

Effects of wet corn distiller's grains with solubles on visceral organ characteristics, trace mineral status, and polioencephalomalacia indicators in individually-fed cattle

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The purpose of this report is to summarize the results of a research project entitled *Effects of wet corn distiller's grains with solubles on visceral organ characteristics, trace mineral status, and polioencephalomalacia indicators in individually-fed cattle* submitted as a collaboration between faculty at Texas AgriLife Research, West Texas A&M University, and New Mexico State University and funded by the Texas Cattle Feeders Association. Information contained herein has not been submitted for publication as of the date of this report and should not be disseminated pending publication.

The objectives of this project as stated in the submitted proposal were as follows:

1. Determine the effect of wet distiller's grains (WDG) and inherent dietary sulfur on indicators of subclinical sulfide toxicosis.
2. Explore the influence of WDG on liver, kidney, and brain trace mineral stores.
3. Characterize carcass yield, and the mass and morphology of visceral organs, intestinal cell growth, cellularity, and vascularity, and digestive tract fill of cattle fed different levels of WDG.

Methods

An individual-animal feeding experiment was initiated in February 2009 at the West Texas A&M Research Feedlot in Canyon, TX, and the in vivo portion completed in August 2009. Feedlot pens were equipped with Calan gates to allow monitoring of individual feed intake.



Twenty-four beef steers were obtained for use in this study and were trained to properly utilize the Calan gates prior to study onset. Steers were weighed and assigned to blocks by weight (mean BW 847 lb) and randomly assigned within block to 1 of 3 treatments. The cattle scale was validated with certified weights before each occasion of use and calibrated as needed. The

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primary treatment of interest was inclusion of WDG at 0%, 30%, or 60% on a DM basis. Diets were formulated to be iso-fat at the 2 lower levels of WDG. Cattle were fed once daily throughout the study. Cattle were allowed ad libitum access to feed and feed refusals were monitored closely. Refused was removed, weighed, and dry matter determined if feed quality declined, and the dry weight of refused feed was deducted from dry feed delivered to determine dry matter intake.

Samples of dietary ingredients were collected 5 days/week (WDG, flaked corn) or weekly (remaining ingredients) for dry matter determination at 60°C for 48 hours. The dry matter used for WDG and flaked corn each week represented an average of the 5 daily dry matter determinations. Ration dry matter was calculated and updated each week using ingredient dry matters. Actual diet composition during the study (Table 1) was determined using actual ingredient dry matters. Samples of each diet were collected weekly from the bunk and composited over the entire study period for nutrient analysis (Table 2). Water tanks were cleaned and drinking water was sampled once/week. Duplicate aliquots of a composite water sample were assayed by a commercial laboratory and contained 464 mg/L TDS, pH of 8.8, 33 mg/L sulfate, 10 mg/L chloride, 2 mg/L Ca, 1 mg/L Mg, 1.5 mg/L K, 190 mg/L Na, 0.03 mg/L Cu, and Fe was not detectable (limit of detection = 0.05 mg/L).

Table 1: Ingredient composition of experimental diets used to achieve targeted WDG inclusion levels on an iso-fat basis.

Ingredient	Percent WDG in Ration on DM Basis		
	0%	30%	60%
Alfalfa hay	8.98	8.97	8.94
Steam-flaked corn	78.27	56.26	28.77
Cottonseed meal	5.58	-	-
Urea	1.06	0.52	-
WDGS	-	30.13	60.16
Supplement	3.16	2.65	2.12
Steep liquor	-	-	-
Yellow grease	4.01	2.00	-

Table 2: Composition of experimental diets (DM basis) derived from weekly samples composited over the entire study period.

Analyzed Diet Composition	Percent WDG in Ration on DM Basis		
	0%	30%	60%
DM, %,	83.31	60.42	47.6
CP, %	13.4	16.75	22.25
Non-protein N, %	2.45	1.4	0.35
Crude fat, %	5.95	5.95	7.75
Ca, %	0.8	0.79	0.68
P, %	0.29	0.38	0.52
S, %	0.14	0.26	0.41
Cu, mg/kg	16	18.5	19
Se, mg/kg	1.7	1.2	1.8

On the first day of the study, cattle were weighed and received an implant containing 200 mg of trenbolone acetate and 40 mg of estradiol (Revalor XS, Intervet / Schering-Plough Animal Health). A sample was drawn from the rumen gas cap for quantification of hydrogen sulfide concentration using commercially available detection tubes (Sensidyne, LP, Clearwater, FL) with a limit of detection of 25 ppm. A percutaneous liver biopsy was obtained and flash frozen in liquid nitrogen for mineral analyses. Cattle were fed for 125, 150, 164, and 192 days for blocks 1 through 4, respectively, to accommodate beef storage capacity at the WTAMU Meat Laboratory. During the feeding period, a steer assigned to the 0% WDG treatment group died on day 18. The cause of death was not readily apparent. A steer in the 60% WDG treatment group developed symptoms consistent with clinical polioencephalomalacia on day 97 of the study including head pressing, cortical blindness, and anorexia. The steer was treated with thiamine hydrochloride and dexamethasone for a period of 3 days. The steer's symptoms resolved and it was able to complete the remainder of the study without further evidence of disease.

Cattle were harvested at the West Texas A&M University Meat Laboratory. Before feeding on the day of harvest, cattle were weighed (± 1 lb) and blood samples were collected for quantification of plasma urea nitrogen and samples of the rumen gas cap were evaluated for hydrogen sulfide concentration as previously described. The first block of cattle harvested on a given day was loaded and delivered to the Meat Laboratory by 0700 and were not offered feed that morning. The second block of cattle was offered feed equivalent to 15% of the feed fed the previous day between the early morning weigh and transport to the Meat Laboratory at approximately 1100. Weights were recorded at the plant both pre- and post-exsanguination (± 1 lb), and the weights were determined (scale resolution of 0.01 to 0.2 lb depending on scale used based on total tissue weight) for the heart, lungs, liver, kidneys, mesenteric fat, pancreas, spleen, omental fat, gastrointestinal organs, gastrointestinal content, sexual organs, and the hot carcass (± 0.5 lb). Tissue samples were collected from the brain, liver, lung, and kidney and were flash frozen in liquid nitrogen pending laboratory analysis. Measurements were recorded for fat

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thickness at the 12/13th rib, LM area, and marbling score. USDA QG and YG scores were calculated using available data based on established formulae.

Samples of liver were submitted for mineral analyses at a commercial laboratory including sulfur, zinc, iron, manganese, copper, and selenium. Samples of brain and kidney were also submitted for determination of copper concentrations. Protein concentration in all tissues was assessed using perchloric acid tissue digestion followed by the Lowry method of protein determination on the resulting extracts (Lowry et al., 1951). Liver, brain, lung, and muscle tissue were evaluated for cytochrome oxidase c activity using commercially available kits (MITOISO1 and CYTOCOX1, Sigma-Aldrich). Briefly, mitochondria were isolated from samples that were washed in a buffer solution consisting of 50 mM HEPES, 1 M mannitol, 350 mM sucrose, and 5 mM EGTA and homogenized in the presence of the same buffer with 2 mg/mL albumin. Muscle samples were treated with 0.25 mg/mL trypsin prior to homogenization. The resulting homogenate from each tissue was centrifuged at 600 x g for 5 minutes. The supernatant was removed and centrifuged at 11,000 x g for 10 min. The pellet was resuspended in the previously described buffer solution and the centrifugation steps were repeated. The final pellet was resuspended in buffer solution consisting of 50 mM HEPES with 1.25 M sucrose, 5 mM ATP, 0.4 mM ADP, 25 mM sodium succinate, 10 mM potassium phosphate, and 5 mM DTT. The resulting mitochondrial extracts were assayed for cytochrome oxidase c activity by estimating the rate of oxidation of ferrocytochrome c to ferricytochrome c using spectrophotometric methods. Sample activity was calculated using the following formula:

$$\frac{(A/min_{sample} - A/min_{blank}) * dilution * 1.1}{volume_{enzyme} * 21.84}$$

The resulting estimate of enzyme activity was expressed as units per gram of tissue protein for analysis.

Glutathione peroxidase activity was assessed using an available kit (Cayman Chemicals) designed to estimate activity indirectly through the reduction of hydroperoxide to oxidized glutathione coupled with restoration of its reduced state by glutathione reductase. Homogenized liver samples were combined with assay buffer and co-substrate and the reaction was initiated by the addition of cumene hydroperoxide. Bovine erythrocyte glutathione peroxidase was used as a positive control. The targeted reaction was monitored over time to estimate the rate of reduction using a spectrophotometer in a microplate format. A regression line was fit to obtained data points and activity was estimated by:

$$\frac{slope}{0.00373} * \frac{0.19}{0.02} * dilution$$

A separate glutathione assay was performed to estimate the selenium-specific component of glutathione peroxidase activity. Modifications of the described procedure included addition of sodium azide and using hydrogen peroxide as the substrate instead of cumene hydroperoxidase.

Superoxide dismutase activity was determined by estimating the dismutation of superoxide radicals generated by xanthine oxidase using a commercially available kit (Cayman Chemicals). Sample homogenate was added to microplate wells containing the free radical detector. The reactions were initiated by adding xanthine oxidase and measuring absorbance using a spectrophotometer. Absorbance values were used to estimate superoxide dismutase activity by comparing to a standard control. Activity was expressed as units per mg of tissue protein. A separate assay was performed by adding potassium cyanide to estimate copper and zinc-dependent superoxide dismutase activity.

Hydrogen sulfide concentration within the rumen gas cap was analyzed using the Kruskal-Wallis rank test for grouped nonparametric data. Remaining data was analyzed using the PROC MIXED procedure (SAS, Cary, NC) with block included as a random effect. For enzyme and protein data, analysis was repeated independently for each tissue. Normality of residuals was tested using the Shapiro-Wilk test and visual interpretation of quantile plots of residuals vs. the normal distribution. For models that violated the assumption of normality, outcome variables were transformed and the analysis was repeated. Pairwise comparisons were made for all treatment groups. Statistical significance was determined at the 90% confidence level.

Results

Twenty-four steers were enrolled in the study. One steer in the 0% WDG treatment group died during the study on day 18. The cause of death was not readily apparent. In addition, there were some steers that were noted to consume feed from unassigned treatments associated with behavior related to use of the Calan gates. We were unable to specifically define the extent to which these behaviors may have modified the assigned dietary treatments with respect to quantity or duration. Therefore, an additional steer was removed from both the 0% and 30% WDG treatment groups and 3 steers were removed from the 60% WDG treatment group. Data analysis was performed on the reduced study population.

Performance and Carcass Data

Final body weight (Table 3) and adjusted final body weight did not differ among treatment groups (Figure 1). Dry matter intake was greater in steers fed 30% WDG relative to cattle fed 0% or 60% WDG. Adjusted average daily gain was greater in cattle fed 30% WDG relative to cattle fed 60% WDG. LM area in steers fed 30% WDG was larger than cattle fed 60% WDG.

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Table 3: Effects of WDG on performance and carcass traits in feedlot cattle

Variable	0%	30%	60%	SE
Number of steers	6	7	5	-
Initial BW, lb ^a	844	844	854	25
Final BW, lb ^a	1296	1334	1274	47
Adj. final BW, lb ^b	1312	1336	1248	56
DMI, lb/d ^{d,e}	16.76	19.68	16.73	1
ADG, lb/d	2.80	3.10	2.67	0.2
Adj. ADG, lb/d ^e	2.88	3.11	2.50	0.25
DMI:ADG	6.10	6.36	6.21	0.31
Adj. DMI:ADG	5.98	6.42	6.59	0.43
HCW, lb	818	833	778	28
Dressing percent	63.1	62.4	61.3	0.9
Fat thickness, in	0.47	0.59	0.43	0.13
LM area, in ² ^e	12.88	13.77	11.88	0.52
Yield grade	3.0	3.0	2.9	0.5
Marbling ^c	375	371	374	28

^a Pencil shrink of 4% was applied

^b Adjusted BW = HCW / 62.27% (the overall average dressing percentage).

^c Marbling score 300 = slight, 400 = small, etc.

^d 0% vs. 30% WDG (P < 0.1)

^e 30% vs. 60% WDG (P < 0.1)

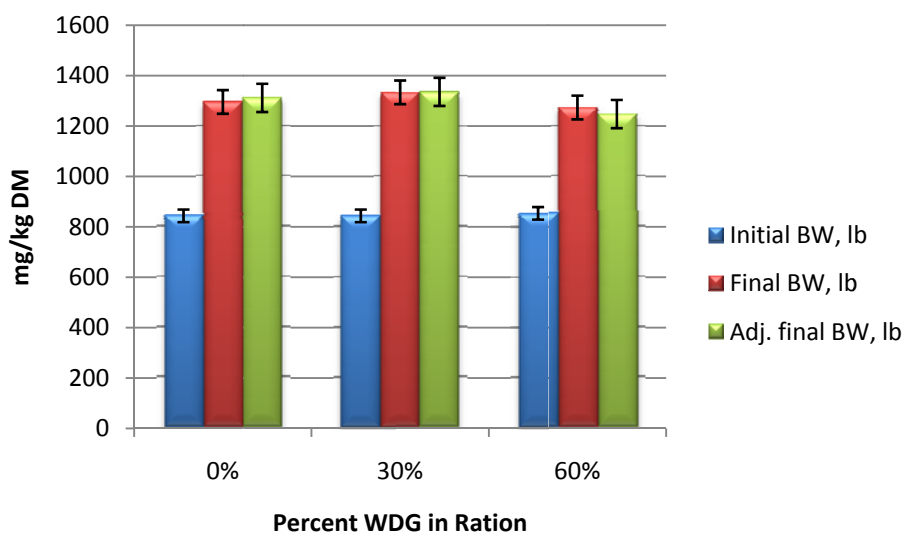


Figure 1: Initial, final, and adjusted final BW of steers by inclusion of WDG in the ration. Bars represent the most conservative standard error across treatments. Adjusted BW was calculated as HCW / 62.27% (the overall average dressing percentage).

Visceral Organ Mass

Visceral organ mass was evaluated based upon empty weight of digestive organs, liver, kidney, omental fat, and mesenteric fat evaluated at harvest (Table 4). Empty body weight was calculated as pre-exsanguination weight – (full weight of all gastrointestinal tract organs – empty weight of all gastrointestinal organs) and was used to express the mass of each visceral organ on a fractional basis scaled to empty body weight. Fractional abomasal and kidney weights were greater in cattle fed 60% WDG compared to cattle fed 30% WDG. Small intestinal weight, portal-drained viscera weight, and total splanchnic tissue weight (gastrointestinal tract plus liver) were greater for cattle fed 30% WDG than for those fed 0% WDG. Total gastrointestinal fill weight was greater in steers fed 30% WDG and 60% WDG compared to steers receiving the 0% WDG diet.

Table 4: Effects of WDG on visceral organ mass

	WDGS, % DM			SE ^a
	0	30	60	
Empty body weight (EBW), kg	568	583	551	19
Reticulum-rumen, g/kg EBW	20.79	23.98	25.44	1.94
Omasum, g/kg EBW	7.11	6.77	8.35	0.89
Abomasum, g/kg EBW ^c	4.93	3.87	5.98	0.79
Small Intestine, g/kg EBW ^b	9.01	10.48	10.23	0.62
Large Intestine, g/kg EBW	9.78	10.76	11.33	1.12
Total gastrointestinal tract, g/kg EBW	51.77	56.31	61.09	2.90
Total fill contents, kg ^b	27.81	35.75	39.6	2.82
Kidneys, g/kg EBW ^c	1.86	1.81	2.23	0.17
Liver, g/kg EBW	11.85	12.21	13.27	0.69
Omental fat, g/kg EBW ^c	21.35	28.09	20.14	3.6
Mesenteric fat, g/kg EBW	13.66	13.62	11.04	1.77
Portal drain viscera, g/kg EBW ^b	89.47	100.15	97.05	5.95
Total splanchnic tissue, g/kg EBW ^b	101.0	112.3	110.7	6.20

^a SE = most conservative mean standard error of the mean (n =6, 7 and 5 for 0, 30 and 60% respectively)

^b 0 vs 30% WDGS (P < 0.1).

^c 30 vs 60% WDGS (P < 0.1).

Chemical Analysis

Hydrogen sulfide concentration of the rumen gas cap was assessed before the morning feeding at the onset of the trial and prior to shipment to harvest. Rumen pressure was also estimated using a digital manometer, but the results were too variable to be considered as a possible covariate in assessing the association between diet and hydrogen sulfide concentration. All steers had concentrations of hydrogen sulfide below the detection limit (25 ppm) for the instrument at the onset of the study. The distribution of hydrogen sulfide concentration varied among diets for measurements obtained on the day of harvest. On the day of slaughter, steers with concentrations below the detection limit were assigned a value of 25 ppm, whereas the initial concentration given by the instrument was adjusted for the dead space within the instrument for remaining steers. Results were analyzed as non-parametric data using a Kruskal-Wallis comparison of group ranks. A significant increase in ruminal hydrogen sulfide concentration was observed in steers fed 60% WDG relative to steers fed 30% WDG diet ($P = 0.05$; Figure 2).

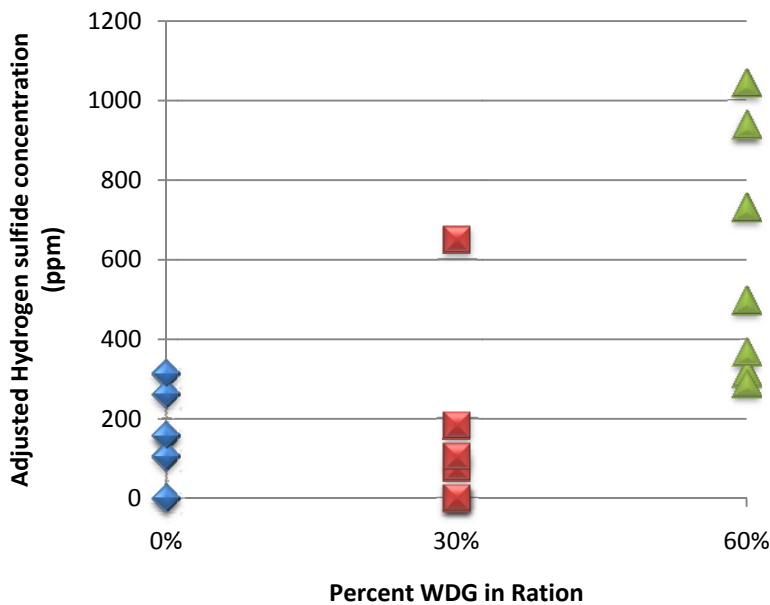


Figure 2: Concentration of hydrogen sulfide in the rumen gas cap prior to feeding on the day of harvest. Raw data were adjusted for dead space in the sampling instrument.

Significant increases in plasma urea nitrogen were observed with increasing proportions of WDG in the diet, as expected (Figure 3). Mineral concentrations in the liver at slaughter were adjusted using initial liver mineral concentration as a covariate, and varied among treatments for sulfur, iron, manganese, and copper (Table 5). Sulfur concentrations were higher for 30% WDG than for 0% WDG steers. Liver iron was lower in the 30% WDG group than in the 0% and 60% WDG groups. Manganese concentrations were greater in the 30% and 60% groups relative to the

0% WDG steers. Liver copper concentrations were lower in the 60% WDG group compared to the 30% WDG group. Significant differences did not exist in liver copper concentrations between the 0% and 30% WDG groups or the 0% and 60% WDG groups. Significant differences were not observed among diets for liver selenium, brain copper, or kidney copper concentrations.

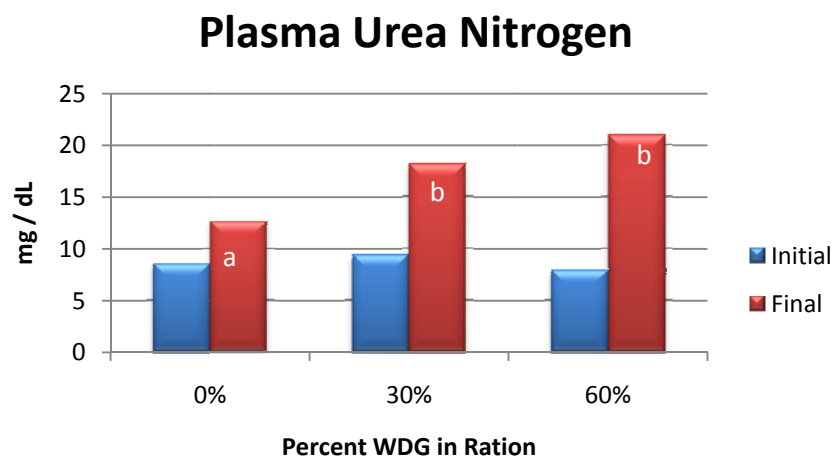


Figure 3: Plasma urea nitrogen concentration at the onset of the study and the day of harvest. Differing superscripts in same category are different ($P < 0.1$)

Table 5: Mineral concentrations for tissues collected on the day of harvest by inclusion of WDG

	WDGS, % DM			SE ^a
	0	30	60	
Liver S, mg/kg of DM ^{b,e}	6234	6594	6466	144
Liver Zn, mg/kg of DM ^b	104	103	99	5.2
Liver Fe, mg/kg of DM ^{b,e,f}	191	121	168	12
Liver Mn, mg/kg of DM ^{b,e}	8.5	10.2	11	0.6
Liver Cu, mg/kg of DM ^{b,f}	224	281	106	33
Liver Se, mg/kg of DM ^b	3.1	8.3	4.2	2.2
Brain Cu, mg/kg DM	14.2	15.7	11.4	3.0
Kidney Cu, mg/kg DM	18.2	19.4	18.8	1.0

^a SE = most conservative mean standard error of the mean (n =6, 7 and 5 for 0, 30 and 60% respectively)

^b initial concentrations were used as covariates for liver trace minerals

^c 0 vs 30% WCDGS (P < 0.1).

^f 30 vs 60% WCDGS (P ≤ 0.1).

Enzyme analysis

Enzyme assays were performed on selected tissues to estimate activity of cytochrome oxidase c, superoxide dismutase, and glutathione peroxidase. Activity of these enzymes was expressed as a function of tissue protein concentration for all analyses. Cytochrome oxidase c is a key enzyme in the electron transport chain. Previous work in mice supports the hypothesis that hydrogen sulfide may inhibit this enzyme and that this pathway may ultimately be the pathogenic mechanism of sulfur-associated polioencephalomalacia. Cytochrome oxidase c activity varied between diets and tissues (Figure 4); a decrease in activity was observed in lung tissue with increasing inclusion of WDG in the ration, which is consistent with respiratory exposure to hydrogen sulfide from the rumen. Cytochrome oxidase c activity in the brain was lower in tissues from steers fed 60% WDG relative to those fed 30%; however, numeric, but not significant differences, were observed between the 0% and 60% WDG groups. Cytochrome oxidase c activity in liver and muscle tissue were not altered by treatment.

Cytochrome oxidase c

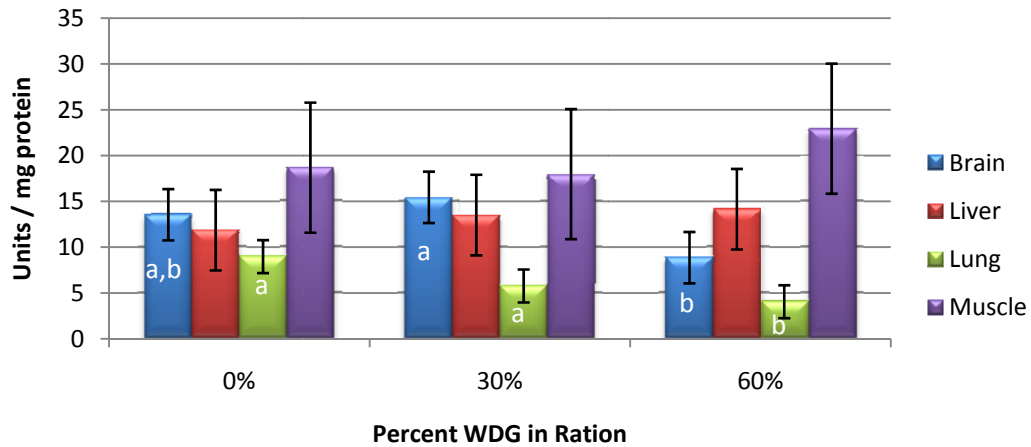


Figure 4: Cytochrome oxidase c activity in selected tissues collected at harvest from steers fed varying levels of WDG. Concentrations are expressed on a per mg of tissue protein basis. Bars represent most conservative standard error across treatments. Differing superscript in same category are significantly different (P < 0.1)

Glutathione peroxidase activity in liver was measured as total activity and in its selenium-specific form to assess the potential effects of feeding WDG and subsequent exposure to levels of sulfur that may interfere with selenium absorption. Higher levels of selenium-specific glutathione peroxidase activity were observed in the 60% WDG group compared to the 0% or 30% WDG groups (Figure 5), but total glutathione peroxidase activity did not differ among treatments.

Glutathione peroxidase

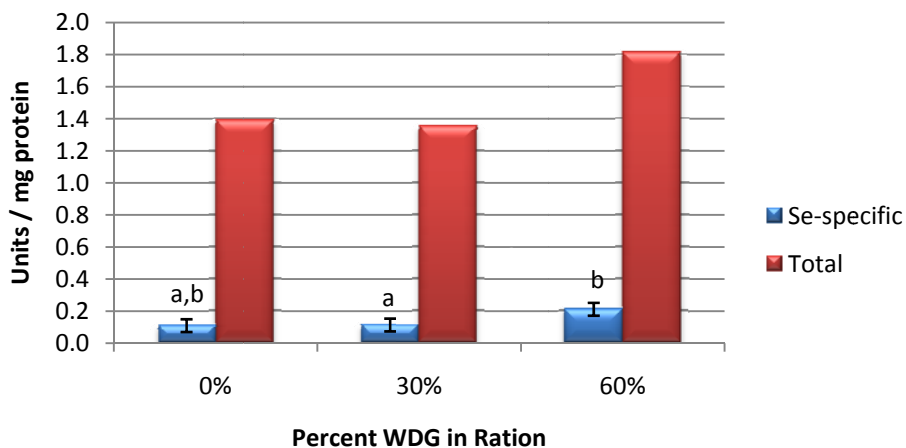


Figure 5: Total and selenium-specific glutathione peroxidase activity in liver samples collected at harvest from steers fed varying levels of WDG. Concentrations are expressed on a per mg of tissue protein basis. Differing superscript in same category are significantly different (P < 0.1)

Superoxide dismutase assays were performed for total enzyme activity, manganese-specific superoxide dismutase, and copper/zinc-specific superoxide dismutase. The copper/zinc component was derived indirectly from total and manganese-specific enzyme activity estimates and resulted in numerous samples with an estimate of no enzyme activity. We expect that this is due to inaccuracies in the approach and did not evaluate these enzymes statistically, although a general trend was observed for decreasing enzyme activity in diets containing WDG. Significant differences were not observed between dietary treatments for total or manganese-specific assays.

Discussion

The results of this study provide insight into the effects of feeding WDG to feedlot cattle with respect to interactions between diet and mineral metabolism, visceral organ mass, and potential mechanisms of sulfur toxicity. Importantly, the number of cattle enrolled in this study was small and should be viewed as a pilot study. Therefore, statistical power to test some targeted associations is limited. We elected to use less stringent criteria for assessing statistical significance, but recognize that additional associations may exist with effects too small to be detected within this project.

The results of this project with respect to organ mass and gut fill are consistent with observed differences in yield associated with feeding WDG. Our data suggest that greater gut fill is the dominant factor in this yield reduction. The lack of any change in feed efficiency when WDG are fed is generally similar to results obtained in this region and elsewhere with respect to feeding ethanol co-products.

The use of WDG in feedlot rations may lead to levels of dietary sulfur that exceed the NRC recommended maximum of 0.4%. Excess dietary sulfur can interfere with absorption of some minerals including copper and selenium and has been associated with polioencephalomalacia. In this study, dietary sulfur was 0.26% and 0.41% in the 30% and 60% WDG groups, respectively, based on weekly samples composited over the study period. Our supplier of WDG for this study closely monitors and regulates the quantity of solubles, the source of the majority of sulfur in WDG, added to the distiller's grains. This may have contributed to the fact that diets fed in this study did not substantially exceed the 0.4% threshold and reduces the risk of sulfur toxicity. Our objective was to slightly exceed the 0.4% 'threshold' in an attempt to challenge animal biology, but not result in excessive clinical sulfur toxicity. However, we did observe one clinical case of polioencephalomalacia which would suggest that either this level was sufficient to cause clinical disease in at least some cattle or temporal variation in sulfur content of the WDG exceeded toxic levels. This assumes, of course, that sulfur toxicity was the cause of the clinical case of polioencephalomalacia. Rumen hydrogen sulfide concentration at the time of diagnosis in the affected steer was 500 ppm. Other lines of evidence that support the cause as sulfur toxicity include liver sulfur concentration being greater in cattle fed 30% WDG than in cattle fed 0% WDG, and liver copper stores being decreased when feeding 60% WDG.

Sulfur compounds in the rumen are metabolized to the sulfide ion, which may be absorbed across the rumen wall or be bound to free hydrogen ions in the rumen to produce hydrogen sulfide gas; this gas is subsequently eructated (Gould, 1998). Hydrogen sulfide gas may be absorbed through the respiratory tract during and following eructation, resulting in toxicosis. The toxic mechanism hydrogen sulfide appears to be the inhibition of cytochrome oxidase c, an enzyme that plays a critical role in driving cellular respiration (Dorman et al., 2002; Gould, 2000; Loneragan et al., 1998). Inhibition of cytochrome oxidase activity in respiratory epithelium has been associated with hydrogen sulfide exposure in mice (Dorman et al., 2002). In our study, rumen hydrogen sulfide concentration was greater in cattle fed 60% WDG. We observed a linear decrease in cytochrome oxidase c activity in pulmonary tissue associated with feeding increasing levels of WDG. This supports the contention that hydrogen sulfide gas eructated from the rumen can be inhaled and absorbed in the respiratory tract. The consequences of cytochrome oxidase c inhibition in pulmonary tissue are unclear. We also observed significantly decreased cytochrome oxidase c activity in brain tissues collected at harvest from cattle fed 60% WDG. This is consistent with the proposed mechanism of sulfur induced polioencephalomalacia.

Truong et al. (2006) found that superoxide dismutase (requires copper and zinc) and glutathione peroxidase (requires selenium), were depleted in liver cells exposed to hydrogen sulfide. These enzymes play an important role in scavenging of free radicals. We observed increasing selenium-specific glutathione peroxidase activity in liver in cattle fed increasing levels of WDG. This is somewhat paradoxical given that selenium concentrations in the liver were numerically lower in the 60% WDG group compared to steers fed 30% WDG. Total glutathione peroxidase activity expressed on a per unit protein basis showed a similar pattern to the selenium-specific glutathione peroxidase activity (data not shown). This may be due to the fact that liver protein concentrations were numerically lower in cattle fed 60% WDG compared to the other treatment groups. Unadjusted total glutathione peroxidase activity demonstrated a pattern that more closely mimicked liver selenium concentration.

The results of this study provide several important insights into the pathogenesis of polioencephalomalacia in feedlot cattle associated with feeding WDG. We have demonstrated that feeding diets with increasing levels of WDG do, as expected, increase dietary sulfur and concentrations of sulfur in the liver. These dietary changes are associated with increased concentrations of hydrogen sulfide in the rumen gas cap. We have also identified effects of increased dietary sulfur on pulmonary and brain cytochrome oxidase c activity. To our knowledge, this is the first time that this proposed mechanism of sulfur-induced polioencephalomalacia has been described in feedlot cattle. We also observed changes in copper concentrations in the liver which are consistent with previous reports of sulfur-induced copper deficiency (Spears, 2003).

The sulfur concentration in the diet containing the highest level of WDG in this study was 0.41%. This is essentially equal to the commonly held threshold for sulfur toxicity, disregarding drinking water. Based on this information, we would expect that commercial feedlots in this region feeding WDG with similar sulfur content would be unlikely to encounter sulfur-induced polioencephalomalacia at common inclusion rates (<30% DM) assuming water was not a substantial source of total consumed sulfur. However, for alternative diets or water sources where total consumed sulfur reaches similar concentrations to what we observed in the 60% WDG diet, the results here would support the potential for subclinical and perhaps clinical effects of sulfur toxicosis through both altered mineral absorption and impaired cytochrome oxidase c activity.

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