AMAFERM® EFFECTS ON THE MORPHOLOGY AND METABOLISM OF THE RUMEN FUNGUS *NEOCALLIMASTIX FRONTALIS* EB188

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AMAFERM stimulated fungal branching and enzyme production.

SUMMARY

DOSE OF AMAFERM USED
0, 7, 25, 35, 50 and 100 µl/ml
(7 µl/ml is equivalent to 3 g/d)

AMAFERM was added to the rumen fungus *Neocallimastix frontalis* EB188 at increasing doses up to 100 µl/ml. Secretions of cellulase and ß-glucosidase were increased in the presence of AMAFERM as well as the acetate:propionate ratio. AMAFERM also increased the morphology of the fungi, illustrated by the increased area of stem, branch and sporangia, and the stimulation of branching.

VALUE

The morphology change of *Neocallimastix frontalis* EB188 by AMAFERM is potentially important for the acceleration of rumen function.

PROTOCOL

Type of Animals/Experimental Units

- In vitro

Number of Animals/Experimental Units

- All cultures were run in replicates of at least 5
- Assays of samples from individual cultures were run in at least triplicate

PROTOCOL (CONTINUED)

Trial Design

- Randomized block design

Treatments

- 0, 7, 25, 35, 50 and 100 µl/ml AMAFERM

Diet Information

- N/A

Data Collection

- Enzyme activity, VFA production, SEM image analysis

DISCUSSION OF RESULTS

- Cellulase increased up to 34% over the Control at 7 µl/ml AMAFERM addition, then decreased until the highest dosage
- The secretion of ß-glucosidase was significantly increased to 31% over the Control at 35 µl/ml AMAFERM level
- Adding 35 µl/ml AMAFERM also stimulated protein secretion by 38% over the Control, then it dropped to the Control level at the highest dosage
- A higher acetate/propionate ratio was observed with AMAFERM addition (Table 1)
- Branch frequency of rhyzoids of *Neocallimastix frontalis* EB188 were tripled with AMAFERM addition, and all cultures up to 50 µl/ml level showed statistical improvement (Table 2)
- Stem and branch area was also increased in AMAFERM treated culture (Table 2). Calculation of the stem/branch ratio indicated that surface area was increased up to 50 µl/ml by AMAFERM
- It took at least 15 hours of ether extraction to obtain material from AMAFERM that stimulated protein secretion, and all the cellulase-stimulating material was removed by 24 hours of extraction

Table 1
VFA production and secretions of culture enzymes and protein in the presence of AMAFERM

Dose (µl/ml)	CMCase, Uml (% of Control)	ß-glucosidase, mU/ml (% of Control)	Protein, µg/ml (% of Control)	Acetate/Propionate ratio
0	0.151 (100)	0.531 (100)	47.1 (100)	3.89
7	0.202 (134)[a]	0.536 (102)	54.7 (116)[a]	8.84[ab]
20	0.181 (120)[a]	ND[1]	53.2 (113)[a]	7.31[a]
35	0.213 (141)[a]	0.696 (131)[ab]	65.2 (138)[ab]	4.32
50	0.170 (113)[a]	0.545 (103)	ND[1]	6.10[a]
100	0.164 (109)	ND[1]	49.1 (104)	ND[1]

[1] Not determined.
Entries which are superscripted differently are significantly different from the Control or other values at $P > 0.05$ or greater.

Table 2
Analysis of fungal morphology in the presence of AMAFERM[1]

Dose (µl/ml)	Stem Area	Branch Area Total area	S/B Ratio	Sporangia Area	Number of Branches
0	73.4	17.6	91.0	68.2	3.7
7	84.9[a]	78.8[ab]	163.7[a]	81.5[a]	14.1[ab]
20	107.6[ab]	69.6[a]	177.2[ab]	99.7[a]	11.6[a]
35	65.5	29.5[a]	95.0	130.3[ab]	7.0[a]
50	73.9	35.2[a]	109.1[a]	ND[2]	8.1[a]
100	75.0[a]	29.8[a]	104.8	ND[2]	4.7

[1] All morphological measurements are determined in microns.
[2] Not determined.
Entries which are superscripted differently are significantly different from the Control or other values at $P > 0.05$ or greater.

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