Numbers in parenthesis represent the differences between control and treatment.

[3] 50C:50F = 50% concentrate:50% forage, SC = yeast culture.

[4] Days in milk.

TABLE 2. EFFECTS OF *Saccharomyces cerevisiae* ON DRY MATTER INTAKE, WEIGHT GAIN AND FEED EFFICIENCY IN GROWING RUMINATNTS

Reference	Animal	Supplement[1]		DM intake[2]	Weight gain[2]	Feed conversion[2]	Diet[3]	Age	Comments
		g/d	% of DM	kg/d	kg/d	feed/gain			
Adams et al., 1981	Steers		0 (5)	10.1	1.34	6.42	50C:50F		
			1.85(5)	11.3(+1.2)	1.39(+.05)	6.32(+1.6%)			
	Lambs		0 (4)	7.7					
			1.85(4)	7.0(−.7)					
Phillips and VonTungeln, 1985	Calves		0 (14)	5.5	0.54	10.2	corn/cottonseed hulls/ soybean meal/molasses/urea	8 month	
			1 (14)	5.9(+.4)	0.86(+.32)	6.9(+32%)			
			2 (13)	6.5(+1.0)	0.80(+.26)	8.1(+21%)			
Fallon and Harte, 1987	Calves	0(20)		1.20	0.63	1.90	barley/soya		Improvement in DM intake on barley/ soya diet.
		+(20)		1.35(+.15)	0.75(+.12)	1.80(+5.3%)	barley/soya + SC		
		0(20)		1.34	0.64	2.09	corn gluten/barley		
		+(20)		1.34(0)	0.68(+.04)	1.97(+5.7%)	corn gluten/barley + SC		
Hughes, 1988	Calves	0(16)		2.08	1.75	1.19		1 wk	
		2(16)		2.29(+.21)	2.00(+.25)	1.15(+3.4%)			
Edwards et al., 1990	Bulls		0 (14)	5.32	1.55	3.44	barley/soybean meal		
			0.15(14)	5.55(+.23)	1.58(+.03)	3.53(−2.6%)			
Wagner et al., 1990	Calves		0 (10)	2.00	0.86	2.31	corn	3 wk	
			0.1 (12)	1.82(−.18)	0.79(−.05)	2.29(+.9%)	corn + SC		
			0 (9)	1.81	0.71	2.54	wheat		
			0.1 (12)	1.87(+.06)	0.76(+.05)	2.44(+3.9%)	wheat + SC		
Edwards et al., 1991	Steers	0(6)		7.42	1.22	6.07	barley/soybean meal/silage		
		10(6)		7.49(+.07)	1.38(+.16)	5.44(+10.4%)			
Cole et al., 1992	Calves		0 (54)	4.55	1.34	3.39	cottonseed hulls/corn/alfalfa /cottonseed meal/molasses		
			0.75(54)	4.53(−.02)	1.41(+.07)	3.21(+5.3%)			
Mutsvangwa et al., 1992	Bulls	0(13)		5.32	1.55	3.43	barley/soybean meal/ molasses	3 month	
		10(13)		5.55(+.23)	1.58(+.03)	3.51(−2.3%)			
Quigley et al., 1992	Calves		0 (9)	0.97	0.33	2.94	corn/soybean meal/soybean hulls/wheat middings/ animal fat/molasses	12 wk	
			0.2 (9)	0.96(−.01)	0.34(+.01)	2.82(+4.1%)			

[1] Numbers in parenthesis represent the number of animals used in the study.
[2] Numbers in parenthesis represent the differences between control and treatment.
[3] 50C:50F = 50% concentrate:50% forage; SC = yeast culture.

production group (32.8 vs. 30.9 kg/d), whereas there was no treatment effect in the low production cows (22.3 kg/d). Fat test was greater in both yeast culture treated groups, averaging 19.4% higher in high producers and 14 % higher in lower producer compared with control cows.

Diet composition also influences responses to yeast culture. Supplemental yeast culture increased DM intake of cows by 1.2 kg/d and increased FCM by 1.4 liters/d (Williams et al., 1991). The effects of yeast culture were greater when cows were fed a diet containing 60:40 concentrate to forage ratio compared with cows fed a diet containing 50:50 (Williams et al., 1991). The extra energy supplied because of the enhanced intake of SC supplemented animals may be sufficient to allow for increases in milk yield that have been observed (Newbold, 1990). Milk production and composition were not always improved by yeast culture supplementation (Erdman and Sharma, 1989; Arambel and Kent, 1990; Alikhani et al., 1992; Swartz et al., 1994).

Growth and feed efficiency

Dietary composition and environmental conditions influence the growth response of animals to yeast culture supplements. Fallon and Harte (1987) reported that the inclusion of yeast culture in a barley/soya diet promoted DM intake by 12.8% and increased live weight gain by 19 % in Friesian male calves (average weight 45 kg). These effects were not observed in corn gluten/barley diets. Williams et al. (1987) demonstrated that lambs fed diets supplemented with yeast culture had higher daily gains than lambs fed a control treatment. These researchers observed an interaction of environmental temperature × diet on daily gain of lambs fed supplemental yeast culture.

Response to yeast culture may not be affected by breed type. Hughes (1988) reported that yeast culture increased daily live-weight gain in both dairy and beef cross calves. However, no differences were found between breed types.

In contrast to the above studies, little or no effects of yeast culture supplementation on weight gain and feed efficiency were found by other researchers (table 2). Adams et al. (1981) reported no significant differences due to supplementation of yeast culture in average daily gains and feed conversions in lambs. Phillips and VonTungeln (1985) conducted an experiment using conditions that simulated the sequence of events found in marketing channels by weaning, fasting, refeeding and fasting a second time. Yeast culture was added to the poststress diet at 1 or 2% of DM to study its effects on DM intake and poststress performance of steer and heifer beef calves. Dry matter intake tended to increase with the addition of yeast culture, but no differences were observed between 1 and 2% yeast culture supplementation. Weight gain was not consistently increased by the addition of yeast culture. Wagner et al. (1990) studied the effect of supplementing corn- or wheat-based diets with yeast culture (1 g/kg DM) on DM intake and weight gain using 48 Holstein calves at approximately 3 weeks of age. Feed intake, weight gain, and feed efficiency were not affected by supplemental yeast. More recently, Mutsvangwa et al. (1992) found that DM intake was significantly greater for bulls given yeast culture than for control bulls, but average daily gain and feed conversion were not improved significantly by yeast culture. Quigley et al. (1992) also found that yeast culture affected blood and ruminal metabolites but did not influence DM intake or daily gain of Holstein calves. Mir and Mir (1992) concluded that supplemental live-yeast did not result in positive effects on feed utilization by steers.

2) *Aspergillus oryzae*

Isolated aspergilli organisms are characterized by great diversity and variability. Therefore, two strains of *Aspergillus oryzae* (AO) may carry the same Latin name, but may be very different in their characteristics and properties. *Aspergillus oryzae* is known as a producer of starch-degrading enzymes (amylases and amylogluco-sidases) and proteolytic enzymes (Fogarty and Kelly, 1979; Boing, 1983). Incorporation of AO into calf, growing cattle, dairy cattle or sheep diets has produced variable results (tables 3 and 4).

Milk production and composition

Feeding AO to dairy cows has resulted in increased milk production or FCM yields (Harris et al., 1983; Kellems et al., 1987; Gomez-Alarcon et al., 1991). In most cases, the magnitude of response was dependent on the stage of lactation. Gomez-Alarcon et al. (1991) reported that milk yields, efficiency of milk production and nutrient digestibility 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. Kellems et al. (1987) reported an increase in 3.5% FCM when cows received 3 g AO/d during 40 to 90 and 91 to 120 DIM periods. No significant increase was observed during the 121 to 150 DIM period. Similar results were reported by Wallentine et al. (1986). In a subsequent study, Kellems et al. (1990) suggested that AO had its greatest effect during early stages of lactation and higher milk production in later stage of lactation was a result of higher initial production,

TABLE 3. EFFECTS OF *Aspergillus oryzae* ON DRY MATTER INTAKE, MILK YIELD AND COMPOSITION IN LACTATING DAIRY COWS

Reference	Supplement[1]	DM intake[2]	Milk[2]				Diet[3]	DIM[4]	Comments on AO effect
	g/d	kg/d	yield, kg/d	FCM, kg/d	protein, %	fat, %			
Harris et al., 1983	0(4)	27.3	26.9	26.6		3.41	65C:35F, pelleted cottonseed hull	Mid-lactation	Increase in FCM and milk fat percent in silage diet but not in cottonseed hulls diet.
	56(4)	26.9(−.4)	26.4(−.5)	24.2(−2.2)		2.99(−.42)			
	0(4)	22.8	24.7	24.7		3.55	78C:22F, sugarcane silage		
	56(4)	24.3(+1.5)	25.3(+.6)	26.0(+1.3)		3.66(+.11)			
van Horn et al., 1984	0(16)	22.7	24.5	24.2	2.93	3.46	By-product feeds/ animal fat	Early to mid-lactation	
	57(16)	23.5(+.8)	26.2(+1.7)	25.3(+1.1)	2.90(−.03)	3.30(−.16)			
Huber et al., 1985	0(6)		18.5				normal conc.	Mid-lactation	Increase in milk yield in normal concentrate diet.
	90(6)		20.2(+1.7)				normal conc. + AO		
	0(6)		19.4				low conc.		
	90(6)		18.7(−.7)				low conc. + AC		
Huber et al., 1986	0(12)	19.0	22.6						
	3(12)	19.9(+.9)	23.5(+.9)						
Wallentine et al., 1986	0(25)			30.1				0- 60	Increase in FCM in early lactation.
	90(25)			33.7(+3.6)					
	0(25)			29.1				61-120	
	90(25)			27.9(−1.2)					
Kellems et al., 1987	0(16)			35.6			earlage/alfalfa silage/rolled corn/barley/whole cottonseed	40- 90	Greater increase in FCM in early stage of lactation.
	3(16)			38.9(+3.3)					
	0(16)			36.1				91-120	
	3(16)			38.2(+2.1)					
	0(16)			33.3				121-150	
	3(16)			34.7(+1.4)					
Gomez-Alarcon et al., 1988	0(23)		38.7				60C:40F	3 to 5wk	Increase in milk production
	+(23)		40.4(+1.7)						
Kellems et al., 1988	0(48)		27.1	28.4		3.67	earlage/alfalfa silage/rolled corn/barley/whole cottonseed	Early lactation	
	3(48)		28.5(+1.4)	29.5(+1.1)		3.63(−.04)			
	90(48)		27.5(+.4)	28.7(+.2)		3.66(−.01)			

TABLE 3. (CONTINUED)

Reference	Supplement[1]	DM intake[2]	Milk[2]				Diet[3]	DIM[4]	Comments on AO effect
	g/d	kg/d	yield, kg/d	FCM, kg/d	protein, %	fat, %			
Kellems et al., 1990	0(70)	21.3	26.0	27.5		3.73	alfalfa silage/ corn earlage/rolled barley/whole cottonseed/corn	Complete lactation	FCM production was increased in latter stage (after 18 wk) of lactation
	3(70)	21.3(0)	27.7(+1.7)	28.8(+1.3)		3.68(−.05)			
	90(70)	21.2(−.1)	26.6(+.6)	27.5(0)		3.72(−.01)			
Gomez-Alarcon et al., 1991	0(12)	19.1	22.3	20.5	3.15	2.97	50C:50F, alfalfa hay/barley/corn	145	Increase in yield on 60C:40F diet
	3(12)	19.9(+.8)	23.2(+.9)	21.4(+.9)	3.20(+.05)	2.93(−.04)			
	0(23)	25.1	37.3	34.1	3.02	3.07	60C:40F, alfalfa hay/cottonseed	3 to 5wk	
	3(23)	25.6(+.5)	39.8(+2.5)	35.9(+1.8)	2.99(−.03)	2.91(−.16)			
Denigan et al., 1992	0(10)	22.6	30.9		2.92	3.74	61C:39F, alfalfa hay/alfalfa cubes/cottonseed hulls/whole cottonseed/ commercial dairy concentrate	75	
	1.5(10)	25.9(+3.3)	30.8(−.1)		2.96(+.04)	3.88(+.14)			
	3(10)	24.1(+1.5)	30.8(−.1)		3.03(+.11)	3.71(−.03)			
	6(10)	23.2(+.6)	30.8(−.1)		2.92(0)	3.85(+.11)			
	0(6)	25.5	26.2		3.42	3.97		140	
	1.5(6)	22.7(−2.8)	25.8(−.4)		3.23(−.19)	3.63(−.34)			
	3(6)	25.4(−.1)	25.7(−.5)		3.21(−.21)	3.89(−.08)			
	6(6)	23.9(−1.6)	26.0(−.2)		3.29(−.13)	4.18(+.21)			
Higginbotham et al., 1993	0(55)		39.3	37.9	3.05	3.32	58C:42F	140	Higher milk protein percent
	3(55)		39.6(+.3)	38.7(+.8)	3.12(+.07)	3.36(+.04)			
Sievert and Shaver, 1993a	0(8)	24.4	35.1	32.8	2.93	3.09	42% NFC	40	NFC by AO interaction for milk fat percent.
	3(8)	25.3(+.9)	35.5(+.4)	32.8(0)	2.98(+.05)	3.03(−.06)	42% NFC + AO		
	0(8)	24.0	34.5	32.3	2.94	3.11	35% NFC		
	3(8)	24.1(+.1)	34.8(+.3)	32.8(+.5)	2.94(0)	3.15(+.04)	35% NFC + AO		
Sievert and Shaver, 1993b	0(12)	23.3	30.9	30.5	3.20	3.46	42% NFC	40	NFC by AO interaction for milk fat. Across NFC levels, AO decreased milk fat percentage.
	3(12)	23.5(+.2)	31.1(+.2)	30.9(+.4)	3.20(0)	3.48(+.02)	42% NFC + AO		
	0(12)	22.4	30.4	30.3	3.20	3.53	36% NFC/BP		
	3(12)	22.7(+.3)	30.3(−.1)	30.1(−.2)	3.18(−.02)	3.45(−.08)	36% NFC/BP + AO		
	0(12)	22.9	31.0	31.0	3.22	3.54	36% NFC/SH		
	3(12)	23.4(+.5)	30.8(−.2)	30.6(−.4)	3.17(−.05)	3.46(−.08)	36% NFC/SH + AO		

[1] Numbers in parenthesis represent the number of animals used in the study.

[2] Numbers in parenthesis represent the differences between control and treatment.

[3] 50C:50F = 50% concentrate:50% forage; AO = *Aspergillus oryzae* fermentation extract; NFC = non-fiber carbohydrate; BP = beet pulp; SH = soy hulls.

[4] Days in milk.

TABLE 4. EFFECTS OF *Aspergillus oryzae* ON DRY MATTER INTAKE, WEANING DATE, WEIGHT GAIN AND FEED EFFICIENCY IN GROWING RUMINANTS

Reference	Animal	Supplement[1]	DM intake[2]	Weaning date[2]	Weight gain[2]	Feed conversion[2]	Diet	Age	Comments on AO effect
		g/d	kg/d	wk	kg/d	feed/gain			
Allison and McCraw, 1989	Steer	0(28) 90(28)			1.06 1.10(+.04)		corn silage/ soybean meal	230 kg	ADG was higher during the first 28-d period but not in later periods.
Herring et al., 1989	Lambs	0(5) 0.3(5) 0.6(5)	2.17 2.16(−.01) 2.17(0)		0.20 0.19(−.01) 0.21(+.01)	10.9 11.4(−4.6%) 10.3(+5.5%)	pelleted alfalfa	60 d	
Rush et al., 1990	Steers	0(48) 113(48)			0.83 0.88(+.05)		pasture	250kg	
Beharka et al., 1991	Heifer calves	0(18) 0.5(18) 1(18) 3(18)	1.11 1.24(+.13) 1.19(+.08) 1.24(+.13)	5.4 4.6(−.8) 4.6(−.8) 4.6(−.8)	0.51 0.54(+.03) 0.54(+.03) 0.59(+.08)	2.18 2.30(−5.5%) 2.20(−0.9%) 2.10(+3.7%)	corn/soybeans/ soybean hulls/ oats/molasses	Neonatal	AO calves were weaned earlier.
Beharka et al., 1991	Bull calves	0(10) 0.5(10) 1(10) 3(10)	1.21 1.24(+.03) 1.26(+.05) 1.14(−.07)	5.5 4.7(−.8) 4.8(−.7) 5.1(−.4)	0.54 0.63(+.09) 0.61(+.07) 0.57(+.03)	2.24 1.97(+12.1%) 2.07(+7.6%) 2.00(+10.7%)			

[1] Numbers in parenthesis represent the number of animals used in the study.

[2] Numbers in parenthesis represent the differences between control and treatment.

which reflected an increased persistency. However, Harris et al. (1983) observed no difference in milk yield of mid-lactation cows fed sugarcane silage treated with AO enzyme product at time of ensiling at rate of 5 kg/ton. Other workers failed to observe improvements in milk yield in AO supplemented cows, even though animals were in early lactation (Denigan et al., 1992; Sievert and Shaver, 1993a,b).

Composition of milk was not affected by AO supplementation in most studies (Kellems et al., 1990; Gomez-Alarcon et al., 1991; Denigan et al., 1992). In contrast, Harris et al. (1983) found higher milk fat percentage and Higginbotham et al. (1993) found higher milk protein percentage for the cows fed AO.

Growth and feed efficiency

Reports on the use of AO supplements in diets fed to growing calves have been inconclusive (table 4). Allison and McCraw (1989) reported that average daily gains of calves were higher for the AO treated group than controls. More recently, Beharka et al. (1991) concluded that AO supplemented calves were weaned 1 wk earlier than unsupplemented calves and had higher numbers of ruminal bacteria and greater ruminal fermentative activity. Wiedmeier (1989) observed greater body weight gains in beef cows and calves fed AO and suggested that AO improved performance of cows with suckling calves when grazing pastures with a high percentage of mature forage. In contrast, Rush et al. (1990) concluded that performance of British crossbred steers did not improve when AO along with vitamin and mineral supplements were fed for 111 d late in the grazing season when forage quality was lowest. Growth and carcass characteristics of fine-wool lambs were not influenced by dietary AO (Herring et al., 1989). Feed efficiency was not improved by AO supplementation (Herring et al., 1989; Beharka et al., 1991; Gomez-Alarcon et al., 1991).

3) Summary of production responses to fungal cultures

Table 5 summarizes the frequency and size of response to fungal cultures when effects were significant. When SC was fed to lactating dairy cows, 2 of 10 studies showed an increase in DM intake with an average of 1.3 kg/d. Average increases in FCM (kg/d), milk fat (%), and milk protein (%) were 2.5, 0.14, and 0.05, respectively. Overall, only 18 to 27% of the studies reviewed showed a positive response to SC supplementation.

Increase in DM intake in response to AO supplementation was observed in 1 of 8 studies (1.8 kg/d). Supplemental AO increased milk production in 6 of 14 studies, averaging 2.03 kg/d. Response of milk fat (%) and milk protein (%) were observed once each with the level of .11 and .07, respectively. From these data, it is apparent that producers who consider feeding fungal additives to dairy cows must be sure to compare cost plus risk factor versus potential benefits.

TABLE 5. SUMMARY OF POSITIVE EFFECTS OF *Saccharomyces cerevisiae* AND *Aspergillus oryzae* ON DRY MATTER INTAKE, MILK YIELD AND COMPOSITION IN LACTATING DAIRY COWS

Item	No. of study	Mean	Range	Comments[1]
Saccharomyces cerevisiae				
Dry matter intake (kg/d)	2/10	1.3	.9 -2.2	Improvements were observed on high (>60%) concentrate diet.
Fat corrected milk (kg/d)	3/11	2.5	1.5 -3.4	
Milk fat (%)	2/11	.14	.14	
Milk protein (%)	2/10	.05	.05- .06	
Aspergillus oryzae				
Dry matter intake (kg/d)	1/ 8	1.8	.6 -3.3	
Milk production (kg/d)	6/14	2.03	1.3 -3.6	Increase in milk production in early lactation with high concentrate diets.
Milk fat (%)	1/12	.11	.11	NFC by AO interaction.
Milk protein (%)	1/ 8	.07	.07	

[1] NFC = non fiber carbohydrate; AO = *Aspergillus oryzae* fermentation extract.

3. Modification of Ruminal Fermentation and Other Changes in the Gastrointestinal Tract

1) Stimulation of ruminal microbial growth

Various studies have indicated that the population of microorganisms in the rumen can be influenced by the addition of fungal culture supplements to ruminant diets. Increased concentrations of total anaerobic and fibrolytic bacteria were observed both *in vivo* (Wiedmeier et al., 1987; Harrison et al., 1988; Newbold et al., 1992a,b) and *in vitro* (Dawson et al., 1990; Beharka and Nagaraja, 1991; Newbold et al., 1991) as a result of fungal culture supplementation. Lactic acid utilizing bacteria also increased with fungal culture supplementation (Beharka and Nagaraja, 1991; Nisbet and Martin, 1991a). Such increases in the numbers of bacterial cells suggest that supplemental fungal cultures can alter the composition of the bacterial population in the rumen and alter ruminal fermentation. Cellulose digestion and lactic acid utilization are most likely to improve with such stimulation.

Numbers of eukaryotes in the rumen microbial population, ciliate protozoa and anaerobic fungi, were not influenced by supplemental AO to the diet of sheep (Newbold et al., 1992a), which is consistent with observations by Newbold et al. (1992b). These results indicate that stimulation of forage degradation in the rumen of animals supplemented with AO is most likely due to a stimulation in the numbers of prokaryotic rather than eukaryotic microbes in rumen fluid.

When supplemented to ruminant diets, fungi may have selective stimulatory effects on specific ruminal bacteria, shifting the microbial population causing an increase in microbial protein synthesis and changing bacterial amino acid profiles (Beharka and Nagaraja, 1991; Dawson and Hopkins, 1991; Erasmus et al., 1992). Dawson and Hopkins (1991) suggested that individual yeast strains may affect each cellulolytic group of ruminal bacteria differently from other strains of yeast. Supporting research by Dawson (1993) indicated that certain strains of ruminal bacteria could be selectively stimulated by certain strains of yeast in cocultures, whereas other strains were not influenced by yeast supplementation. Similar results were reported by Beharka and Nagaraja (1991) with AO supplementation. The addition of AO to the growth medium increased growth rate of *Ruminococcus albus* and *Fibrobacter succinogenes* but not other fibrolytic bacteria. Numbers of lactate utilizing bacteria (*Megasphaera elsdenii* and *Selenomonas ruminantium*) were also stimulated by AO.

Improved microbial protein synthesis stimulated by ruminal microbial growth in response to fungal culture could supply specific limiting amino acids required by high producing animals. A recent study (Erasmus et al., 1992) indicated that supplementation of yeast culture tended to increase microbial protein synthesis in dairy cows and significantly altered the amino acid profile of duodenal digesta. The duodenal flow of methionine increased from 41 to 58 g/d. However, animals may not be as responsive to fungal culture supplementation when dietary feed ingredients are high in nutrient availability. In general, bacterial growth responses to fungal supplementation have varied with the quality of rations, strains of fungi and other factors which have not yet been identified. Other researchers found no differences in ruminal microbial population with supplemental SC (McLeod et al., 1990) or AO (Oellermann et al., 1990; Varel and Kreikemeier, 1994).

Whether viable fungal cells are necessary in preparations to obtain a benefit is questionable. Several studies have been conducted to answer this question. Two main criteria were used in these studies. The first criteria was whether or not fungal cells could grow in the rumen. Most yeast strains that were examined had limited ability to grow under anaerobic conditions (Hession et al., 1992), but early studies using rumen simulators suggested that SC could grow in the rumen (Dawson, 1987). Recently, yeast numbers have been measured in the rumen of sheep fed diets continuously from a belt feeder and offered SC twice daily (Newbold et al., 1990). Counts of viable yeast cells in the rumen increased 1 h after SC addition, but the increase was of a similar magnitude to the number of live cells added in the SC supplement. Therefore, growth of SC in the rumen was considered negligible. This observation was supported by other studies (Arambel and Tung, 1987; Fiems et al., 1993). However, lack of growth in the rumen does not exclude the possibility of metabolic activity. Ingledew and Jones (1982) demonstrated that brewers yeast could be metabolically active in ruminal fluid up until 6 h incubation at 39℃. In addition, Fiems et al. (1993) demonstrated that yeast cells survived passage through the digestive tract. Microscopic examination of digesta reaching the duodenum of AO treated cows demonstrated attachment of *Aspergillus* fungus to fiber particles (Wanderley et al., 1985). Survival of SC and AO in duodenal digesta suggests that postruminal effects of these organisms cannot be excluded.

The second criteria was whether or not fungal cells that were killed or inactivated by heating, autoclaving or irradiation could stimulate ruminal bacterial growth and activity. Studies using heat killed or live yeast in rumen-simulating cultures indicated that stimulation of cellulolytic bacteria was either dependent on the activities

of viable yeast cells or some heat labile component of yeast culture (Dawson et al., 1990). Lactate-utilizing bacteria were stimulated by a fermentation product found in filter-sterilized, cell-free extracts from both yeast cultures (Nisbet and Martin, 1991a) and AO (Beharka and Nagaraja, 1991; Nisbet and Martin, 1991b). Therefore, the mechanism for stimulating growth of lactate-utilizing bacteria is different from those for stimulating other anaerobic and cellulolytic bacteria (Dawson, 1993). Influence of autoclaved or irradiated AO fermentation extract on fermentation was examined by Newbold et al. (1991) using a rumen simulation technique (Rusitec). Results from their study indicated that the mode of action of AO on ruminal fermentation was dependent on a heat-labile component, possibly a nutrient or an enzyme and thus viable AO cells were not required to stimulate bacterial growth and activity. Further studies are required to clarify this issue.

2) Nutrient flow to the small intestine

Fungal culture can influence the contributions of microbial protein synthesis to the nutrient profile of digesta supply to the small intestine. Williams et al. (1989) reported that the apparent absorption of dry matter (DM) and non-ammonia nitrogen (NAN) between the duodenum and terminal ileum increased by 35 and 23%, respectively when SC was added to the diet of sheep. The presence of SC tended to increase the flow of DM and NAN at the duodenum, but flow at the terminal ileum was unchanged. These investigators suggested that this increased flow and absorption of NAN probably represented an increase in the flow of useful microbial protein to the small intestine. Addition of AO to the diet of dairy cows increased total N and bacterial N flow to the duodenum (Gomez-Alarcon et al., 1986; Gomez-Alarcon et al., 1987). Recently, Carro et al. (1992) found an increase in the flow of undegraded feed N to the duodenum of dairy cows with supplementation of SC. However, duodenal flow of NAN and microbial N did not increase significantly.

The amino acid profile of duodenal digesta greatly influences the amounts of individual amino acids available for milk yield and protein production in dairy cows. Although there is no agreement on the limiting amino acids for milk production, combinations of undegradable protein sources have been used to manipulate the amino acid profile of duodenal digesta. Manipulation of the amino acid profile is difficult because amino acid composition of bacterial protein synthesized in the rumen is relatively constant when recommended amounts of undegradable protein are fed. Erasmus et al. (1992) found that amino acid concentrations in digesta of yeast culture supplemented cows were higher for four of the seventeen amino acids analyzed (Met, Cys, Thr, and Ser) and lower for glutamic acid. Because the same basal diet was fed to the cows, it was speculated that any change in the duodenal amino acid profile was a consequence of changes in the amino acid profile of bacterial protein. These results suggest that yeast culture can influence the amino acid profile of the bacterial protein flowing out of the rumen, presumably by selective stimulation of growth of certain species of anaerobic bacteria (Dawson et al., 1990; Erasmus et al., 1992). Caton et al. (1993) found that total duodenal essential and nonessential amino acid flows increased in steers grazing cool-season pasture supplemented with AO compared with control steers. This response was likely a result of increased N intake by steers supplemented with AO. Because of limited data, extreme caution must be exercised in interpretation and relating changes in the amino acid profile of duodenal digesta to possible changes in the profile of bacteria. Further investigation is necessary to confirm change in amino acid profile as a mode of action of yeast culture.

Ruminal liquid and particulate outflow rates have been measured with or without fungal supplementation (Wiedmeier et al., 1987; Harrison et al., 1988; Caton et al., 1993). Data suggest that ruminal liquid outflow rate increases with fungal culture supplementation although the magnitude of response is low and unlikely to be significant with the small number of animals used in each experiment.

3) Ruminal fermentation

Research has shown that fungal cultures can alter the pattern of ruminal fermentation. Volatile fatty acid (VFA) production by ruminal bacteria was used to measure the stimulatory effects of yeast culture on ruminal fermentation (Gray and Ryan, 1988; Martin et al., 1989). Increases in total VFA concentration (Edwards et al., 1991; Nisbet and Martin, 1991a; Varel and Kreikemeier, 1994) and molar proportions of individual VFA (Harrison et al., 1988; Nisbet and Martin, 1991a) were observed. But, in most other *in vivo* and *in vitro* studies, fungal cultures had no effect on total VFA concentration and molar proportions of individual VFA (Carro et al., 1992; Caton et al., 1993; Fiems et al., 1993; Higginbotham et al., 1994).

Methane production was reduced slightly by SC in the gastrointestinal tract of cattle (Williams, 1988) and by AO using the rumen simulation technique (Frumholtz et al., 1989a,b). Although methane production can represent up to 12% loss of dietary energy, the small changes noted

after supplementation with fungal cultures are unlikely to represent a major energy saving. However, this change is important in confirming changes in fermentation stoichiometry (Williams and Newbold, 1990).

Several studies showed that fungal culture can reduce the concentration of ruminal ammonia (Dawson, 1987; Williams and Newbold, 1990). Stimulation of microbial growth due to yeast culture supplements is often associated with an increase in ammonia utilization by ruminal microorganisms. Reduced ruminal ammonia levels were not associated with decreased protein degradation or deamination (Williams and Newbold, 1990) and appeared to be related to increased ammonia utilization by ruminal microorganisms. Uptake and assimilation of ammonia into protein by the yeast-stimulated microbial population could explain this decrease in ruminal ammonia concentrations. However, such decrease have not been consistently observed (Wiedmeier et al., 1987; Carro et al., 1992; Hession et al., 1992). Such disparities may be influenced by differences in nitrogen availability in feeds and by the nitrogen recycling mechanisms in the animals (Dawson, 1993).

Yeast culture supplementation to high concentrate rations has been reported to decreases ruminal lactic acid concentrations and moderate ruminal pH. Williams et al. (1991) demonstrated that yeast culture consistently lowered ruminal lactic acid concentrations in steers fed a hay plus barley diet. Lower lactic acid concentrations were associated with higher ruminal pH and lower concentrations of oligosaccharides. Nisbet and Martin (1991a,b) demonstrated that extracts prepared from yeast culture and AO increased lactate uptake by *Selenomonas ruminantium*. Presence of dicarboxylic acid L-malate in AO fermentation extract may partially be responsible for the increased lactic acid utilization by *S. ruminantium* (Nisbet and Martin, 1990; Martin and Streeter, 1994). This suggests a stimulatory mechanism which enhances microbial activity in the rumen by increasing the rate of substrate uptake by lactic acid-utilizing bacteria. Edwards (1991) reported greater concentrations of lactic acid-utilizing bacteria in the rumen of cattle receiving a high energy finishing ration supplemented with yeast culture. Stabilization of ruminal pH due to a reduced ruminal lactate concentration, arising from decreased production and (or) increased uptake, may explain the increase in bacterial population when fungal cultures are included in the diet. Stimulation of lactic acid utilization and control of ruminal pH by fungal cultures suggest a significant role of these supplements in high concentrate diets. In contrast, other data do not confirm these findings (Beharka et al., 1991; Newbold et al., 1991; Newbold et al., 1992b).

4) Digestibility of the diet

Fungal cultures can increase ruminal (Campos et al., 1990; Gomez-Alarcon et al., 1990) and total tract (Ayala et al., 1992; Erasmus et al., 1992) digestibilities of dry matter, NDF, ADF, cellulose, hemicellulose and crude protein. Some of these enhanced digestive activities may be directly related to fungal stimulation of microbial growth and activity (Wiedmeier et al., 1987; Edwards, 1991).

Increased rate of digestion (Fondevila et al., 1990) or decreased lag time of fiber digestion (Chademana and Offer, 1990) were observed with fungal culture supplementation. Williams et al. (1991) demonstrated an increase in fiber digestion measured using Dacron bags during the first 24 h in the rumen of cattle receiving yeast culture, but overall digestion after 48 h was not affected by the supplement. A similar trend was observed by Carro et al. (1992). Dawson et al. (1990) also reported that the initial stages of fiber digestion by cultures of individual ruminal bacteria were enhanced by certain strains of yeast. However, the majority of reports indicate that the addition of fungal cultures to diets fed to ruminants had no effect on total tract digestibility (Wohlt et al., 1991; Denigan et al., 1992). These studies indicate that fungal culture supplementation may affect the time course of digestive processes in the rumen, but total tract digestibility may not differ from untreated animals because of hindgut fermentation.

Research to date indicates that the effect of fungal cultures on production response and corresponding modifications of ruminal fermentation and other changes in gastrointestinal tract are dependent upon many factors including phase of lactation, level of production, nature of the diet, forage to concentrate ratio of the diet, management, strains used in culture products, environmental conditions, etc. Therefore, inconsistencies among studies can be explained by different experimental conditions. For example, measurements of digestibility have been made in lactating (Gomez-Alarcon et al., 1990) and non-lactating (Wiedmeier et al., 1987) dairy cows fed restricted (Williams et al., 1991) and *ad libitum* (Gomez-Alarcon et al., 1990) diets. Studies have also been performed with a variety of dietary components (Fiems et al., 1993), while concentrate to forage ratios have been high (Chardemana and Offer, 1990; Gomez-Alarcon et al., 1990; Williams et al., 1991) and low (Wiedmeier et al., 1987). To identify the optimum conditions for maximizing animal performance in response to fungal cultures, carefully designed experiments should be conducted both quantitatively and qualitatively.

5) Other observations

Yeast culture supplements increased retention of nutrients in growing animals. Edwards et al. (1991) reported that supplemental yeast culture improved nitrogen retention in steers fed silage and concentrates. Cole et al. (1992) reported that lambs fed yeast culture had greater N balance and tended to have greater Zn and Fe balance than control lambs and suggested that supplementation of morbid calves with yeast culture can have beneficial effects (fewer sick days, higher feed intakes) mediated by improved N, Zn and Fe metabolism. Petersen et al. (1987) noted that yeast culture supplementation reduced urinary mineral excretion and increased total dairy metabolizable mineral and retention of K, Cu and Zn in lambs. These results suggest that yeast culture can have a positive effect on mineral metabolism in lambs.

Fungal cultures may help to alleviate heat stress, although the mechanism of action is unclear. Studies summarized by Huber et al. (1994) showed that cows fed AO often have lower rectal temperatures and respiration rates than companion controls in hot weather (Marcus et al., 1986; Gomez-Alarcon et al., 1991; Higginbotham et al., 1993). In addition, researchers in Arizona, U.S. (Gomez-Alarcon et al., 1987; Gomez-Alarcon et al., 1990; Gomez-Alarcon et al., 1991) consistently observed higher ruminal or duodenal fiber digestion when animals were fed diets supplemented with fungal culture, suggesting that animals may respond better to supplemental fungal cultures under stressed environmental conditions. However, no other studies have been conducted to examine the interaction between fungal cultures and physiological or environmental stress.

4. Model for the Action of Fungal Cultures in Ruminants

Newbold (1990), Dawson (1993) and Wallace (1994) have proposed models to explain the effects of fungal cultures on animal performance. Figure 1 is a conglomerate from these models. The basis of this model is the ability of fungal cultures to stimulate growth and activities of specific groups of rumen bacteria. The exact mechanisms for stimulating microbial activities are not completely understood. Removal of oxygen in ruminal fluid by yeast (Newbold et al., 1993) may prevent toxicity to the ruminal anaerobes. The stimulation of lactate-utilizing bacteria could account for enhanced lactate utilization and corresponding stabilization of ruminal pH in animals receiving high concentrate diets. Moderation of ruminal pH could enhance growth of other groups of bacteria sensitive to acidic conditions, increasing the microbial population in the rumen. In addition, a greater microbial population could utilize more ruminal ammonia and synthesize more microbial protein. Stimulation of microbial growth could be expected to increase digestion of nutrients ingested. Increases in microbial protein synthesis and digestibility of nutrients could increase feed intake and supply more substrate to the small intestine for further digestion and absorption. The net result of these effects could increase production responses by the animal.

5. Future Aspects

Previous studies have indicated that effects of fungal cultures on production responses and other changes in the gastrointestinal tract were influenced by many factors. Therefore, the optimal conditions for obtaining a response from these supplements are difficult to identify. Because the stimulation of specific strains of bacteria is the basis of the mode of action of fungal cultures, identification of the mechanisms is essential. Data from Dawson et al. (1990) and Nisbet and Martin (1991a) indicate that the mechanism for stimulating the growth of one group of bacteria is different from that associated with the stimulation of other groups of bacteria. Therefore, identification of the specific mechanism of stimulation by fungal culture on specific strains of bacteria that are important under certain dietary conditions and associated production situations would be useful.

At the present time, it is not clear whether the effects of fungal cultures are due to the activity of the fungi or via the metabolites produced by the fungi (vitamins, enzymes, etc.) during the preparation of cultures. Purification of metabolites and testing of each metabolite separately could provide the answer. Once determined, the conflicting issue of viability of fungal cultures may be clarified.

Data from Dawson (1993) suggest that different strains of yeast have different characteristics. Certain strains of yeast appear to be more effective at stimulating certain groups of bacteria and ruminal fermentation than others. This hypothesis is supported by other researchers (Tapia and Herrea-Saldana, 1989; Williams and Newbold, 1990). In addition, a mixture of several different strains of fungal cultures had an advantage over the use of a single strain of fungal culture (Wiedmeier et al., 1987). Therefore, research is needed to identify the specific strain or mixture of strains of fungal culture for maximizing their metabolic activity under specific conditions.

As the results from the studies by Arizona researchers indicated, a relationship between fungal cultures and stress (or management) could exist. Currently, many farmers

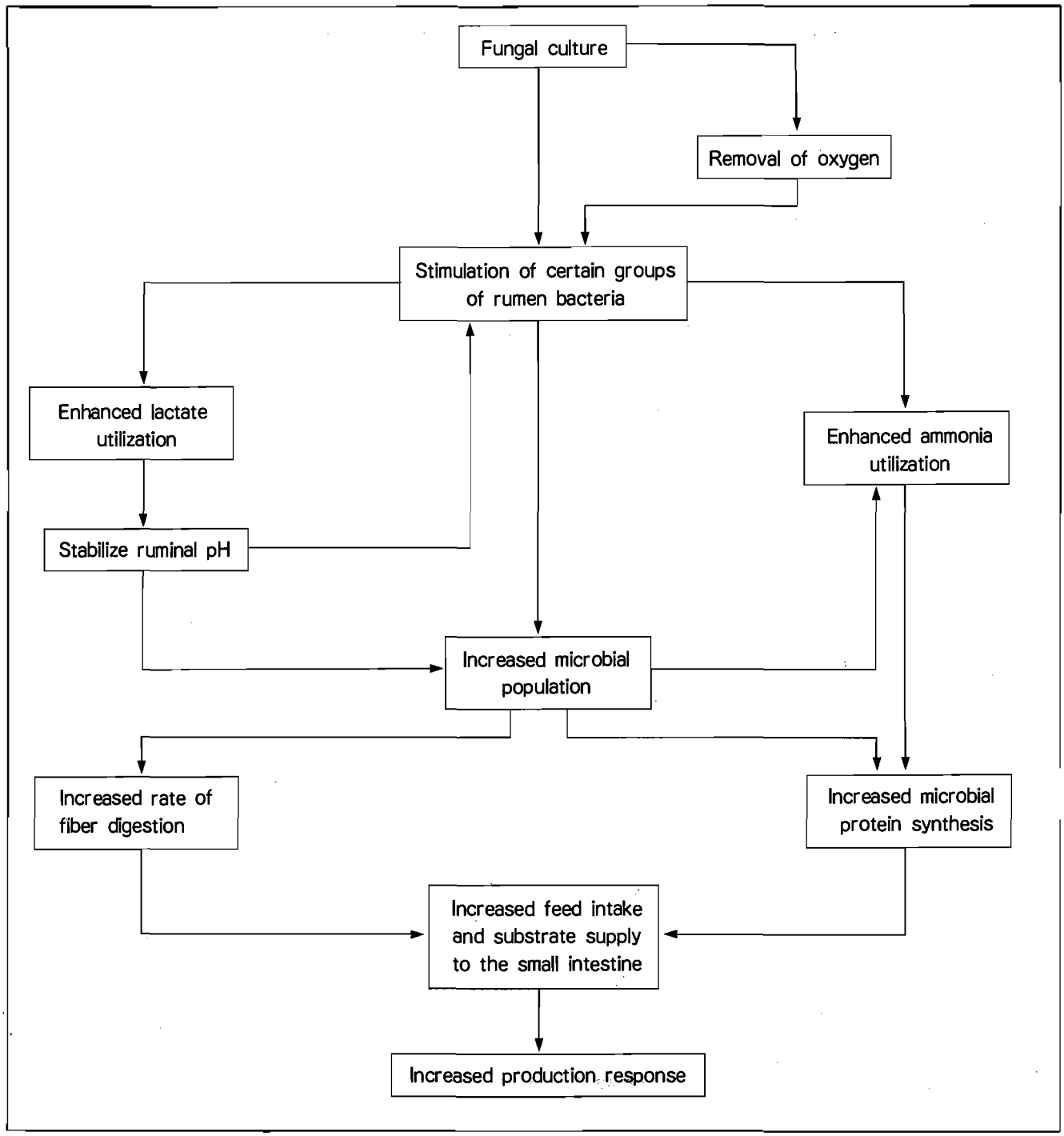

Figure 1. Model depicting the action of fungal cultures in ruminants.

supplement fungal cultures to diets for dairy cows two weeks prepartum to improve animal health. However, scientific data supporting this practice are limited. Studies to examine animal health response to supplemental fungal cultures are needed.

New technology such as molecular techniques could be applied to alter the activities of fungal cultures in the gastrointestinal tract. Fungal cells in feed supplements may provide an economical vehicle for implementing the benefits of recombinant DNA technology to ruminal fermentation (Wallace, 1994). A complete understanding of the activity of fungal cultures in the rumen is necessary to provide a basis for designing and engineering the most appropriate strains of fungi for maximal production responses.

Lactic Acid Bacteria

1. History

At the beginning of the century, Metchnikoff (1908) proposed that the longevity of the Bulgarians was due, in part, to their consumption of a fermented milk product. He postulated that *Lactobacillus bulgaricus* present in the fermented product prevented detrimental putrefaction by the disease producing intestinal microorganisms. Since then, the therapeutic value of *Lactobacillus spp.* has been studied by a number of investigators (Vincent et al., 1959; Hamdan and Mikolajcik, 1974; Barefoot and Klaenhammer, 1983; Newman et al., 1989). According to Stern and Storrs (1975) the early popularity of *L. acidophilus* therapy in this country reached its peak by about the middle thirties and then faded. Following World War II, antibiotics came into use and were often so efficient that they destroyed most of the intestinal bacteria, both good and bad (Mannheim, 1951). The net effect was an increase in the incidence of "antibiotic diarrhea" and related side effects. Acidophilus therapy for restoration of the normal intestinal flora began to be recalled. Since then, there has been a slow but steady increase in the application of acidophilus to humans and animals. But production responses of growing and lactating animals and corresponding changes in ruminal fermentation due to *L. acidophilus* have not been examined until recently.

2. Production Response

Very few studies have been conducted to examine the production response by ruminants to lactic acid bacteria. *Lactobacillus acidophilus* has been used as a means to establish and maintain a normal intestinal flora in young calves or stressed animals (weaned or shipped) rather than as a production stimulant. Because only limited data are available, caution should be used when interpreting the effect of *L. acidophilus* on animal performance.

1) Milk yield and composition

Increased milk yield, but no changes in milk composition, due to *L. acidophilus* have been reported in limited studies. Jaquette et al. (1988) reported that milk production was higher for cows fed a diet containing *L. acidophilus* compared with those fed a control diet. Milk fat and protein percentages were not affected by *L. acidophilus*. Similar results were observed in two California herds consisting of 500 and 600 cows (Ware et al., 1988a). During the 180 d experiment, each animal in the treatment group received 2.0×10^9 CFU (colony forming unit) *L. acidophilus* per day. Daily milk production was higher with the addition of *L. acidophilus*, but dry matter intake, milk fat percent or SNF percent were not affected. Colenbrander et al. (1988) found that treatment of alfalfa silage with *L. acidophilus* did not improve dry matter intake, milk yield and composition of dairy cows, but efficiency of milk production was significantly improved.

2) Growth and feed efficiency

Greater average daily gain was observed in crossbred feeder calves supplemented with various levels of *L. acidophilus* compared with controls (Orr et al., 1988). Feed intake and feed efficiency did not differ among treatments. Ware et al. (1988b) reported that *L. acidophilus* increased average daily gain and improved feed conversion in yearling steers fed high concentrate rations compared with controls. *Lactobacillus acidophilus* did not affect DM intake, USDA yield grade, USDA quality grade, dressing percentage, marbling score and liver abscess incidence. Beeman (1985) used fifty-two-Holstein male calves that had a history of diarrhea and antibiotic therapy, to evaluate the effects of *Lactobacilli spp.* on weight gain of calves convalescing from neonatal diarrhea. All animals were treated with antibiotics for 3 days before the study began. At the two-week evaluation, calves treated with lactobacilli gained an average of 8 kg whereas control calves gained an average of 3.5 kg. By day 56 of the study, average weight gains were 47.3 kg and 37.8 kg for the treated and control groups, respectively. In contrast, Kercher et al. (1986) showed no beneficial effects of *L. acidophilus* supplementation to calf diets. Inoculation of calves via a dose syringe or adding a live culture of *L. acidophilus* to the feed did not influence feed intake, gains, feed efficiency or health status of beef calves during the first four weeks post weaning.

3. Effect on Ruminal Fermentation

Effects of *L. acidophilus* on ruminal fermentation were not studied until recently. Dawson et al. (1990) reported various effects of *L. acidophilus* (1.2 to 2.3 $\times$ 10^9 CFU/g) on ruminal fermentation of steers fed a fescue hay-based roughage diet with or without a mixed microbial supplement containing *L. acidophilus* plus yeast and enterococci. Ruminal pH decreased and ruminal isoacid concentration increased in the treated group. In addition, cellulolytic ruminal bacterial numbers increased in steers fed the mixed microbial supplement. In an *in vitro* study (Dawson and Newman, 1988), the concentrations of anaerobic bacteria, cellulolytic bacteria and lactobacilli

were not influenced by the addition of the mixed microbial supplement. Total VFA concentration and molar proportions of propionate and butyrate were greater, while molar proportion of acetate was lower in fermenters receiving the supplement. No differences in fermentation patterns were observed when the roughage content of the diet was altered from 40% to 78% fescue hay.

Huffman et al. (1992) suggested that *L. acidophilus* may modify subacute ruminal acidosis. Ruminally fistulated steers were fed a 50% concentrate diet for 12 days. On d 13, steers were dosed with a 100% concentrate diet via a ruminal cannula to induce subacute acidosis. Feeding *L. acidophilus* at 5×10^8 CFU/d reduced the amount of time that ruminal pH was below 6 compared with the control. Recently, Van Koevering et al. (1994) reported that ruminal concentrations of D-lactate and total lactate were lower in steers fed *L. acidophilus*. These data suggest that *L. acidophilus* can decrease the severity of subacute acidosis. Attempts have also been made to reduce the incidence of acidosis by inoculation of lactate utilizing bacteria (Hession and Kung, 1992; Robinson et al., 1992).

4. Other Observations

1) Antibacterial effect

Many lactobacilli have demonstrated inhibitory activity against pathogens. *Lactobacillus acidophilus* has been shown to be antagonistic toward enteropathogenic *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Clostridium perfringens* (Gilliland and Speck, 1977). Mann et al. (1980) showed that a strain of *E. coli*, which caused illness and death when it was the sole microbial species in young lambs could be tolerated in the presence of lactobacilli. Ellinger et al. (1980) observed that calves fed whole milk treated with *L. acidophilus* had a linear decrease in coliforms. Similar responses were observed in pigs (Muralidhara et al., 1977). These antagonistic responses vary with various strains of *L. acidophilus*. Hydrogen peroxide produced by the lactobacilli appears to be partially responsible for the antagonistic interaction (Gilliland and Speck, 1977). In addition, a number of reports suggest that antimicrobial proteins or bacteriocins either mediate or facilitate antagonism by *L. acidophilus* (Vincent et al., 1959; Hamdan and Mikolajcik, 1974; Gilliland and Speck, 1977; Barefoot and Klaenhammer, 1983).

2) Antitumor properties

According to Friend and Shahani (1984), epidemiological evidence and dietary studies have shown that the consumption of dairy products fermented by lactobacilli may reduce the risk of colon cancer in animals and humans. Specific strains of lactobacilli also possess activity against a number of transplanted and chemically-induced cancers in animals. The mechanism of action of these organisms has not been fully elucidated. Lactobacilli may inhibit carcinogenesis by inactivating or inhibiting formation of carcinogenic compound in the gastrointestinal tract or may suppress promotion of cancer by stimulating or enhancing the immune properties of the host.

3) Immune response

Enhanced immunity has been observed in animals fed *L. acidophilus*. Perdigon et al. (1986) reported increased activities of macrophages and lymphocytes in mice following oral inoculation or intraperitoneal injection of lactobacilli. Pollmann et al. (1980) observed increased white blood cell counts in *L. acidophilus* inoculated pigs. However, the extent to which lactobacilli act as adjuvants in the immune defense system of the host is uncertain.

4) Anticholesterolemic effects

Grunewald (1982) showed that fermented milk induced a lower serum cholesterol concentration than untreated milk and suggested that fermented milk contained bacterial metabolites which inhibited cholesterol synthesis by the body. Feeding trials conducted by Gilliland et al. (1985) showed that certain strains of lactobacilli reduced cholesterol levels in serum of pigs fed cholesterol but other strains did not.

5) Competitive attachment

Surface action through attachment to the intestinal wall is necessary for enterotoxin-producing strains of *E. coli* to induce diarrhea. Attachment is believed to support proliferation and reduce peristaltic removal of organisms. Muralidhara et al. (1977) found that homogenates of washed intestinal tissue collected from piglets dosed with *L. lactis* had markedly higher numbers of attached lactobacilli and lower *E. coli* counts than scouring or normal control pigs.

5. Future Aspects

Limited data indicate that addition of lactobacilli may increase milk production and alter ruminal fermentation to some extent, however there is no clear scientific evidence that exists to explain whether these observations are ruminal effects, post-ruminal effects or both. More research is needed to obtain a quantitative data base and to define the mode of action of lactic acid bacteria.

Conclusions

This review has explored production responses that have been observed under various conditions with supplemental DFM and has presented details of the corresponding modification of ruminal fermentation in ruminant animals. Supplementation of DFM as a tool for manipulating ruminal microbial fermentation was not always effective and showed various results. Less than 40 % of the studies reviewed demonstrated a positive production response to the DFM supplementation. The optimal conditions for the use of DFM have not been determined. A complete understanding of the activity of DFM in the rumen is necessary to provide a basis for determining optimal conditions for their use. However, the potential effect of DFM on postruminal metabolism should not be excluded.

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