

Effects of replacing soybean hulls with molasses during the receiving period of feedlot calves

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Introduction: Forage is a necessary purchase in most cattle feeding operations in the US; however, it is one of the most expensive dietary ingredients when considering the cost per unit of energy (Z. K. Smith, 2021). It is during the entrance to the feedlot that cattle are generally offered diets with the greatest inclusion of forage. These receiving diets are formulated to get cattle started on total mixed rations with moderate gains as they transition to final, high-grain diets. This receiving phase is often overlooked to attempt to garner greater performance from calves prior to starting on the high-grain rations. Due to an increase in nutrient utilization, mainly expressed by greater fiber degradation and consequent greater growth performance (Ciriaco et al., 2015), as well as an improved diet consumption (Havekes et al., 2019), the inclusion of molasses into receiving diets of feedlot steers has the potential to mitigate the stressors associated with cattle transportation by inducing a healthier physiology of digestion and immunological response.

When considering impacts of molasses on ruminal fermentation, one must also consider the changes in fermentative pathways. When utilized in the rumen, molasses increased concentrations of ruminal butyrate (Ciriaco et al., 2016), whereas fermentation of fiber, such as soybean hulls, usually lead to increases in the concentration of acetate. In the rumen, for every mol of acetate produced by bacteria, there is 1 mol of CO₂ and H₂ produced as byproducts. On the contrary, butyrate production actually utilizes 1 mol of H₂ and produces 1 mol of CO₂. Carbon dioxide and H₂ are the substrates for methane. Therefore, by replacing a high energy ingredient, such as soybean hulls, in the receiving diets of growing steers, it is likely that enteric methane production would decrease due changes in the fermentative pathways of ruminal bacteria.

The increase in butyrate associated with the addition of molasses can have lasting impacts on gut health and development. These include greater epithelial cell growth since epithelial cells utilize butyrate as an energy source. The growth in epithelial cells leads to improved papillae morphology increasing absorption of nutrients in the rumen. Furthermore, with greater gut health, there is likely a reduction in inflammatory responses from cattle experiencing stress, such as shipping to feedlots. Reduction in inflammatory responses can lead to greater overall health and performance of growing steers.

The rationale for designing an experiment to replace soybean hulls with molasses in receiving diets is to develop strategies for producers in the Southeast to retain ownership throughout the finishing phase of the beef industry. Since 2020 when the COVID pandemic began, there has been a rapidly growing demand for locally raised beef throughout the US. In Georgia in particular, the state government is using funds to develop small slaughter facilities throughout the state to fulfill the consumer demands. This will require that producers in the Southeast begin to finish cattle in their own environments with the commodities available to

them, hence the usage of soybean hulls rather than corn in the proposed receiving diet. Both molasses and soybean hulls are readily available in the Southeast and are likely candidates for growing cattle operations.

Specific Objectives:

1. *Determine the impact of replacing soybean hulls with molasses in the receiving diets of feedlot weaned steer-calves on growth performance, nutrient digestibility, enteric methane emissions, and blood health marker.* We hypothesize that steers consuming diets with molasses will have greater fiber digestibility leading to greater growth performance. Furthermore, we hypothesize that, due to the fermentative pathways of molasses leading to greater butyrate concentration, enteric methane emissions will be reduced
2. *Quantify the effects of replacing soybean hulls with molasses in receiving diets on blood inflammatory markers.* We hypothesize that steers will have lesser gastro-intestinal tract dysfunctions due to positive impacts of butyrate on gut health. This will in turn lead to fewer inflammatory responses, such as LPS-binding protein.

Procedures: All procedures involving animals will follow standard approval from the UGA Institutional Animal Care and Use Committee (IACUC). A brief description of major activities follows.

Experimental design, animals, diet and treatments: 36 Angus-crossbred steers, from the University of Georgia – Tifton Beef Center, will be used in a randomized complete block design with 2 treatments to investigate the effects of replacing soybean hulls in feedlot receiving diets with liquid molasses. Diet will be considered treatment with the main difference being high-energy source. The control diet will comprise (DM basis) 30% soybean hulls, 48% corn silage, 19.5% dried distillers grains plus solubles, 0.5% urea, and 2% vitamin and mineral packet (NEg = 0.51 Mcal/lb; CP = 15.07%; RDP = 65.46%). The treatment diet will comprise 27% molasses, 48% corn silage, 20% dried distillers grains plus solubles, 3% corn gluten meal, and 2% vitamin and mineral packet (NEg = 0.55 Mcal/lb; CP = 14.48%; RDP = 63.48%). The diet was formulated to meet or safely surpass requirements published by the Nutrient Requirements of Beef Cattle (NASEM, 2016).

Experimental period: The experiment (approximately 90 days) will consist of 2 periods (45 days each) for data. Each period will have a different cohort of steers (18 head per period). Briefly, on days -2 and -1, steers will be weighed and the average of the 2 days will be considered initial body weight. On day 1, after weighing, steers will be transported via truck and trailer for 12 hours (6 hours in one direction then 6 hours back to the facility) to induce stress experienced by calves when shipped to another location as is standard in the beef industry. Steers will be provided hay the evening they return to the facilities. Dietary treatments will commence on day 0 of the experimental periods. Other body weight measurements will be recorded on days 9, 18, 27, 36, 44, and 45. Final body weight will be considered the average of two consecutive weights recorded on days 44 and 45. Blood samples will be collected via venipuncture on days corresponding to body weight measurements. Feed intake data will be collected daily by measuring dry matter of feed offered and orts refused. From day 14 - 20, breath samples will be

collected, using the sulfur hexafluoride technique, to determine enteric methane production; during the breath sample collection periods, feces will be collected to determine nutrient digestibility. This experimental period will be replicated with the 2nd group of steers in Period 2.

Feed intake measurements: Feed bunks will be evaluated visually each day of the experiment to determine the quantity of feed to offer to each steer. The bunk management approach will be designed to allow between 3 to 5% of feed remaining in the feed bunk daily. Diets will be mixed in a tractor-pulled mixer mounted on load cells and delivered to the pens by hand. Samples of dietary ingredients will be taken weekly during the experiment to determine nutrient content. Fresh subsamples will be composited within weigh periods for further nutritional analyses. Daily, feed bunks will be swept, and any feed remaining in the bunks (if any) will be weighed and its dry matter content determined (when greater than 5% of amount offered). Samples of feed delivered to the bunks and refusals (if greater than 5%) will be analyzed for nutrient content.

Apparent total tract digestibility: To determine apparent total tract digestibility of dry matter (DM), organic matter (OM), and fiber components (NDF and ADF), feed and fecal samples will be collected daily for 4 consecutive days during the methane collection period. Fecal samples will be taken twice daily at 0800 h and 1600 h via rectal grab. Feed and fecal samples will be stored frozen at -20°C. Feed and fecal samples will be composited within animal and day, dried at 55°C in a forced-air oven for 72 h, ground in a Willey mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2-mm sieve for further analyses of nutrient content and digestibility marker concentration. Indigestible NDF (iNDF) will be used as an internal marker to determine digestibility.

Enteric methane emissions: In vivo CH₄ emissions will be measured using the SF₆ tracer technique (Johnson et al., 1994; Henry et al., 2015; Henry et al., 2020). On day 0, steers will be dosed, intraruminally, using a balling gun with a brass permeation tube containing approximately 2.5 g of SF₆ designed to release SF₆ at a rate of approximately 7-9 mg per day. Continuous collection of breath samples for analysis of CH₄ and SF₆ will be taken for at least 5 continuous days. To collect the samples, steers will be fitted with a nylon halter, specifically designed for gas collection experiments, with tubing that connects to a polyvinyl chloride (PVC) collection canister, which is placed around the neck of the steer. Each canister will be under vacuum (25 in Hg) to ensure a continuous collection of sample for 24 h.

Laboratory analyses: For feed and fecal DM and OM, samples will be weighed into ceramic crucibles and dried at 100°C for 4 h. After weighing, crucibles will be placed in an ashing oven at 600°C for 4 h.

For fiber analyses (NDF and ADF), previously dehydrated and ground feed and fecal samples will be placed in duplicate F57 bags (Ankom Technology, Macedon, NY) and analyzed in an Ankom²⁰⁰ Fiber Analyzer using a heat-stable α -amylase, sodium sulfite, and adjusting for final residual ash. Subsequent ADF analysis will be performed (Van Soest et al., 1991).

Crude protein of feed and fecal samples will be determined by combustion using a LECO TruMac N analyzer (LECO, St. Joseph, MI) following official method 992.15 (AOAC, 1995).

Concentration of iNDF in feed and fecal samples will be determined as proposed by Cole et al. (2011) with modifications by Krizsan and Huhtanen (2013). Samples will be placed in F57 bags and incubated for 288 h in the rumen of a ruminally-cannulated steer. Samples will then be rinsed, air-dried and analyzed for NDF concentration as described above (without discounting final ash residue).

Blood samples will be analyzed for the inflammatory marker LPS binding protein utilizing test kits. Samples will be analyzed according to manufacturer directions.

Calculations and Statistical analysis: Total tract digestibility of DM, OM, NDF, and ADF will be calculated as follows: $100 - 100 \times [(\text{marker concentration in feed consumed} / \text{marker concentration in feces}) \times (\text{nutrient concentration in feces} / \text{nutrient concentration in feed consumed})]$. All data will be analyzed as a generalized randomized block design using the MIXED procedure of SAS. The model will include the fixed effect of treatment and the random effect of period. Steer within period will be considered the experimental unit. Significance will be declared at $P \leq 0.05$.

Literature cited

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Budget: We are requesting \$6,000 in funding to cover partial funds for a graduate student who will manage the project, coordinate, and conduct data collection, preparation, sample processing and analyzing throughout the duration of the project. Materials and supplies (\$12,000) will include, but are not be limited to: field and lab consumables used in field evaluations including sample bags, tags, needles, syringes, test kits, labels, paper, pens, clipboards, notebooks, ink, markers, computer supplies, thumb drives, and fees associated with cloud-based file storage; personal protection equipment including, but not limited to, sunscreen, gloves, bug spray, water, coolers, Gatorade mix, and uniforms to protect from heat and sun damage during data collection; general supplies for beef cattle management; supplies for collection of breath samples; feed sample analyses, breath sample analysis, including all reagents and bottles to conduct measurements; diesel fuel and gasoline used by UGA vehicles and equipment in the research and extension efforts. We also request travel expenses (\$2,000) to be dedicated for this project, including funding for the extension efforts that may necessitate overnight travel for one or more of our team members, and funding for one of our team members to attend a professional meeting so that we can present the results in a professional setting and obtain peer-review of this research effort. Thus, our total request for this project is \$20,000.

| Item | Price |
|------------------------|-----------------|
| Materials and supplies | \$12,000 |
| Labor | \$6,000 |
| Travel expenses | \$2,000 |
| TOTAL | \$20,000 |