Evaluation of a mixture of crude glycerol and molasses as an energy supplement for beef cattle consuming bermudagrass hay

Phase 1 Final Report

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INTRODUCTION

Beef and dairy cattle have a crucial competitive advantage over non-ruminant livestock due to their ability to transform forages into animal protein. Of the solar energy captured by the earth's biomass, only 5% is available as a direct source of food for humans (Russell and Gahr, 2000). Therefore, the ability to transform part of that biomass into animal protein is important. Cellulose is the most abundant carbohydrate on earth; however, it cannot be digested by mammalian enzymes. The symbiotic relationship existing in ruminant animals, by which microorganisms harbored in the gastrointestinal tract ferment cellulose and other structural carbohydrates releasing volatile fatty acids that serve as an energy source for the host, is the key to the success of beef and dairy production when compared to non-ruminant species. However for cellulose to be digested efficiently by the rumen microorganisms, a timely balance between energy and protein needs to exist. Achieving this balance can be challenging under most beef cattle production systems.

The Southeastern region of the United States presents several characteristics that make it unique when compared to other beef cattle regions. The states that represent the southeast and Gulf Coast regions account for 48% of all beef cows in the United States. In the current context of increasing feed input costs, the abundant forage production in the Southeastern U.S. provides an opportunity to decrease the cost of production, considering that feeding is the largest cost in a cattle operation. Unfortunately the predominant forages in this region can be of limited nutritive and often not sufficient to support high levels of production. As a result, there are some critical periods during the year in which there is a need for supplementation with energy and/or protein in cow/calf operations in the Southeast (Hersom et al., 2011). Combined with the abundance of forages, another advantage of beef production in the Southeastern U. S. is the availability of several byproducts and coproducts from diverse industries, which can have a great nutritional value for cattle and can provide an excellent opportunity to correct nutritional imbalances a through strategic supplementation.

The sugar industry is very strong in the state of Florida and as a result, byproducts such as molasses have been fed to cattle for decades (Pate and Kunkle, 1989; Kunkle et al., 2000).

Molasses provides an excellent complement to the forages found in Florida as it complements the often low energy (TDN) content of warm season forages. The excellent palatability of molasses can be a double-edged sword: in one hand provides an opportunity to stimulate intake, especially in feeds or forages of low palatability; however, due to the same reason, can lead to overconsumption when offered free choice. Overconsumption of molasses can have a negative effect on rumen fermentation by producing excess amount of volatile fatty acids (VFA) and lactic acid, which in turn can decrease pH, depress fiber digestion, or cause acidosis (Kovacik et al., 1986; Owens et al., 1998). It is for this reason that the search for cost-effective intake limiters that can be added to free choice supplements continues to be a research priority.

Crude glycerin (or crude glycerol) is a generally recognized as safe (GRAS) animal food ingredient and is generally produced as a coproduct of the soap making industry. More recently, the increase in production of biodiesel has prompted the use crude glycerol, which contains the contaminant methanol, for cattle feeding (Sellers, 2008). The U.S. Food and Drug Administration established a limit of 150 ppm of methanol in crude glycerol in order to be considered safe for animal feeding. Several studies in the recent years have reported the use of glycerol as a replacement for corn in finishing diets (Krehbiel, 2008; Bach et al., 2009; Parsons et al., 2009), however few studies have addressed the use of glycerol in forage-based diets (Hess, 2008). One of the benefits of glycerol when added to forage based diets may be related to the ruminal fermentation profile. The main end products of glycerol fermentation in the rumen are propionic and butyric acid (Hess, 2008; Krehbiel, 2008). The fermentation of glycerol may lead to a reduced concentration of ruminal lactate when compared to other supplemental feeds such as grain byproducts or molasses which may depress fiber digestibility by a decrease in ruminal pH mediated by lactate accumulation. However the main disadvantage of glycerol feeding is that at high inclusion rates can depress intake and digestibility (Hess, 2008; Parsons et al., 2009). In forage-based diets, Hess (2008) found that up to 13.3% inclusion of glycerol did not affect the digestibility of forages in vitro.

The concept of complementarity of fermentation profiles is very commonly exploited in feedlot nutrition by the inclusion of mixtures of cereal grains with different grain processing methods in the same diet. The rate of fermentation of starch in different processing methods produces a more sustained release of energy, favoring overall nutrient synchrony of finishing diets. Applying this concept to liquid feeds, ***we hypothesize that the inclusion of a mixture of liquid supplement may provide a more sustained release of energy during ruminal carbohydrate fermentation, favoring forage digestion***. Feeding up to 2.3 kg (5 lb) per day of a 50:50 mixture of glycerol:molasses to a cow consuming approximately 10.9 kg (24 lb) of hay DM/d should maintain the inclusion level of glycerol under 13% of the diet DM, which has been identified as the threshold above which the digestibility of fiber is affected (Hess, 2008).

The objectives of this study are: 1) to determine the effects of different levels of supplementation with a 50:50 mixture of molasses:crude glycerol on bermudagrass hay intake and digestibility of nutrients in the total tract, and 2) to determine the effects of different levels of supplementation with a 50:50 mixture of molasses:crude glycerol on ruminal in situ digestibility of the fiber fraction and in vitro ruminal fermentation parameters.

MATERIALS AND METHODS

Phase 1: in vivo nutrient digestibility

Phase 1 of this study was conducted in April, 2013 at the University of Florida Feed Efficiency Facility (FEF) located at the North Florida Research and Education Center (NFREC) in Marianna, FL. Liquid supplement was provided by Westway Feed Products (New Orleans, LA). A total of 24 Angus crossbred heifers (380 ± 31 kg) were used in the study. On d 0, heifers were weighed after 16-h feed withdrawal, stratified, and blocked by initial BW (2 blocks: lightest and heaviest), and randomly assigned to one of 4 treatments: 0, 0.45, 1.36, and 2.27 kg/d (as fed basis) of a 50:50 mixture of crude glycerol:molasses (equivalent to 0, 1, 3, and 5 lb/d, respectively). Heifers were housed in individual pens at the FEF for 28 days and had ad libitum access to Tifton 85 bermudagrass hay, which was ground and placed in the feed bunk. The amount of liquid supplement corresponding to each treatment was weighed and offered daily in a plastic container inside the pen to each individual animal. Any unconsumed amount of supplement was weighed and recorded for the first 7 d. By day 8, all heifers were consuming the entire amount of liquid supplement every day, thus no orts recording was needed. After the 14-d adaptation to diets and facility, heifers were weighed and collection of daily feed intake and intake behavior data started. Each pen at the FEF was equipped with two GrowSafe tubs (GrowSafe System Ltd., Airdrie, Alberta, Canada) to record intake by weight change measured to the nearest gram. Beginning on d 22 and d 23, feed (hay and liquid) and fecal samples were collected, respectively, for 4 consecutive days to determine apparent total tract digestibility of DM, OM, CP, NDF, and ADF. Feed samples were collected once a day immediately after delivery of liquid supplement. Fecal samples were collected twice a day at 0800 h and 1600 h from the ground, inside the pen, right after the animal defecated. Feed and fecal samples were pooled within heifer and indigestible NDF (iNDF) was used as an internal indigestible marker. On d 28 heifers were weighed again after withholding feed for 16 h, for evaluation of performance during the entire period. Concentrations of iNDF in feed and feces were determined as described by Cole et al. (2011) with the following modification: in vitro incubations were conducted for 288 h instead of 96 h using the Daisy II incubator (Ankom Technology, Macedon, NY) to ensure complete digestion of potentially digestible NDF in hay as reported by Krizsan and Huhtanen (2013).

Statistical analyses. Data on Phase 1 was analyzed as a generalized randomized block design using the MIXED Procedure of SAS (SAS Institute Inc., Cary, NC). Animal was considered the experimental unit and the model included the fixed effects of treatment, and the random effect of block. Orthogonal polynomial contrasts were conducted to determine the effects of supplementation level on nutrient digestibility or animal performance. Significance will be determined at $P \leq 0.05$ and tendencies will be considered when $0.10 > P > 0.05$.

RESULTS (Phase 1 only)

Table 1. Analyzed[1] chemical composition of Tifton 85 bermudagrass hay and liquid supplement fed to heifers during the experiment.

Item	Hay	Liquid supplement[2]
DM, %	91.2	74.83
OM, %	85.6	86.44
CP, %	13.0	5.55
NDF, %	71.5	-
ADF, %	40.6	-
TDN, %	56.0	79.00
Calcium, %	0.41	0.39
Phosphorus, %	0.29	0.08
Magnesium, %	0.32	0.23
Potassium, %	1.63	3.09
Sodium, %	0.09	1.37
Sulfur, %	0.49	0.44
Methanol[3], ppm	-	<100

[1]Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY).
[2]50:50 mixture of molasses:crude glycerol.
[3]Analyzed by SDK Laboratories (Hutchinson, KS).

Table 2. Effects of supplementing increasing levels of a 50:50 mixture of molasses:glycerol on performance by heifers fed Tifton 85 bermudagrass hay ad libitum for 28 d.[1]

	Treatment[1]						
Item	CTRL	SUP1	SUP3	SUP5	SEM[2]	*P*-value[3]	Contrast[4]
Initial BW, kg	379	376	386	379	7.9	0.85	NS
Final BW, kg	415	415	425	423	8.8	0.81	NS
ADG, kg	1.31	1.37	1.39	1.56	0.075	0.11	Linear*
Total DMI, kg/d	5.4[a]	5.58[ab]	6.31[bc]	6.62[c]	0.312	0.04	Linear*
Hay DMI, kg/d	5.4	5.24	5.29	4.92	0.313	0.74	NS
Hay DMI, % of BW	1.36	1.33	1.29	1.23	0.066	0.52	NS
Total G:F[5]	0.243	0.244	0.226	0.237	0.0121	0.70	NS
Hay G:F[6]	0.243[a]	0.260[a]	0.274[a]	0.319[b]	0.0157	0.02	Linear*

[a,b,c]Within a row, means without common superscript differ ($P \leq 0.05$).
[1] CTRL: Tifton 85 bermudagrass hay fed ad libitum; SUP1: Tifton 85 bermudagrass hay fed ad libitum + 0.45 kg/d of a 50:50 molasses:glycerol mixture; SUP3: Tifton 85 bermudagrass hay fed ad libitum + 1.36 kg/d of a 50:50 molasses:glycerol mixture; SUP5: Tifton 85 bermudagrass hay fed ad libitum + 2.27 kg/d of a 50:50 molasses:glycerol mixture.
[2]SE of treatment means; n = 6 heifers/treatment.
[3]*P*-value for the overall treatment effect.
[4]Orthogonal contrasts: Linear = linear effect of liquid feed supplementation level. *$P \leq 0.05$; NS = $P \geq 0.10$.

[5]Gain:feed ratio calculated using total DMI.
[6]Gain:feed ratio calculated using hay DMI only.

Table 3. Effects of supplementing increasing levels of a 50:50 mixture of molasses:glycerol on nutrient intake and apparent total tract digestibility by heifers fed Tifton 85 bermudagrass hay ad libitum for 28 d.

	Treatment[1]						
Item	CTRL	SUP1	SUP3	SUP5	SEM[2]	*P*-value[3]	Contrast[4]
Intake[5], kg/d							
DM	6.74	6.10	5.79	5.80	0.315	0.14	Linear*
OM	6.36	5.74	5.40	5.38	0.301	0.10	Linear*
CP	0.87^{c}	0.74^{bc}	0.65^{ab}	0.56^{a}	0.047	0.002	Linear*
NDF	5.13	4.62	4.41	4.42	0.234	0.13	Linear*
ADF	2.54	2.30	2.19	2.18	0.115	0.12	Linear*
Digestibility, %							
DM	48.5^{a}	51.2^{a}	55.1^{b}	59.0^{c}	1.24	<0.001	Linear*
OM	50.0^{a}	52.3^{a}	55.4^{b}	59.2^{c}	1.12	<0.001	Linear*
CP	51.0^{b}	48.9^{b}	46.3^{b}	38.2^{a}	2.57	0.01	Linear*
NDF	55.1^{a}	57.4^{a}	61.4^{b}	65.5^{c}	0.86	<0.001	Linear*
ADF	52.7^{a}	55.5^{b}	60.6^{c}	64.9^{d}	0.92	<0.001	Linear*

[a,b,c] Within a row, means without common superscript differ ($P \leq 0.05$).
[1] CTRL: Tifton 85 bermudagrass hay fed ad libitum; SUP1: Tifton 85 bermudagrass hay fed ad libitum + 0.45 kg/d of a 50:50 molasses:glycerol mixture; SUP3: Tifton 85 bermudagrass hay fed ad libitum + 1.36 kg/d of a 50:50 molasses:glycerol mixture; SUP5: Tifton 85 bermudagrass hay fed ad libitum + 2.27 kg/d of a 50:50 molasses:glycerol mixture.
[2]SE of treatment means; n = 6 heifers/treatment.
[3]*P*-value for the overall treatment effect.
[4]Orthogonal contrasts: Linear = linear effect of liquid feed supplementation level. *$P \leq 0.05$; NS = $P \geq 0.10$.
[5]Intake during the digestibility measurement period of the experiment (d 22 to 26).

FUTURE WORK

Phase 2

A total of 8 steers were cannulated on August 28, 2013 to be used during Phase 2 of the study. Phase 2 will start in October of 2013 and will consist on a 4 x 4 duplicated Latin square with 4 experimental periods of 28 d each. The sample collection schedule for each experimental period is described in Figure 1. Each experimental period will have 14-d adaptation to diets, a 7-d sample collection period and a 7-d washout period. During which the 7-d sample collection period, the following measurements will take place:

- Ruminal pH: continuously measured for 48 h
- Ruminal VFA concentrations, measured every 2 h for 24 h
- Ruminal NH_3-N concentrations, measured every 2 h for 24 h
- Ruminal in situ DM, CP, and NDF degradability of hay (same cut as that used in Phase 1)
- Blood samples collected every 3 h the determine plasma urea nitrogen concentrations

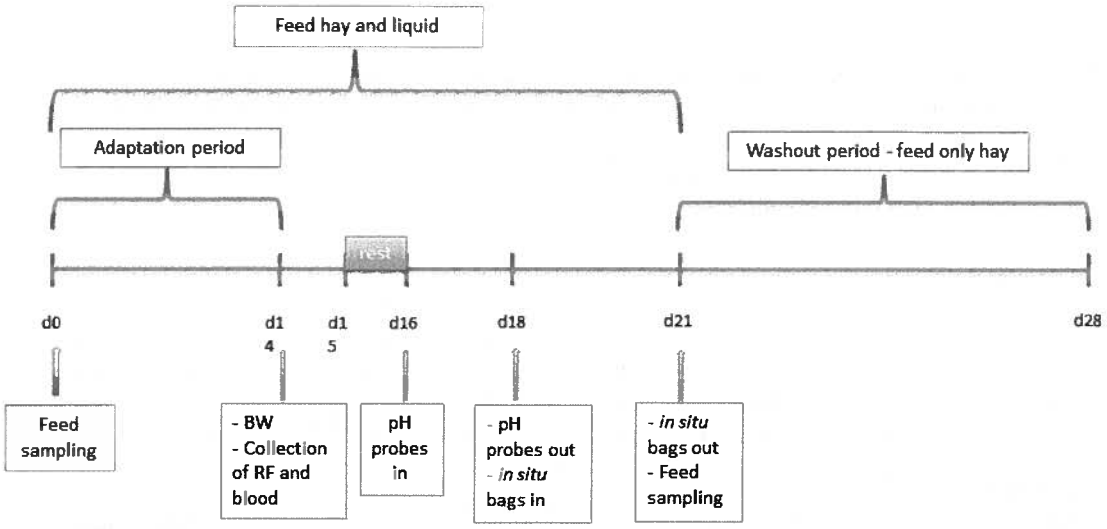

Fig. 1. Sampling schedule for each experimental period in Phase 2.

Phase 3

In vitro batch culture incubations will be conducted to determine the effects of the treatments tested in Phase 1 and 2 on ruminal fermentation parameters, gas production kinetics and total methane production.

Two ruminally cannulated steers will be used as rumen fluid donors for the in vitro incubations. The steers will be fed a diet comprised of 100% Tifton 85 bermudagrass hay (same hay used in Phases 1 and 2) for at least 14 d prior to the collection of ruminal fluid to perform the in vitro incubations. In vitro incubations will be performed in three separate days (replicates) using a substrate comprised of the same 4 diets tested in Phase 1 and considering the hay intake observed to determine the ratio of hay to liquid supplement. The substrate will be mixed with the inoculum (3:1 mixture of McDougall's buffer:ruminal fluid) and duplicate bottles per treatment will be incubated in each day replicate.

The in vitro incubations will be conducted in 250-mL bottles and gas production kinetics will be recorded using the Ankom Gas Monitoring System (Ankom Technologies, Macedon, NY). Bottles containing 1.4 g of substrate and 100 mL of inoculum will be incubated for 24 h at 39°C and volatile fatty acid concentrations will be measured at the end of the incubation period along with NH_3-N concentrations, and final pH. Methane produced during the fermentation will be measured by capturing total gas produced in a Tedlar sample bag and analyzing methane concentration by gas chromatography.

A measurement of in vitro dry matter digestibility will be performed by incubating a separate set of duplicate 100-mL scintillation flasks for 24 h at 39°C in each of the replicate days. These flasks will contain 0.7 g of substrate and will be inoculated with 50 mL of a 3:1 McDougall's buffer:ruminal fluid mixture.

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