

# EFFECTS OF AMAFERM® ON IN VITRO EQUINE CECAL FERMENTATION

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In a horse, supplementation with AMAFERM provides growth factors to the cecal microorganisms and stimulates production of most VFA. AMAFERM increases the fermentation capacity of the microbial ecosystem in the cecum of the horse, thereby assisting its digestive health.

#### SUMMARY

DOSE OF AMAFERM USED 2g and 20g per head, per day equivalent\*

Results demonstrate that high levels of AMAFERM influenced the mixed cecal microorganism fermentation of soluble starch, amino acids, Bermuda grass and alfalfa hay, and appear to provide growth factors to cecal microorganisms.

\*NOTE: Current recommended feeding rate for a mature horse is 10g/d AMAFERM.

#### VALUE

Amaferm increased the fermentation capacity of the digestive ecosystem, thereby providing digestive health to the horse.

#### PROTOCOL

## **Type of Animals/Experimental Units**

• Cecal contents from a cecally fistulated horse incubated in anaerobic media for 24 or 48 hours

## Number of Animals/Experimental Units

 Each substrate tested 4 times (starch, trypticase, bermudagrass hay and alfalfa hay)

# **PROTOCOL (CONTINUED)**

## **Trial Design**

Completely randomized design. Statistical model contained effects due to the level of the addition of AMAFERM. Both trials had same experimental design, substrates and data collected

Trial 1: Horse unadapted to AMAFERM

Trial 2: Horse adapted to AMAFERM for 4 weeks before cecal fluid collection

#### Treatments (both Trial 1 and Trial 2)

- Control
- 0.07 g/L AMAFERM (equivalent to 2 g/d in horse)
- 0.7 g/L AMAFERM (equivalent to 20 g/d in horse)

#### **Diet Information**

 70:30 forage:concentrate in donor horse. Different substrates were tested in vitro including no substrate added; and starch, trypticase, bermuda grass hay and alfalfa hay added

## **Data Collection**

• VFA production, ammonia production, methane production, hydrogen production, in vitro dry matter digestibility, ADF digestibility, NDF digestibility

# **DISCUSSION OF RESULTS**

#### **Experiment 1 (unadapted horse)**

- Total VFA production and ammonia production were not changed significantly with 0.07 g/L of AMAFERM, but were increased with 0.7 g/L AMAFERM treatment in the absence of added substrate
- Acetate to propionate ratio decreased with 0.7 g/L AMAFERM treatment and no added substrate, indicating a greater response on propionate than acetate (data not shown)
- When soluble starch was the substrate, there was not a significant change in VFA with 0.07 g/L AMAFERM, but acetate, propionate and ammonia all increased with the 0.7 g/L AMAFERM treatment (Table 1)
- The addition of Trypticase as the substrate increased butyrate, isobutyrate, isovalerate, valerate and ammonia when compared with soluble starch and no added substrate. The increase in branched-chain VFA and a substantial amount of the ammonia can be attributed to the fermentation of branched-chain amino acids by the mixed cecal organisms (data not shown)



# **DISCUSSION OF RESULTS (CONTINUED)**

- None of the fermentation products were affected (P > 0.05) by 0.07 g/L of AMAFERM. The addition of 0.7 g/L of AMAFERM increased concentrations of acetate, propionate, butyrate and total VFA (Table 2)
- When Coastal Bermuda grass was used as the substrate, there was little response to 0.07 g/L AMAFERM, but a 0.7 g/L treatment increased acetate, propionate, butyrate, total VFA and NH3, and decreased the acetate:propionate ratio
- IVDMD, NDF digestibility and ADF digestibility were decreased by the 0.7 g/L treatment level (Table 3)
- When alfalfa hay was used as a substrate, response was similar to the Coastal Bermuda grass with increased VFA and decreased digestibility of fiber and dry matter (Table 4)

#### **Experiment 2 (adapted horse)**

- In Experiment 2, Trypticase as a substrate increased acetate, propionate and total VFA, with ammonia tending to increase at the 0.7g/L treatment level (Table 5)
- pH was decreased with the higher level of AMAFERM when both starch and Trypticase were used as substrates, indicating an increase in fermentation acids (data not shown)
- When Bermuda grass and alfalfa hay were used as substrates, there were no significant differences in VFA production or ratio (data not shown).
- Ammonia production increased with the 0.7 g/L treatment on Bermuda grass and alfalfa hay

	Table 1	Treatment	CH <sub>4</sub> , mM	AC. mM	Pr, mM	Bu, mM	TVFA, mM	Ac:Pr	Lactate, mg/L	NH3, mg/L
Infl AN	Influence of AMAFERM on the fermentation of	Control	0.26	38.77 <sup>d</sup>	27.19 <sup>d</sup>	9.62	75.5	1.53	128.4 <sup>b</sup>	8.8 <sup>b</sup>
	soluble starch by	0.07 g/L	0.26	41.84 <sup>d</sup>	30.88 <sup>d</sup>	9.04	82.3	1.38	151.6 <sup>b</sup>	8.1 <sup>b</sup>
	microorganisms	0.7 g/L	0.00	45.45°	34.67 <sup>e</sup>	7.09	87.5	1.55	1168.8°	33.2°
(EXP.1)	( <i>LXµ</i> .1)	SE	0.06	1.64	1.49	0.81	3.23	0.94	94.89	3.47
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<sup>b,c</sup> Means within a column lacking a common superscript letter differ (P < 0.001)

<sup>d,e</sup> Means within a column lacking a common superscript letter differ (P < 0.05)

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DAIRY	BEEF	POULTRY	SWINE	EQUINE	MULTI-SPECIES	PET	DIGESTIBILITY	MODE OF ACTION	

Table 2	Treatment	CH <sub>4</sub> , mM	AC. mM	Pr, mM	Bu, mM	TVFA, mM	Ac:Pr	NH3, mg/L
Influence of AMAFERM on	Control	0.39	33.48 <sup>⊳</sup>	12.31 <sup>b</sup>	13.14 <sup>b</sup>	64.6 <sup>b</sup>	2.73 <sup>d</sup>	2119.7
the fermentation of Trypticase by	0.07 g/L	0.61	42.42 <sup>b</sup>	15.38 <sup>b</sup>	15.32 <sup>b</sup>	81.4 <sup>b</sup>	2.76 <sup>d</sup>	3090.5
mixed cecal microorganisms	0.7 g/L	0.43	69.05°	36.40°	23.86°	138.9°	1.90°	2454.0
(EXP.1)	SE	0.16	3.47	2.41	1.15	5.48	0.18	634.4

<sup>b,c</sup> Means within a column lacking a common superscript letter differ (P < 0.001)

<sup>d,e</sup> Means within a column lacking a common superscript letter differ (P < 0.05)

<b>Table 3</b> Influence of	Treatment	CH <sub>4</sub> , mM	AC. mM	Pr, mM	Bu, mM	TVFA, mM	Ac:Pr	IVDMD	NDF	ADF
AMAFERM on the fermentation of	Control	1.49°	31.86 <sup>f</sup>	14.24 <sup>h</sup>	5.75 <sup>f</sup>	53.1°	2.24°	35.00°	50.95°	47.96°
Coastal Bermuda grass hay by	0.07 g/L	1.45°	35.29 <sup>f</sup>	16.12 <sup>h</sup>	5.87 <sup>f</sup>	58.6°	2.19°	31.63°	43.33 <sup>d</sup>	39.06 <sup>d</sup>
mixed cecal microorganisms	0.7 g/L	0.65 <sup>d</sup>	65.12 <sup>g</sup>	38.43 <sup>i</sup>	13.23 <sup>g</sup>	120.1 <sup>d</sup>	1.69 <sup>d</sup>	19.65 <sup>d</sup>	29.55 <sup>e</sup>	18.49°
(Exp.1)	SE	0.09	6.05	3.45	0.26	7.85	0.06	1.56	1.22	1.83

 $^{c,d,e}$  Means within a column lacking a common superscript letter differ (P < 0.001)

<sup>f,g</sup> Means within a column lacking a common superscript letter differ (P < 0.05)

<sup>h,i</sup> Means within a column lacking a common superscript letter differ (P < 0.01)

<b>Table 4</b> Influence of	Treatment	CH <sub>4</sub> , mM	AC. mM	Pr, mM	Bu, mM	TVFA, mM	Ac:Pr	IVDMD	NDF	ADF
AMAFERM on the fermentation	Control	3.09°	46.02 <sup>e</sup>	19.34°	6.71 <sup>e</sup>	74.0 <sup>e</sup>	2.38°	68.00°	79.17 <sup>e</sup>	76.87º
of alfalfa hay by mixed cecal	0.07 g/L	2.80°	48.55°	21.14°	7.82°	80.0 <sup>e</sup>	2.31°	62.58°	70.07 <sup>e</sup>	66.31°
microorganisms (Exp.1)	0.7 g/L	1.90 <sup>d</sup>	72.71 <sup>f</sup>	41.24 <sup>f</sup>	14.81 <sup>f</sup>	133.2 <sup>f</sup>	1.77 <sup>d</sup>	32.75 <sup>f</sup>	28.98 <sup>f</sup>	37.30 <sup>f</sup>
	SE	0.21	2.43	1.93	0.78	5.00	0.09	2.84	2.76	3.88

<sup>c,d</sup> Means within a column lacking a common superscript letter differ (P < 0.01)

<sup>e,f</sup> Means within a column lacking a common superscript letter differ (P < 0.001)

TI						P	OWER UP PER MAXIMIZED	FORMANCE.	Y.
DAIRY	BEEF	POULTRY	SWINE	EQUINE	MULTI-SPECIES	PET	DIGESTIBILITY	MODE OF ACTION	

Table 5	Treatment	CH <sub>4</sub> , mM	AC. mM	Pr, mM	Bu, mM	TVFA, mM	Ac:Pr	NH3, mg/L
Influence of AMAFERM on	Control	0.21	36.48 <sup>b</sup>	12.01 <sup>f</sup>	14.74	68.5 <sup>b</sup>	3.04	1019.6
the fermentation of Trypticase	0.07 g/L	0.21	35.04 <sup>b</sup>	11.43 <sup>f</sup>	14.21	65.8 <sup>b</sup>	3.07	1052.2
by mixed cecal microorganisms	0.7 g/L	0.27	39.32°	13.20 <sup>g</sup>	15.34	73.9°	2.98	1461.5
(Exp.2)	SE	0.03	1.13	0.18	0.48	1.31	0.09	178.6

<sup>b,c</sup> Means within a column lacking a common superscript letter differ (P < 0.05)

<sup>d,e</sup> Means within a column lacking a common superscript letter differ (P < 0.01)

<sup>f.g</sup> Means within a column lacking a common superscript letter differ (P < 0.001)

Table 5	Treatment	NH <sub>3</sub> , mM				
Influence of AMAFERM on		Coastal Hay	Alfalfa Hay			
the ammonia production	Control	260.8°	408.0 <sup>e</sup>			
of forages by mixed cecal	0.07 g/L	266.6°	434.6 <sup>ef</sup>			
microorganisms (Exp. 2)	0.7 g/L	405.7 <sup>d</sup>	545.1 <sup>f</sup>			
	SE	21.22	42.83			

<sup>c,d</sup> Means within a column lacking a common superscript letter differ (P < 0.001)

<sup>e,f</sup> Means within a column lacking a common superscript letter differ (P < 0.05)

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