DETERMINATION OF THE OPTIMAL LEVEL OF DRIED DISTILLERS GRAIN INCLUSION FOR METHANE MITIGATION; AN IN VITRO STUDY

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Determine the Optimal Level of Dried Distillers Grain Inclusion for Methane Mitigation; An Vitro Study

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The negative effects of high level of methane emissions from cattle operations are of particular concern to producers. Current management practices call for a variety of feed additives to be used to help mitigate methane production and enhance overall animal efficiency. However growing consumer concern and increasing regulations have led to the requirement of new management practices. Dried distillers grain (DDG) in both whole and defatted forms has shown promising effects on methane mitigation, however an optimal level of inclusion has yet to be determined for either form of this byproduct. By using an *in vitro* gas production (IVGP) technique to model the rumen environment, different percentages and forms of DDG inclusion can be compared, and an optimal level and form can be determined by comparing the gas chromatography (GC) results of methane concentration following the incubation of each diet containing a different level and form of DDG inclusion.

DEDICATION

I dedicate this thesis to Dr. Josie Ann Coverdale (1977-2016) – a phenomenal researcher, horsewoman, and mentor. Thank you for being the one to first spark my love and passion for animal nutrition and for making such a lasting impact on not only my life, but also on the lives of countless other students.

ACKNOWLEDGMENTS

I would like to thank Dr. Luis Tedeschi and Ms. Whitney Crossland for their invaluable help and guidance throughout this project and the writing of this thesis.

NOMENCLATURE

DDG	dried distiller's grains
IVGP	*in vitro* gas production
GC	gas chromatography
DM	dry matter

CHAPTER I

INTRODUCTION

History of greenhouse gas emission by beef cattle production

Greenhouse gasses are currently being investigated to determine how detrimental their effect is on overall climate change trends. Methane (CH_4) is among the greenhouse gasses currently being studied in regards to climate change. This is of particular concern to producers in the agriculture sector as this gas is emitted by ruminants, especially beef and dairy cattle. Methane is naturally produced by cattle through the rumination process due to their inherent microbial fermentation. The Environmental Protection Agency (EPA) has reported a consistently high methane emission rate from beef cattle since 2005[1]. In addition to the concern over greenhouse gas emissions, there is also a growing concern by consumers over feed additives usage in cattle. This poses a problem for beef cattle producers, as current production methods primarily utilize feed additives to increase the efficiency of beef cattle, which in turn also work to mitigate methane production—to a limited degree. The ideal solution would be to identify a management practice that would utilize the ideal inclusion of a feed ingredient at optimal levels that would allow the producer to maintain maximum production efficiency, as well as assuage the EPA's concerns by decreasing levels of overall methane production.

Precedent for research into DDG and methane mitigation

Results from the Texas A&M Ruminant Nutrition Laboratory have shown promising results with replacement of corn with dried distillers grain (DDG) in beef cattle rations in both *in vitro* as well as *in vivo* studies (Figure 2; Tedeschi et al, unpublished, figure attached in References). It is

believed that this is directly due to the fat found in DDG that aid in methane mitigation. Dietary fat, which is high in medium-chain fatty acids and/or polyunsaturated fatty acids, has been shown to have anti-methanogenic activity. However, the level of anti-methanogenic activity is heavily influenced by dosage as well as the physical composition of the fat[2,3]. DDG also contains high levels of these medium-chain and polyunsaturated fatty acids. To further confirm, the results from the aforementioned Texas A&M Ruminant Nutrition Laboratory research showed a nonlinear decrease in methane emissions with a 0.005 L/kg/d decrease in methane emissions for each % dry matter (DM) increase of DDG in the diet (P=0.0027). Additionally, there has been further research that has shown that placing growing and finishing feedlot cattle on a diet high in DDG (>50% inclusion rate) results in no loss of average daily gain when compared to cattle fed a traditional all corn growing/finishing ration.[6] This suggests that there would likely be no loss of production viability when optimal levels of DDG are included in terminal beef cattle diets. DDG is also a more inexpensive feedstuff than most corn diets, thus production value would in fact likely be positively impacted by optimal inclusion of DDG. This is in addition to the reduced production of CH_4 emissions, which also indicates greater feed efficiency, as well as minimizing the environmental impact of large-scale cattle productions.

However, there has yet to be further research to effectively determine the optimal level of DDG inclusion, or the best form of DDG, whether whole or in defatted form, in feedlot beef cattle diets for the highest level of methane mitigation, without hindering overall animal performance. The purpose for also testing the defatted form of DDG would be to effectively test the hypothesis of whether it is the medium chain fatty acids found in whole DDG that is having the

methanogenic effect, or if there is another factor at play in the feedstuff such as sulfur content that causes this effect.

CHAPTER II

METHODS

Twelve different diets with different levels of DDG inclusion, and either defatted or whole DDG, and a control diet containing the baseline feeds without any DDG additives were compared for their methane mitigation capabilities. These diets are shown in Table 1. The diets were compared using the *in vitro* gas production (IVGP) technique described by Tedeschi et al[4], which is also further, explicitly described in the body of this report for clarity.

Table 1. DDG Type and Inclusion Levels for Test Diets

Whole/Defatted DDG	% DM of DDG Inclusion	Test Diet ID
Defatted	50	A
	40	B
	30	C
	20	D
	10	E
	0 (CONTROL)	F
Whole	50	G
	40	H
	30	I
	20	J
	10	K
	0 (CONTROL)	L

An explanation of *in vitro* gas production (IVGP) technique

An incubator chamber, with a multi-plate stirrer apparatus, was utilized for this project to simulate the environment consistent with the internal temperature and the kinetic activity of the rumen environment. Each sample was placed in a 125-mL Wheaton bottle and each bottle was fitted with a stir bar to allow for the facilitation of the desired, simulation of the kinetic activity

of the rumen. Feed samples, each with a mass of 200mg was the transferred to the Wheaton bottles after weighing and 14 mL of rumen media and 10mL of distilled water was then added. Rumen media has been consistently used by the Texas A&M Ruminant Nutrition Laboratory in past nutrition *in vitro* trials to more closely simulate the natural buffering capacity of the rumen that is normally completed via the ruminating and re-mastication process. The bottles were then continuously filled with CO_2 to prime the bottles and create a favorable environment for ideal microbial ecology prior to being sealed with airtight stoppers and crimping metal lids to keep gases from escaping. Ruminal fluid inoculum was then added to the bottles using injection syringes with 20-gauge needles. The inoculum provided the most similar microbial environment to what would be found in the rumen environment. The ruminal fluid inoculum was obtained from rumen-cannulated steers with free access to medium-quality mixed grass forages and fed once daily with a commercial ration for non-lactating steers, formulated by the Texas A&M Nutrition and Physiology Center. The bottles containing the five different test diets (Figure 1), plus the control were then placed in the incubator chamber for 48 hours. When the incubation chamber reached 39° C, the ideal temperature of the rumen environment, the 48 hour incubation period began. Each test diet, as well as the control, were then completed in duplicate for each 48 hour incubation period, referred to as a "run". This allowed for maximum data collection and maximum utilization of the incubation chamber during each test run.

Gas production measurement technique

Following the 48-hour incubation period, the IVGP sample bottles were allowed to cool to room temperature to allow for the gases within the bottles to mix fully, thus allowing for accurate gas concentration measurement results. Once the bottles cooled to room temperature, they were taken to the Agricultural Research Services building on F&B Road in College Station, TX. The methane emissions for each sample were measured using a GowMac thermal conductivity series 580 gas chromatograph (GC, Gow Mac Instrument, Bridgewater, NJ) equipped with a Porapak Q column (60°**C,** 20 mL/min of N_2 carrier gas). A gas sample of 0.5 mL was removed from each of the IVGP sample bottles using a 0.5 mL glyercolubricated syringe and inserted into the GC. Each sample was analyzed for hydrogen (H_2), carbon dioxide (CO_2) and CH_4 concentrations.

After obtaining all of the measurement readouts for each of the sample bottles, the peaks for each of the gases extruded was measured using a digital Vernier caliper and the heights of the peaks were recorded in mm. The information for the CO_2 gas production was not recorded, as this emission did not have bearing on the study conducted. The heights of the peaks were then transferred to the GasFit modeling system, developed by Dr. Luis O. Tedeschi, to allow for the test diets to be compared based on their methanogenic activity. The gas data was also run through the SAS program using the PROC MIXED in a 2 x 6 factorial arrangement in duplicate to check the results for statistical significance.

CHAPTER III

RESULTS

Analysis of overall gas production using GasFit and SAS programs

Upon initial analysis of gas pressure data collected during the 48-hour incubation period, it was apparent that there was a significant loss of pressure in a majority of the bottles. Due to this equipment error, statistical analysis became more difficult to carry out because convergence of the gas data in the GasFit program was not possible. However, using the raw gas data, estimations were determinable for use in further analysis by the SAS program.

Based upon the results obtained by the SAS program, there was no statistically significant effect on total gas production when forms were compared, that is, there was no difference between whole DDG and defatted DDG. There was also no statistically significant effect of inclusion rate of either form of DDG on total gas production.

Table 2. Statistical comparison of overall gas production

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Form	1	12	3.05	0.1061
Inclusion Level	5	12	1.78	0.1916
Form*Level	5	12	1.70	0.2095

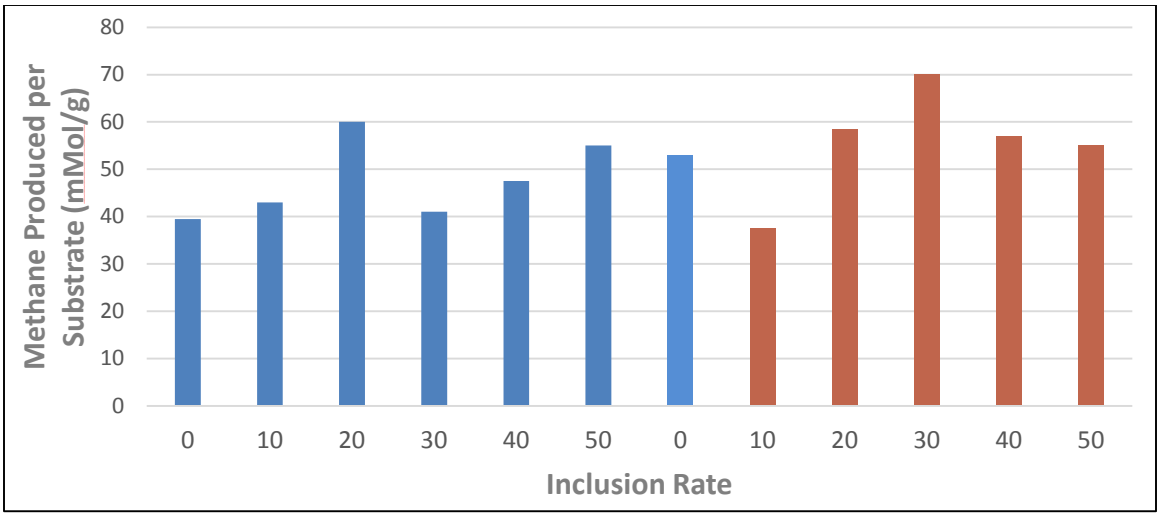

Figure 1. Comparison of overall gas production by form and inclusion level

Comparison of whole DDG and defatted DDG in methanogenic capability using GasFit

Using the same hypothetical convergence estimates provided by the GasFit program and the same factorial arrangement in the SAS program, methane production was analyzed for statistically significant effects of either form or inclusion rate. Initially, it was observed that the results were the same for form. There was no significant effect of varying the level of inclusion for either of the forms of DDG on reducing the amount of methane that was produced.

However, when form of DDG was compared for methane mitigation effects there was a significant reduction in the amount of methane produced in those diets that included deffated DDG than those that included the whole form of DDG. The methane produced by diets that included whole DDG yielded an average of 0.74 mMol of CH_4 per gram of substrate whereas defatted DDG significantly reduced that amount to 0.48 mMol of CH_4 per gram of substrate.

Table 3. Statistical comparison of CH_4 production

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Form	1	12	14.53	0.0025
Inclusion Level	5	12	1.29	0.3304
Form*Level	5	12	2.09	0.1377

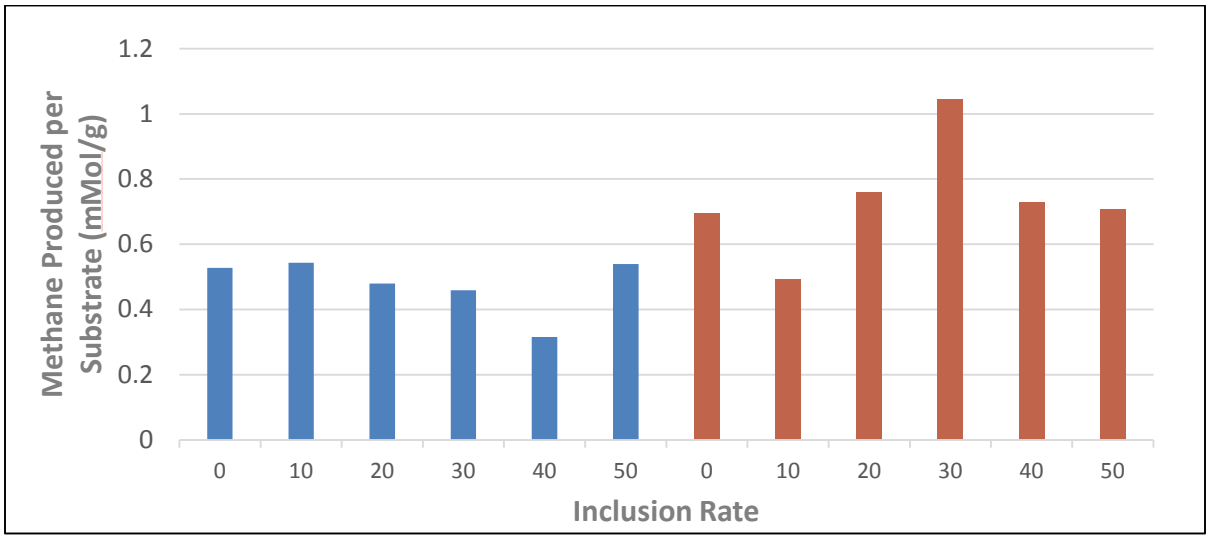

Figure 2. Comparison of CH_4 production by form and inclusion level

This indicated that there is some intrinsic component to defatted DDG that makes it a better source of CH_4 mitigation. One potential is that defatted DDG contains a potential hydrogen sink. A hydrogen sink being a way that hydrogen is utilized in the fermentation process by binding with other nutrients instead of carbon so that methane is not produced in such high amounts.

Whole DDG has high amounts of unsaturated fatty acids, which is a known hydrogen sink, however it is apparently not as effective as the hydrogen sink contained in defatted DDG as it did not yield less CH_4. Based upon the nutritional analysis in Table 4 below, there is a much greater percentage of sulfur in defatted DDG than in whole DDG. This is due to a relative increase in the concentration of all nutrients when the ether extract (fat) fraction is removed from the DDG.

Table 4. Sulfur content of DDG diets in different forms and inclusion levels

Inclusion Level of DDG	**Sulfur Content (% DM)**	
	Whole DDG	**Defatted DDG**
50%	0.375	0.404
40%	0.352	0.366
30%	0.329	0.341
20%	0.306	0.316
10%	0.283	0.291
Recommended[7]	**≤ 0.5**	

When hydrogen is exposed to lager amounts of sulfur during the fermentation process the product hydrogen sulfide, H_2S, can be formed, thus, sulfur provides a hydrogen sink. Therefore, based upon the greater CH_4 mitigation abilities of the defatted DDG, this would indicate that sulfur may be a better hydrogen sink than unsaturated fatty acids.

CHAPTER IV

CONCLUSION

Dried distillers' grain has many benefits as a feed additive in the beef cattle industry. It is an easily attainable and very inexpensive feed additive as it is a by-product of ethanol production. Based upon the results of this study, low amounts of DDG, specifically defatted DDG, can be fed to still get maximal CH_4 mitigation effects, as there was no observable effect by increasing inclusion level. There was also no measurable effect on fermentative capability of the different inclusion levels based upon the overall gas production data. DDG products that are subjected to greater oil extrusion—defatted DDG—may also be more beneficial in regards to both nutrient density and methane mitigation due to relative increase of all other nutrients, specifically sulfur. Therefore, the primary consideration when feeding defatted DDG for sulfur inclusion should be to stay below the recommended DM threshold for dietary sulfur ($\leq$ 0.5 % DM)[7].

REFERENCES

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(6) Nuñez, A. J. C., et al. "Effect of Dried Distillers Grains with Solubles Or Corn in Growing Cattle Diets, Followed by a Corn-Based Finishing Diet, on Performance of Feedlot Cattle." *Animal Feed Science and Technology* 207 (2015): 267-73. Web

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APPENDIX A

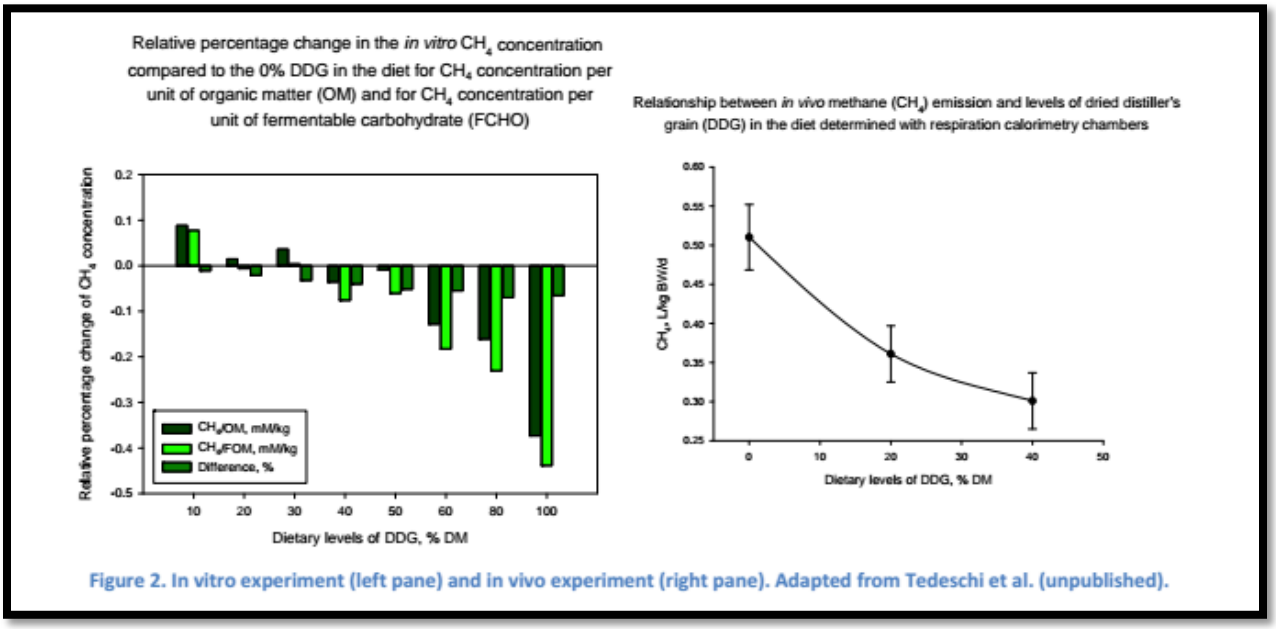

Figure 2. In vitro experiment (left pane) and in vivo experiment (right pane). Adapted from Tedeschi et al. (unpublished).

(2) Tedeschi, L.O., et al. Unpublished