BiOWiSH™ TECHNOLOGIES

Research Study

Effects of BiOWiSH® on Methane Production and Digestibility in Beef Cattle Diets

Summary:

Enteric methane, which is produced through fermentation in the digestive system, represents a major loss of energy consumed by ruminants, as well as a significant input of greenhouse gas emissions sourced from agricultural sectors. The introduction of beneficial microbial populations to the rumen through direct-fed microbials is hypothesized to reduce methane production and promote non-methanogenic microorganisms, thereby enhancing rumen efficiency. The potential to mitigate methane production in beef cattle consuming an energy-dense, dairy transition diet was investigated for a commercial probiotic ,BiOWiSH® MultiBio 3PS.

Treatments (control 0g BiOWiSH®/mT of feed vs. 100g of BiOWiSH®/mT of feed) were examined in a feeding trial using seven steers fitted with ruminal cannulas over four 21-day (d) periods. Rumen fluid was collected two hours post-feeding for in vitro incubation, and subsequent gas chromatography analysis.

Materials and Methods:

Feeding Trial:

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University and the research was conducted by Dr. Tryon Wickersham, Texas A&M AgriLife Research.*

Seven steers fitted with ruminal cannulas were used in an incomplete replicated 4 × 4 Latin square design to determine effects of BiOWiSH® MultiBio 3PS on in vitro methane production.

Treatments consisted of: control (no BiOWiSH®) and BiOWiSH® included at 100g/mT. Intake was limited to 2.5% of body weight, and a growing diet was offered to represent the broadest cross-section of diets fed in beef cattle systems and create a sufficient response surface to test for a response. The BiOWiSH® treatment was dissolved in 100 mL of H_2O, and hand mixed into the ration (1.51 Mcal of NE_l/kg), which was fed daily at 0700. The diet consisted of bermudagrass hay (43.1%), alfalfa hay (14.8%), cracked corn (22.8%), dried distillers' grains (8.4%), soybean meal (4.2), and molasses (5.0%; Table 1). Each experimental period consisted of a 13 d adaptation to treatments (d 1 -13), 6 d to determine intake and digestion (d 14 – d 19), 1 d to determine ruminal fermentation (d 20), and 1 d to measure ruminal methane production (d 21). A 21 d adaptation period was implemented to ensure the ruminal microbial population was stabilized. Steers were housed in individual pens with ad libitum access to water.

Sample Collection:

Intake and digestion observations were made on d 14 through 19. Samples of diets were collected on day 14 through 18 to correspond with fecal samples obtained on d 15 through 19. Titanium dioxide (TiO2) was hand mixed into diet (10 g) daily beginning 5 days prior to and continued through intake and digestion sample collection (d9-d19). Daily fecal production (kg DM) was estimated as:

$$\frac{\text{Ti intake (g)}}{\text{\% Ti in feces}} \text{ /1000}$$

A ruminal fluid sample was collected 2 hours (h) after feeding on d 21 to determine methane-producing activity. This sample was obtained by removing ruminal contents from three locations within the dorsal and ventral sacs of the rumen and squeezing through four layers of cheesecloth into insulated containers. Containers were filled with ruminal fluid and a small amount of ruminal contents subsequently capped, to reduce oxygen exposure, and immediately transported to the laboratory. In the laboratory, ruminal fluid and contents were blended using an IKA Lab Egg (Wilmington, NC) to dislodge microorganisms attached to the fibrous mat, then aliquots were apportioned for analyses of methane-producing activity.

Ruminal fermentation profile was characterized on d 20 of each period. A suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) was used to collect rumen fluid samples prior to feeding (h 0) and 4, 8, 12, and 18 h after feeding. Rumen fluid pH of each sample was measured by a portable pH meter with a combined electrode (VWR Symphony) immediately after each sample time. Subsamples of rumen fluid were collected for analysis of VFA concentrations, with 8 mL of rumen fluid added to 2 mL of 25% m-phosphoric acid and subsequently frozen at -20°C.

*References to Texas A&M University and Texas A&M AgriLife do not constitute endorsement of BiOWiSH® products.

Sample Analysis:

Samples of diet, orts, and feces were weighed, dried at 55°C in a forced-air oven for 96 h, allowed to air equilibrate, and weighed again to determine partial DM. Samples were then ground to pass a 1 mm screen using a Wiley Mill. Diet samples were composited on an equal weight basis within period, whereas ort and fecal samples were composited by steer across days within period. Samples were dried at 105°C for 24 h to determine DM. The loss of dry weight during combustion at 450°C for 8 h was used for determination of organic matter. Analysis of NDF and ADF was performed using an Ankom Fiber Analyzer with sodium sulfite admitted and without correcting for residual ash (Ankom Technology Corp., Macedon, NY). Gross energy was also determined by with a Parr 6300 model bomb calorimeter (Parr Intstrument Co., Moline, IL) to test for changes in digestible energy intake.

Rumen fluid samples were thawed and centrifuged at 20,000 g for 20 min at room temperature. Concentrations of VFA using a gas chromatograph were measured according to the methods described by Vanzant and Cochran (1994).

In vitro ruminal methane-producing activity was determined by incubation of 5 mL freshly collected rumen fluid with 5 mL anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate and 0.2 g finely ground alfalfa as described by Anderson et al. (2006). Triplicate 18 × 150 mm crimp top culture tubes flushed with 50% H2-50% CO_2 were sealed using rubber stoppers and aluminum crimps and incubated for 3 h at 39°C. Upon conclusion of incubation period, tubes were allowed to cool to room temperature. One mL gas sample was drawn from the headspace of each tube and analyzed for CH_4 by gas chromatography on a Gow Mac thermal conductivity series 580 gas chromatograph (Gow Mac Instrument, Bridgewater, NJ) equipped with a HaySep Q column (60°C, 25 mL/min of Argon carrier gas). Methane production rate is reported as µmol CH_4 produced per mL incubation fluid, per hour.

Calculations:

Nutrient digestibilities were calculated as: **[1-(output of nutrient/input of nutrient)] × 100**

Statistical Analysis:

Intake, digestion, and nutrient balance were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Treatment and period were terms in the model and steer was included as the random effect. Fermentation variables were ran using the MIXED procedure. Terms in the model were treatment, period, hour, and treatment × hour. Steer and treatment × period × steer were included as the random terms. For analysis of methane production rates, treatment means were calculated using LSMEANS and analyzed with differences.

Results:

Table 1. Composition and nutrient analysis of growing ration.

Ingredient	% of Dry Matter
Bermudagrass Hay	43.1
Alfalfa Hay	14.8
Cracked Corn	22.8
Dried Distillers' Grains	8.4
Soybean Meal	4.2
Molasses	5.0
Limestone	1.5
Magnesium Oxide	0.3
Nutrient Composition	**% of Dry Matter**
OM (Organic Matter)	92.9
NDF (Neutral Detergent Fiber)	56.1
ADF (Acid Detergent Fiber)	28.4
GE, Mcal/kg DM (Gross Intake Energy and Dry Matter)	4.0

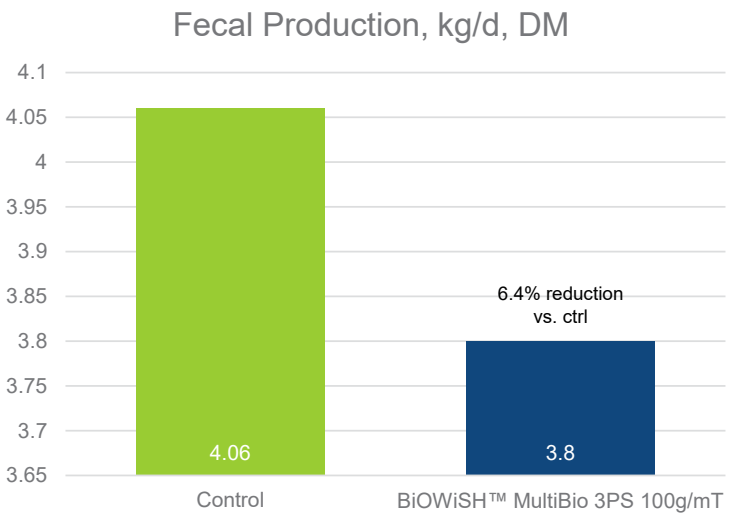
Table 2. Effect of BiOWiSH® on intake, digestibility, and rate of methane production

	0 (control)	BiOWiSH® MultiBio 3PS 100g/mT	SEM	P-Value			
				Control vs. Treatment	Linear	Quadratic	Cubic
No. observations	7	7					
Intake, kg/d							
DM	9.45	9.50	0.082	0.180	0.157	0.364	0.988
OM	8.78	8.83	0.077	0.169	0.159	0.359	0.941
NDF	5.29	5.33	0.048	0.142	0.151	0.332	0.867
ADF	2.68	2.70	0.024	0.138	0.236	0.315	0.658
GE, mcal/d	37.54	37.73	0.327	0.178	0.137	0.366	0.957
Fecal production, kg/d, DM	4.06	3.80	0.137	0.125	0.267	0.447	0.401
Total digestion, %							
DM	56.94	59.98	1.431	0.091	0.206	0.386	0.385
OM	59.53	62.43	1.407	0.096	0.235	0.395	0.356
NDF	52.73	56.45	1.751	0.111	0.369	0.292	0.369
ADF	45.39	49.35	2.095	0.153	0.484	0.325	0.387
GE	56.27	59.32	1.557	0.086	0.234	0.452	0.272
DE (Digestable Energy), mcal/kg	2.23	2.36	0.062	0.086	0.233	0.450	0.274
Total digestible OM intake	5.24	5.51	0.135	0.108	0.240	0.405	0.398
Total digestible NDF intake	2.80	3.01	0.097	0.112	0.348	0.287	0.398
Total DE intake, mcal/d	21.17	22.37	0.627	0.099	0.239	0.460	0.317
No. observations	5	5					
CH4 production rate,							
µmol CH4/mL incubation fluid/hr	2.08	1.21	0.443		0.307	0.039	0.946

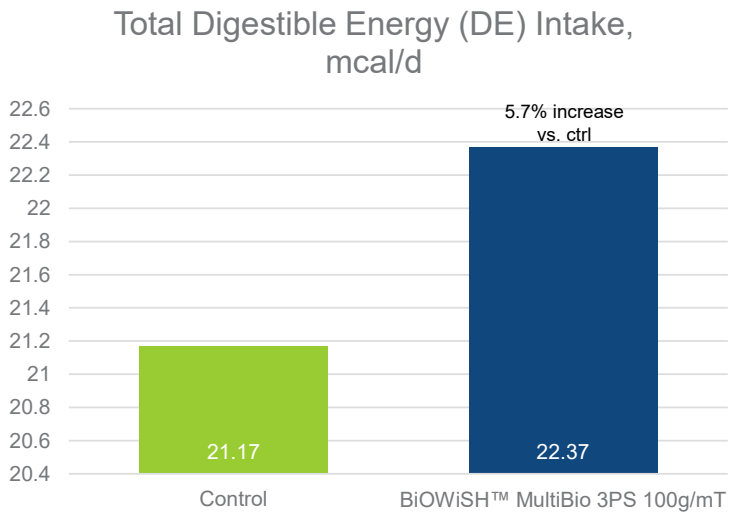
Intake:

Intake, kg/d was greater for the BiOWiSH® Treatment vs. the control. Additionally, fecal production, kg/d, DM was reduced in the BiOWiSH® treatment by as much as 6.4%.

Digestibility:

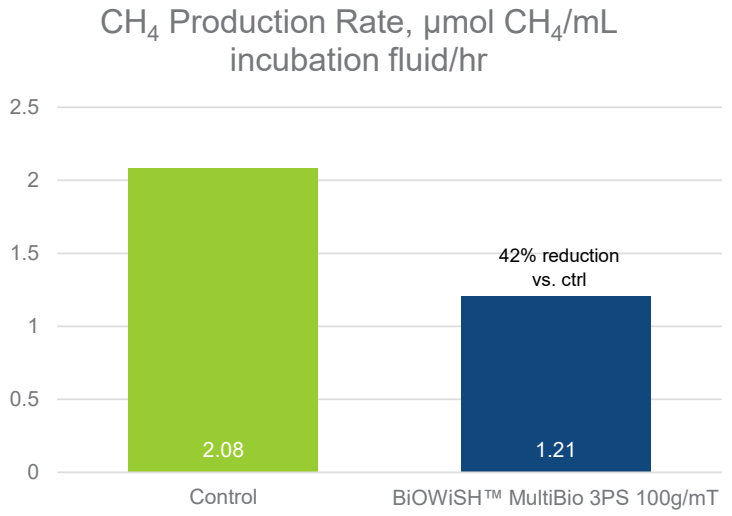


Digestibility in the BiOWiSH® Treatments was also greater than the control. Total Digestible Energy (DE) intake for the 100 g/mT was 5.7% higher than the control. This data could be interpreted as increased nutrient utilization by steers using BiOWiSH®.



Methane Production:

The rate of methane produced was greatly decreased in the BiOWiSH® 100 g/mT treatment vs. the control at 42%.



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