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EFFECTS OF INTERACTIONS BETWEEN *ASPERGILLUS ORYZAE* EXTRACT (AMAFERM¹) AND ANTIMICROBIAL COMPOUNDS ON THE GROWTH OF RUMINAL BACTERIA

A. A. Beharka and T. G. Nagaraja

Summary

The effect of Amaferm, with or without antimicrobial compounds, was determined on the growth rate of pure cultures of predominant ruminal bacteria. Adding Amaferm to media containing chlortetracycline or neomycin tended to diminish the negative effects of those compounds on the growth rate of some ruminal bacteria, even when they had shown no positive response to Amaferm alone. However, adding Amaferm to media containing tylosin decreased the growth rate of *Selenomonas ruminantium* D. These results indicate that Amaferm interacts both positively and negatively with certain antimicrobial compounds.

(Key Words: *Aspergillus oryzae* Extract, Rumen Bacteria, Antimicrobial Compounds.)

Introduction

The use of fungal supplements in ruminant diets date back to 1924. However, results of those early studies were inconclusive. Partly because of concerns about antibiotics, interest has been renewed in using microbial products as feed additives in ruminant diets. Microbial feed additives contain either microorganisms (yeasts, molds, or bacteria); their dry products; the medium in which they grew; and/or the residues of their metabolism.

One commercially available product is Amaferm, a fermentation extract of the mold *Aspergillus oryzae*. Amaferm supplementation was shown to increase ruminal microbial activity *in vitro* and *in vivo* as evidenced by

increased VFA concentration and bacterial numbers, particularly those of fiber-digesting bacteria. Initial experiments with pure cultures of several predominant rumen bacteria indicated that Amaferm can effect the growth and metabolic activity of some (fiber digesters and lactate-utilizers), but not all, ruminal bacteria. Increased microbial activity may partially explain the reports of increased digestion of dry matter, fiber, and crude protein *in vivo* and *in vitro* with Amaferm supplementation.

Antimicrobial feed additives, such as monensin and tylosin, are widely used to increase cattle performance and reduce disease incidence. Microbial products currently do not fall under the FDA feed additive regulations and therefore, can be fed in combination with any other approved compound. However, data on the combined use of microbial feed additives and antimicrobial compounds are limited. Therefore, our objective was to determine the effect of Amaferm in combination with antimicrobial feed additives on the growth rate of selected pure cultures of ruminal bacteria.

Experimental Procedures

Pure cultures of *Selenomonas ruminantium* D (lactate utilizer), *Megasphaera elsdenii*, (lactate utilizer), and *Ruminococcus albus* (fiber-digester), which had previously demonstrated increased growth rate with the addition of Amaferm, and *Prevotella* (*Bacteroides*) *ruminicola* (fiber-digester), *Bacteroides amylophilus* (starch digester), and *Selenomonas ruminantium* HD4 (lactate utilizer), which had previously not been affected by Amaferm,

¹Biozyme Enterprises, Inc., St. Joseph, MO 64504

were grown in anaerobic, complete carbohydrate, rumen fluid medium to determine the effect of Amaferm with or without selected antimicrobial compounds. The compounds included monensin, tylosin, monensin + tylosin, bacitracin, neomycin, chlortetracycline, and oxytetracycline. Amaferm was filter-sterilized and included at 5%. The medium was inoculated with late-log-phase cultures, and growth was monitored by measuring absorbance.

Results and Discussion

Adding Amaferm to the medium increased ($P < .1$) the growth of *Selenomonas ruminantium* D (growth rate .71 vs .43/h) (Figure 1), *Megasphaera elsdenii* (growth rate .32 vs .43/h), and *Ruminococcus albus* (growth rate .35 vs .26/h) but had no effect on the other bacteria tested.

Selenomonas ruminantium HD4 and *Megasphaera elsdenii* grew slower ($P < .1$)

when neomycin and chlortetracycline were added to the media. Adding of Amaferm to the growth media containing neomycin and chlortetracycline increased ($P < .1$) the growth rate of both strains (Figure 1). However, growth rate never reached that of the control. Surprisingly, although Amaferm addition alone had no effect on the growth rate of *Selenomonas ruminantium* HD4 and *Bacteroides amylophilus*, these bacteria grew faster in the presence of Amaferm and neomycin than with neomycin alone ($P < .1$).

In contrast, when *Selenomonas ruminantium* D was grown in combination with tylosin and Amaferm (Figure 2), the growth rate was slower than when it was grown in tylosin alone (growth rate .69 vs .57/h), indicating a possible negative interaction between the two compounds. The combination of tylosin and Amaferm had no effect on the growth rate of *Selenomonas ruminantium* HD4.

Amaferm diminished the negative effect on bacterial growth associated with some antimicrobial compounds, but appeared to have a negative interaction with tylosin.

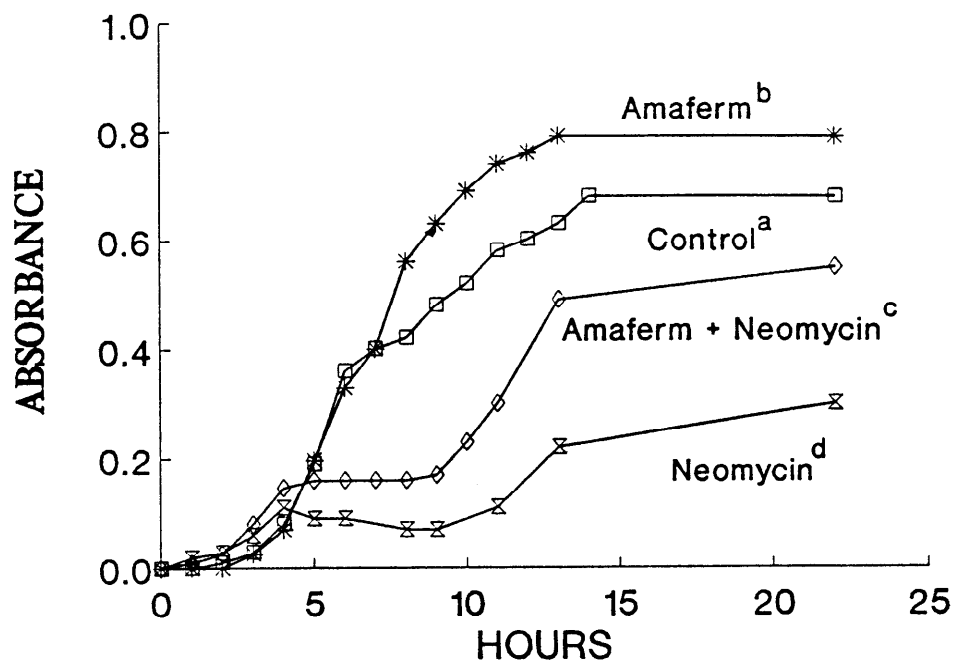


Figure 1. Effect of Amaferm and Neomycin on the Specific Growth Rate of *Selenomonas ruminantium*. Lines with Different Superscripts Differ (P < 1).

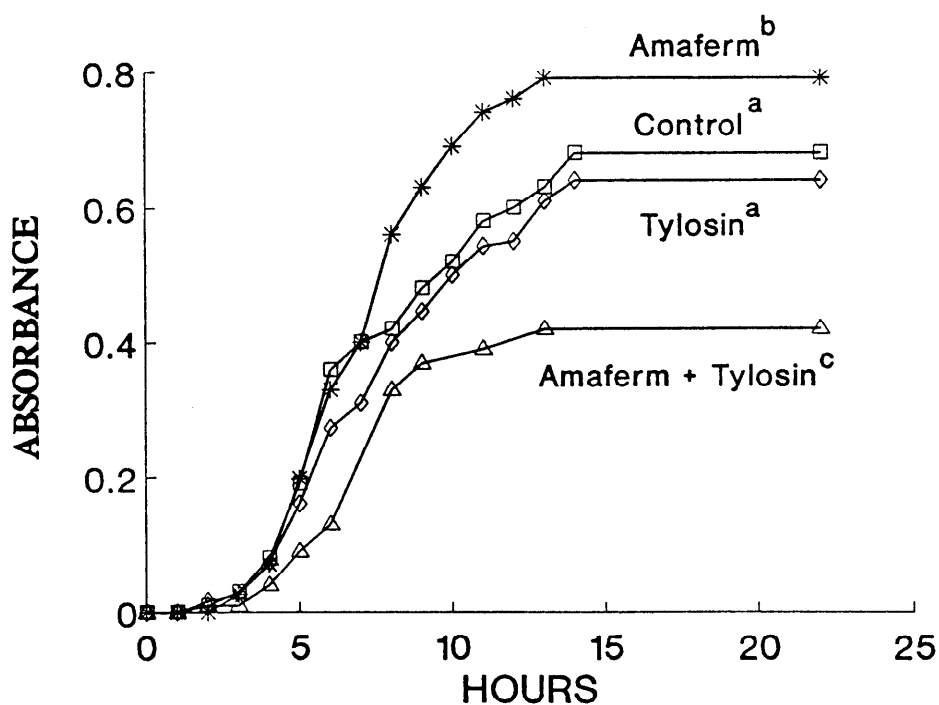


Figure 2. Effect of Amaferm and Tylosin on the Specific Growth Rate of *Selenomonas ruminantium*. Lines with Different Superscripts Differ (P < .1).