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# Determination of potential human health benefits from diets containing corn distiller's coproducts

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## INTRODUCTION

The rapid growth of the U.S. ethanol industry has resulted in a large supply (over 35 million metric tonnes) of corn dried distiller's grain with solubles (DDGS). Currently, about 1 million metric tonnes of DDGS are exported to other countries, with the remainder being used domestically in animal feeds. However, there is significant interest in developing new markets and uses for these coproducts to add value. One potentially large, undeveloped market for these coproducts is the human food/nutraceutical market. Unfortunately, there is limited information on the effects of distiller's co-product consumption on human health. Distillers coproducts have many nutritional components that give it the potential for use as a functional food in human nutrition. Some of the important nutritional components found in corn distiller's coproducts include unsaturated fatty acids, antioxidants/phenolic acids, dietary fiber, and xanthophylls.

Cardiovascular disease (CVD) is a major cause of death in North America. Several components of corn (i.e. arabinoxylans, phytosterols, and xanthophylls) have been shown to be effective in lowering cholesterol [1-3]. Corn and corn coproducts are higher in natural antioxidants (e.g. ferulic acid) than other grains [4] and thermal processing (which occurs during ethanol production) releases the bound phenolic acids which have even higher antioxidant activity [5]. These antioxidants have been shown to be effective in reducing colon cancer [6] and controlling type 1 diabetes [7]. However, no studies have been conducted to determine the bioavailability of ferulic acid, the primary phenolic acid in corn and corn coproducts [8]. Corn and corn coproducts are high in carotenoid pigments called xanthophylls. Two of these pigments, lutein and zeaxanthin, are uniquely concentrated in the macular region of the retina and are associated with preventing macular degeneration, several types of cancer, and coronary artery disease [8]. However, no studies have been conducted to determine the bioavailability of xanthophylls or phenolic acids in corn coproducts [8].

The overall goal of this research project is to identify potential therapeutic uses for corn-based distiller's coproducts, thereby increasing the value of these coproducts. The value of distiller's coproducts will be increased if we can demonstrate that they can be used as a functional food/nutraceutical ingredient for human health rather than being strictly used as an animal feed ingredient.

## MATERIALS AND METHODS

### Experiment 1. DDGS Characterization

**Objective.** The objective of this experiment was to characterize DDGS samples obtained from a number of different ethanol production plants for the antioxidant capacity of the DDGS's, the concentration and variation of antioxidants (i. e., tocopherols and tocotrienols), the degree of oxidation, the total and free ferulic acid, and color.

**Analyses.** An in vitro assay (DPPH) was used to determine the amount of antioxidant capacity in DDGS samples and corn bran. Tocopherols (vitamin E) and tocotrienols were determined by HPLC. The degree of oxidation was measured as thiobarbituric acid reactive substances (TBARS) and peroxide value in the oil extracted from DDGS. Free versus conjugated forms of ferulic acid were distinguished by pre-treatment with  $\beta$ -glucuronidase and sulfatase, followed by quantitation using HPLC. Sample color was determined using a Minolta colorimeter.

## Experiment 2. Development of Atherosclerosis

The objective of this experiment was to measure the impact of feeding diets with distillers coproducts on concentration of cholesterol in liver and serum samples along with development of atherosclerotic lesions of the arterial system.

**Diets, feeding, and experimental design.** Male C57BL/6J apoE-deficient mice (5 weeks old; Jackson Laboratory, Bar Harbor, ME, USA), a common animal model used for study atherosclerosis, were fed 4 diets (n = 10/group). A control diet was formulated to contain no corn coproducts (Positive control). Diet 2 contained 20% corn bran (Bran fraction), diet 3 contained 20% of DDGS (DDGS), and diet 4 contained 20% of the soluble fraction (Soluble fraction) The composition of the diets is shown in Table 1. Diets were formulated to contain similar concentrations of protein, total carbohydrates, fat, and total dietary fiber.

Diets were fed for 5 months.

**Data collection, sampling, and analyses.** Body weights and 24 hour food disappearance were measured weekly. Blood samples were collected after 1 and 3 months feeding diets. Blood samples were analyzed for the concentration of cholesterol in serum [9]. At 5 months, mice were anaesthetized with isoflurane. Blood was collected by cardiac puncture. The circulatory system was perfused with PBS and fixed in 10% Formalin. The arterial system was stained with Oil-Red O and pinned onto black wax. The whole section of the arterial system was photographed and the atherosclerotic area was determined using ImageJ Software [10]. Livers were removed and liver cholesterol extracted and measured as described by Gallaher et al. [11].

## Experiment 3. Bioavailability of Xanthophylls and Ferulic Acid

**Objective.** The relative bioavailability of the xanthophylls lutein and zeaxanthin and of ferulic acid from the three coproducts used in Experiment 2 will be estimated in rats, as well as a number of parameters indicative of potential health benefits, including liver cholesterol, fecal bile acid excretion, and urinary excretion of lipid oxidation products.

**Experimental design.** A control diet was formulated to contain no corn coproducts (Positive control). Diet 2 contained 20% corn bran (Bran fraction), diet 3 contained 20% of DDGS (DDGS), and diet 4 contained 20% of the soluble fraction (Soluble fraction). The composition of the diets is shown in Table 1. Diets were formulated to contain similar concentrations of protein, total carbohydrates, fat, and total dietary fiber. The diets were fed for 3 weeks, then blood collected and tissues harvested for sample analysis. Plasma was separated from whole blood. Plasma and tissues were stored at -80° C until analyzed.

**Data collection, sampling, and analyses.** Plasma and tissue concentrations of lutein and zeaxanthin were determined by LC-MS. Urinary ferulic acid was determined by HPLC. In addition, liver cholesterol, bile acid excretion, cecal weight and cecal contents pH, and fat pad weight (as a measure of adiposity) were determined.

## RESULTS

### Experiment 1. DDGS Characterization

The antioxidant capacity as measured by the DPPH assay (measured as tocopherol equivalents, TE), concentration of free and bound ferulic acid, and tocopherols varies considerably among sources of DDGS. The source with the greatest concentration of TE was 2.1 times greater than the source with the least concentration (Figure 1). The average concentration was 4,000 TE/100 g DDGS. The source with the greatest concentration of TE was also the source with the greatest concentration of free ferulic acid (Figure 2). This concentration was 4.5 times greater than the source with least concentration of ferulic acid. However, the concentration of total ferulic acid was not associated with the concentration of total ferulic acid, which was 950 mg/100 g in the source with greatest concentration. All sources of DDGS had greater concentration of tocopherols and tocotrienols than corn and the concentration varied among sources of DDGS, with 1.8 times difference between the source with the greatest and the least concentration (Figure 3).

The degree of lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) (Figure 4) and as peroxide value (Figure 5). The TBARS values varied from less than 1 to as high as 5 ng MDA eq./mg oil. However, with the exception of sample #10, values ranged from 1-2 ng MDA eq./mg oil. Peroxide values were all close to the value expected for fresh vegetable oils with the exception of sample #10, which had a very high peroxide value, indicating a high degree of lipid peroxidation.

Hunter color is a color scale frequently used in the food industry to examine color of foods.  $L^*a^*b^*$  scores describes all the color visible to the human eye.  $L^*$  is a measure of lightness; a greater value indicates a lighter color, and  $a^*$  and  $b^*$  are color-opponent dimensions. Generally, the greater the positive  $a^*$ , the greater the redness, whereas the greater the  $b^*$ , the greater the yellow color. Color values were similar for all samples except sample #10, which had lower  $L^*$  and  $b^*$ , indicating a darker sample with less yellow color.

## **Experiment 2. Development of Atherosclerosis**

The body weight of mice fed the diet with soluble fraction and the bran fraction was greater than the body weight of mice fed the positive control diet (Figure 7). The growth of mice fed the DDGS diet was not different from that of mice fed the soluble fraction through the middle period of the experiment, but it was less at the end of the experiment. Therefore, the final body weight of mice fed DDGS was less than that of mice fed the soluble fraction and the bran fraction.

The concentration of serum cholesterol of mice fed diets with the DDGS and the soluble fraction was greater than bran fraction after 30 days of feeding (Figure 8). The concentration of serum cholesterol of mice fed the control diet was intermediate. However, at the end of the study, there were no differences in serum cholesterol concentrations among diets. The % atherosclerotic lesions in the arch arterials of mice fed the DDGS diet was less than that of mice fed the soluble fraction and not different from mice fed the control diet, or the bran fraction (Figure 9). The % atherosclerotic lesions in the arterial tree were not different among the diets.

## **Experiment 3. Bioavailability of Xanthophylls and Ferulic Acid**

Experiment 3 is currently in progress. The animal feeding trial has been concluded and plasma, urine, and tissue samples collected and stored in the appropriate freezers. Sample analysis has begun. Results from these experiments will be provided as a supplement to this final report.

## DISCUSSION

The antioxidant capacity of DDGS may be greater than in corn because antioxidants may be concentrated during the production process, but we also observed differences among sources of DDGS. The differences in antioxidant capacity among sources of DDGS are likely due to both the antioxidant capacity of the source corn used in the fermentation as well as the drying conditions used. It is known that high temperature drying can produce Maillard reaction products that have high antioxidant capacity. Therefore, in some circumstances, a high antioxidant capacity could actually be an indicator of thermal abuse.

The concentration of free ferulic acid also varied among sources of DDGS. Free ferulic acid is well absorbed in the small intestine. Since greater absorption of ferulic acid is associated with a number of positive health benefits, including cholesterol lowering and improved blood glucose control (Youn & Gallaher, unpublished results), greater free ferulic acid content is a positive attribute. The difference between the total and free ferulic acid represents bound ferulic acid. Bound ferulic acid is generally not well absorbed. However, the degree to which it is absorbed is influenced by the source material (corn in this case) and processing. Thus, the fermentation of the corn to produce the DDGS may lead to greater absorption of the bound ferulic acid than would be found in corn. This will be examined in experiment 3 that is currently in progress.

The degree of lipid peroxidation was measured in two independent ways – TBARS and peroxide value. Except for sample #10, values were low by both measures, indicating a low degree of lipid peroxidation. Sample #10 appears to be highly peroxidized, likely due to thermal abuse.

The color of the DDGS samples was evaluated using the industry standard Hunter color scale. Again, except for sample #10, the lightness ( $L^*$ ) and color ( $a^*$  and  $b^*$ ) appear similar among the samples. Sample #10 was darker (lower  $L^*$ ) and less yellow (lower  $b^*$ ), again consistent with thermal abuse.

A primary objective of this project was to determine the effect of DDGS and related coproducts on the development of atherosclerosis in an animal model that develops atherosclerosis at a rapid rate, and that exhibits atherosclerotic lesions highly similar to those seen in humans. This was accomplished using the apoE-knockout mouse. After 30 days of feeding there was a trend for greater serum cholesterol in the mice fed DDGS and the soluble fraction, but this difference was not present at the end of the experiment. There was a trend for a reduction in atherosclerotic lesion area in the aortic arch, but not the arterial tree, in mice fed DDGS, suggesting that components within the DDGS may slightly retard the development of atherosclerosis at the aortic arch. Since considerable evidence suggests that high levels of oxidative stress promote atherosclerosis, antioxidant compounds in the DDGS, either tocopherols or ferulic acid, may have contributed to this. Evaluation of the degree of atherosclerosis in sections of the aorta, which is now underway, may clarify the ability of DDGS to reduce atherosclerosis.

The bioavailability of xanthophylls and of ferulic acid from DDGS and related products is unknown. Our initial objective was to determine bioavailability by measuring the concentration of xanthophylls in plasma over time after a meal containing DDGS or related coproducts. However, we were unable to detect xanthophylls in plasma after such meals using HPLC. We increased our sensitivity of detection by using an LC-MS system, but were still unable to detect xanthophylls in the plasma. Consequently, we have changed our approach to a long-term

feeding trial, which will allow xanthophylls to accumulate in plasma and tissues, and therefore make detection easier. We are hopeful this will allow us to determine xanthophyll concentrations and consequently estimate xanthophyll bioavailability. Further, by conducting this study as a long-term feeding trial, we have the opportunity to examine the effect of DDGS and related coproducts on ferulic acid bioavailability as well as several health-related parameters, such as cholesterol lowering, bile acid excretion, adiposity, and degree of fermentation.



## REFERENCES

1. Lopez, H.W., et al., Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats. *J Nutr Biochem*, 1999. **10**(9): p. 500-9.
2. Wilson, T.A., et al., Corn fiber oil lowers plasma cholesterol levels and increases cholesterol excretion greater than corn oil and similar to diets containing soy sterols and soy stanols in hamsters. 2000. **11**(9): p. 443-449.
3. Ribaya-Mercado, J.D. and J.B. Blumberg, Lutein and zeaxanthin and their potential roles in disease prevention. *J Am Coll Nutr*, 2004. **23**(6 Suppl): p. 567S-587S.
4. Adom, K.K. and R.H. Liu, Antioxidant activity of grains. *J Agric Food Chem*, 2002. **50**(21): p. 6182-6187.
5. Dewanto, V., X. Wu, and R.H. Liu, Processed sweet corn has higher antioxidant activity. *J Agric Food Chem*, 2002. **50**: p. 4959-64.
6. Wargovich, M.J., et al., Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression. *Carcinogenesis*, 2000. **21**(6): p. 1149-55.
7. Balasubashini, M.S., et al., Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytother Res*, 2004. **18**: p. 310-4.
8. Plate, A.Y. and D.D. Gallaher, The potential health benefits of corn components and products. *Cereal Foods World* 2005. **50**(6): p. 415-423.
9. Warnick, G.R., J. Benderson, and J.J. Albers, Dextran sulfate-Mg<sup>2+</sup> precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem*, 1982. **28**(6): p. 1379-88.
10. Rasband, W.S., ImageJ. 1997-2012, U.S. National Institutes of Health: Bethesda, Maryland USA.
11. Gallaher, D.D., C.A. Hassel, and K.J. Lee, Relationships between viscosity of hydroxypropyl methylcellulose and plasma cholesterol in hamsters. *J Nutr*, 1993. **123**(10): p. 1732-8.

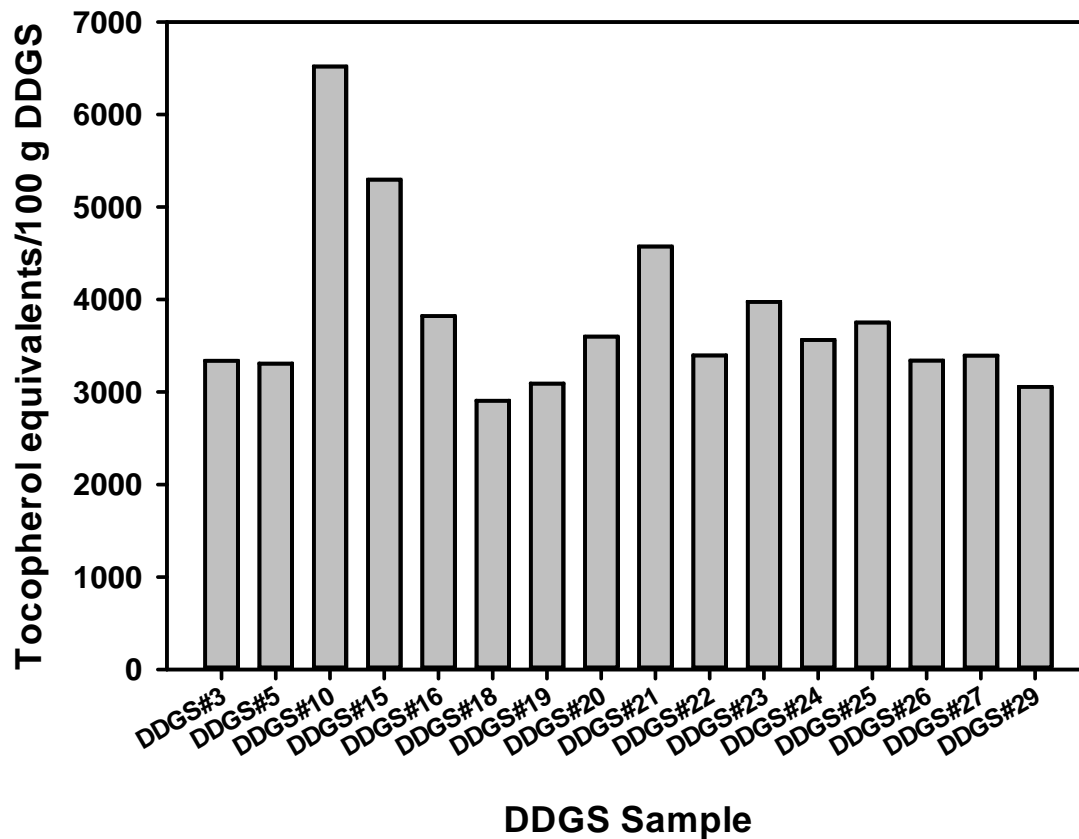
**Table 1.** Diet composition for Experiments 2 and 3.

Diet Ingredient <sup>1,2</sup>	AIN-93G	DDGS	Corn Bran Fraction	Dried Solubles Fraction
	g/kg	g/kg	g/kg	g/kg
<b>Distillers Co- Product</b>		<b>200</b>	<b>200</b>	<b>200</b>
<b>Carbohydrate</b>				
Corn Starch	<b>300.5</b>	<b>241.6</b>	<b>232.3</b>	<b>204.6</b>
Dextrinized Corn Starch	<b>115.2</b>	<b>115.2</b>	<b>115.2</b>	<b>115.2</b>
Sucrose	<b>87.3</b>	<b>87.3</b>	<b>87.3</b>	<b>87.3</b>
<i>Total Carbohydrate</i>	<i><b>484.6</b></i>	<i><b>467.7</b></i>	<i><b>464.3</b></i>	<i><b>483.6</b></i>
<b>Protein</b>				
Casein (>85% protein)	<b>200.0</b>	<b>140.5</b>	<b>178.8</b>	<b>161.8</b>
<i>Total protein</i>	<i><b>174.0</b></i>	<i><b>174.0</b></i>	<i><b>174.0</b></i>	<i><b>174.0</b></i>
<b>Fat (15%)</b>				
Oil (Corn)	<b>150.0</b>	<b>124.4</b>	<b>133.2</b>	<b>112.2</b>
0.15% Cholesterol	<b>1.5</b>	<b>1.5</b>	<b>1.5</b>	<b>1.5</b>
<i>Total Fat</i>	<i><b>152.0</b></i>	<i><b>151.4</b></i>	<i><b>151.8</b></i>	<i><b>151.6</b></i>
<b>Total Fiber (9.5%)</b>				
Cellulose	<b>95</b>	<b>39</b>	<b>1.2</b>	<b>66.8</b>
<i>Total fiber</i>	<i><b>95</b></i>	<i><b>95</b></i>	<i><b>95</b></i>	<i><b>94.8</b></i>
Mineral Mix (93G-Mx)	<b>35</b>	<b>35</b>	<b>35</b>	<b>35</b>
Vitamin Mix (93G-Vx)	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>
L-Cystine	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>
Choline Bitartrate	<b>2.5</b>	<b>2.5</b>	<b>2.5</b>	<b>2.5</b>
TBHQ	<b>0.014</b>	<b>0.014</b>	<b>0.014</b>	<b>0.014</b>
	<b>1000.000</b> <b>0</b>	<b>1000.000</b> <b>0</b>	<b>1000.0000</b>	<b>1000.0000</b>

<sup>1</sup>Numbers in blue are diet inclusion rates.

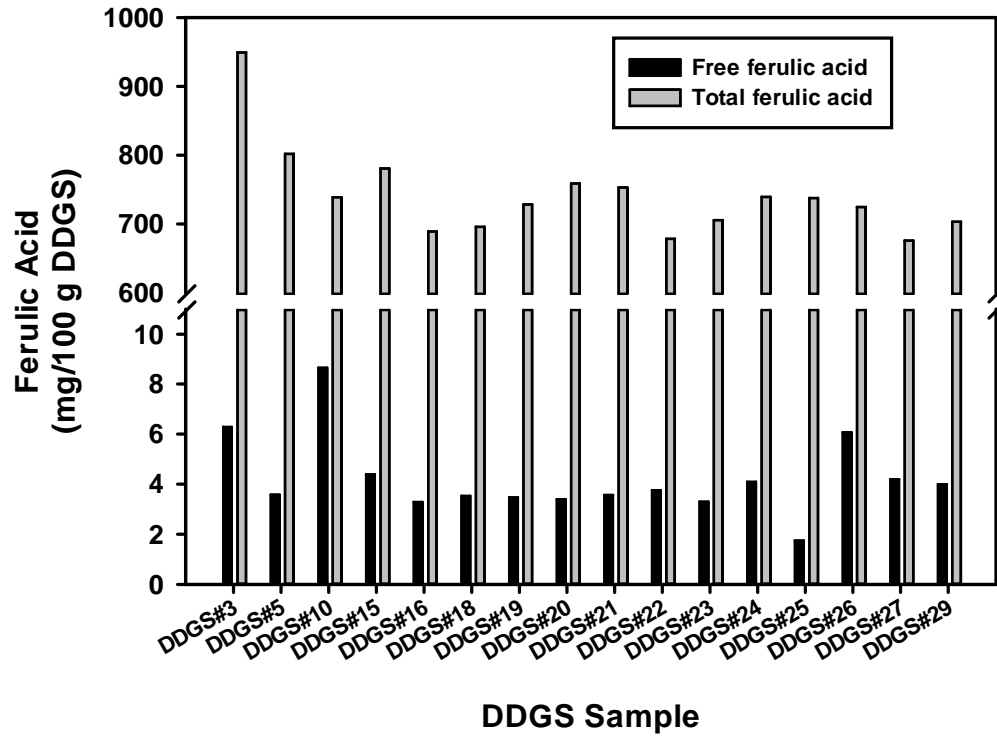
<sup>2</sup>Numbers in red italics are the sum of that nutrient class - includes carbohydrate from vitamin and mineral mixes and takes into account water content of starch, maltodextrin, and casein.

## Antioxidant Capacity of DDGS Samples



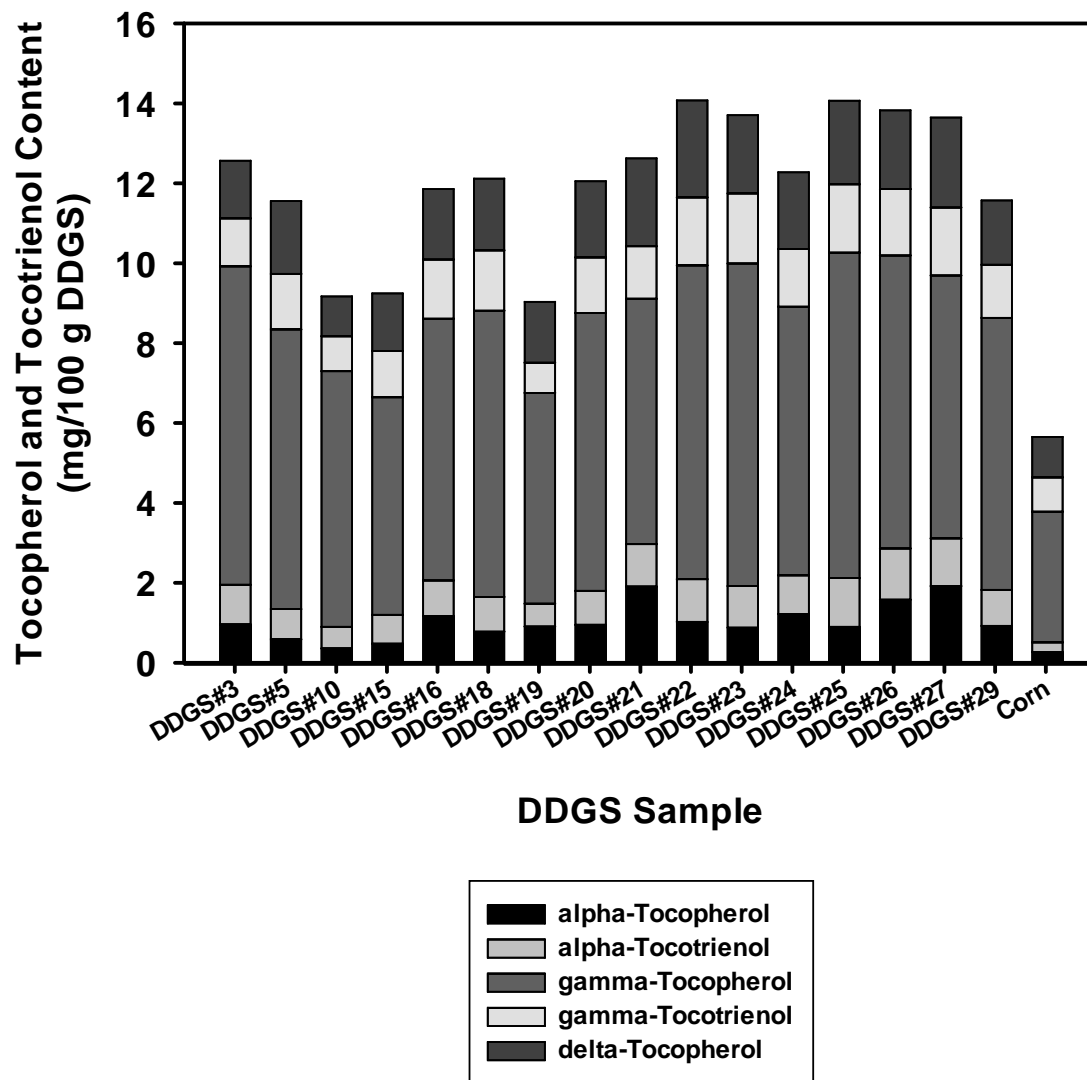
**Figure 1.** Antioxidant capacity of DDGS samples obtained from various ethanol production plants across the United States. The greater the value, the greater the ability of the sample to resist oxidation.

## Free and Total Ferulic Acid in DDGS Samples



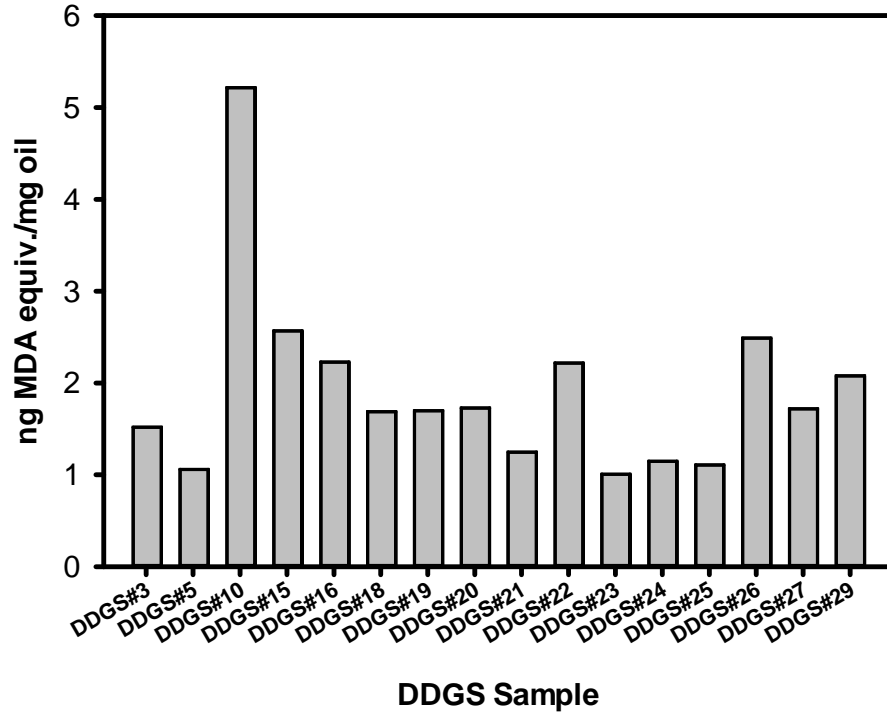
**Figure 2.** Free and total ferulic acid content of DDGS samples obtained from various ethanol production plants across the United States. Ferulic acid is the major phenolic compound in corn. Free ferulic acid is readily absorbed. However, bound ferulic acid (total – free) is variably absorbed, depending on the food or feed material and how it has been processed.

## Tocopherol and Tocotrienol Content of DDGS Samples



**Figure 3.** Tocopherols (vitamin E) and tocotrienols in DDGS samples obtained from various ethanol production plants across the United States and corn. All tocopherols and tocotrienols have the same antioxidant activity in the sample. However, their biological antioxidant activities differ.  $\alpha$ -Tocopherol is the most potent biological tocopherol. However, other tocopherols and tocotrienols may have other types of biological activity.

## Thiobarbituric Reactive Substances (TBARS) in Various DDGS Samples

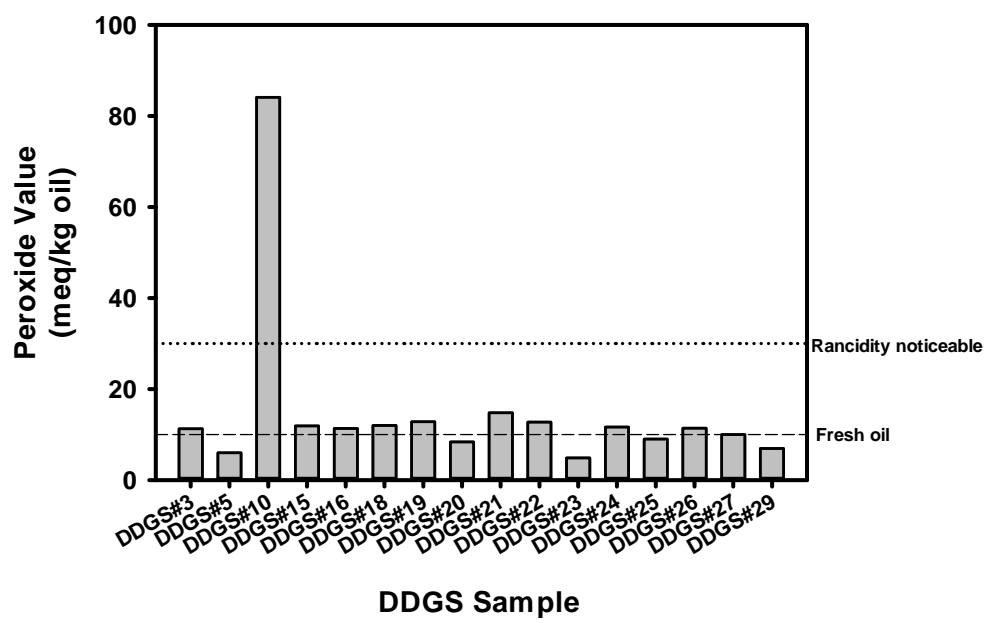


**Figure 4. T**

United States and corn. TBARS is a measure of lipid peroxidation. A greater value indicates greater lipid peroxidation.

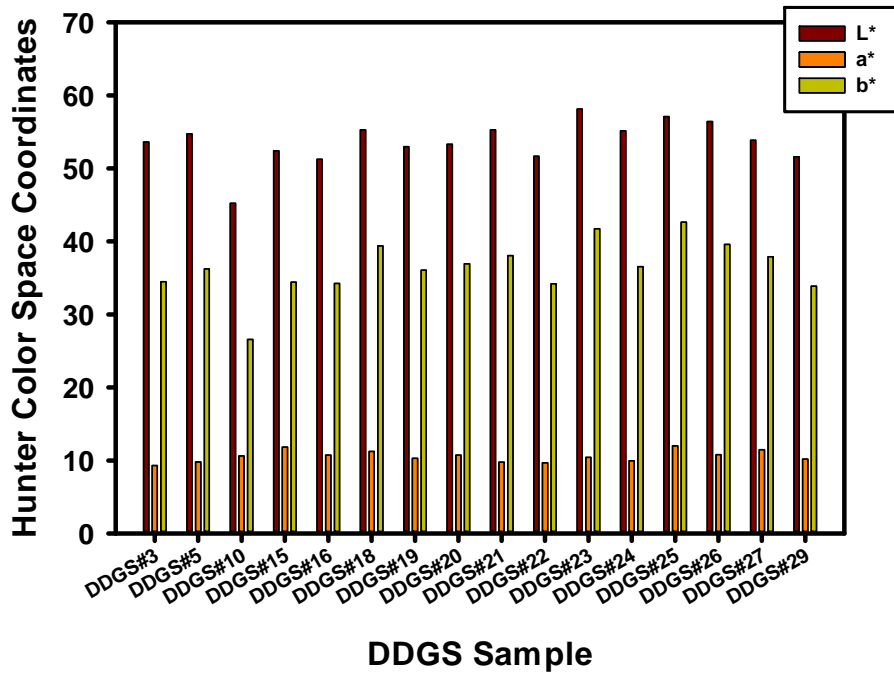
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### Peroxide Value of Various DDGS Samples



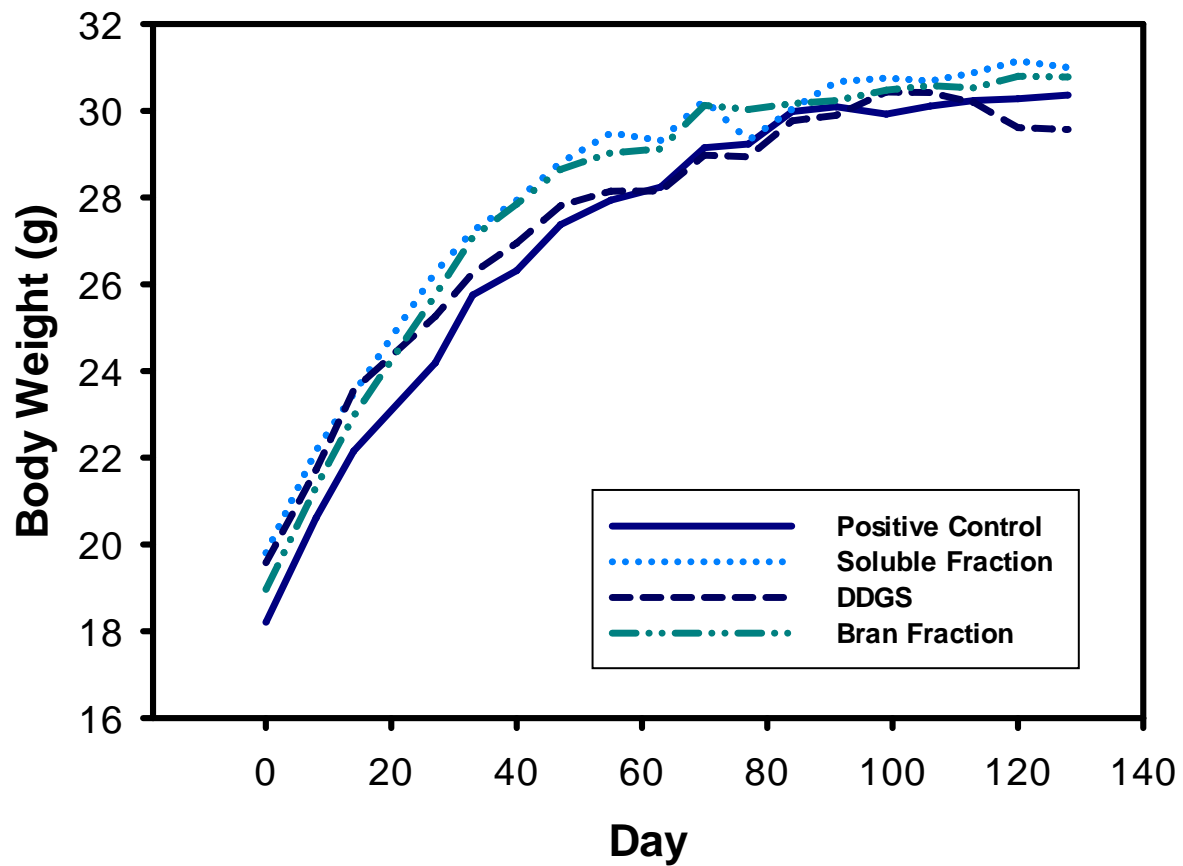
**Figure 5.** Peroxide value in DDGS samples obtained from various ethanol production plants across the United States and corn. Peroxide value is a measure of lipid peroxidation. A greater value indicates greater lipid peroxidation.

## Hunter L\*a\*b\* Color Space Coordinates of Various DDGS Samples

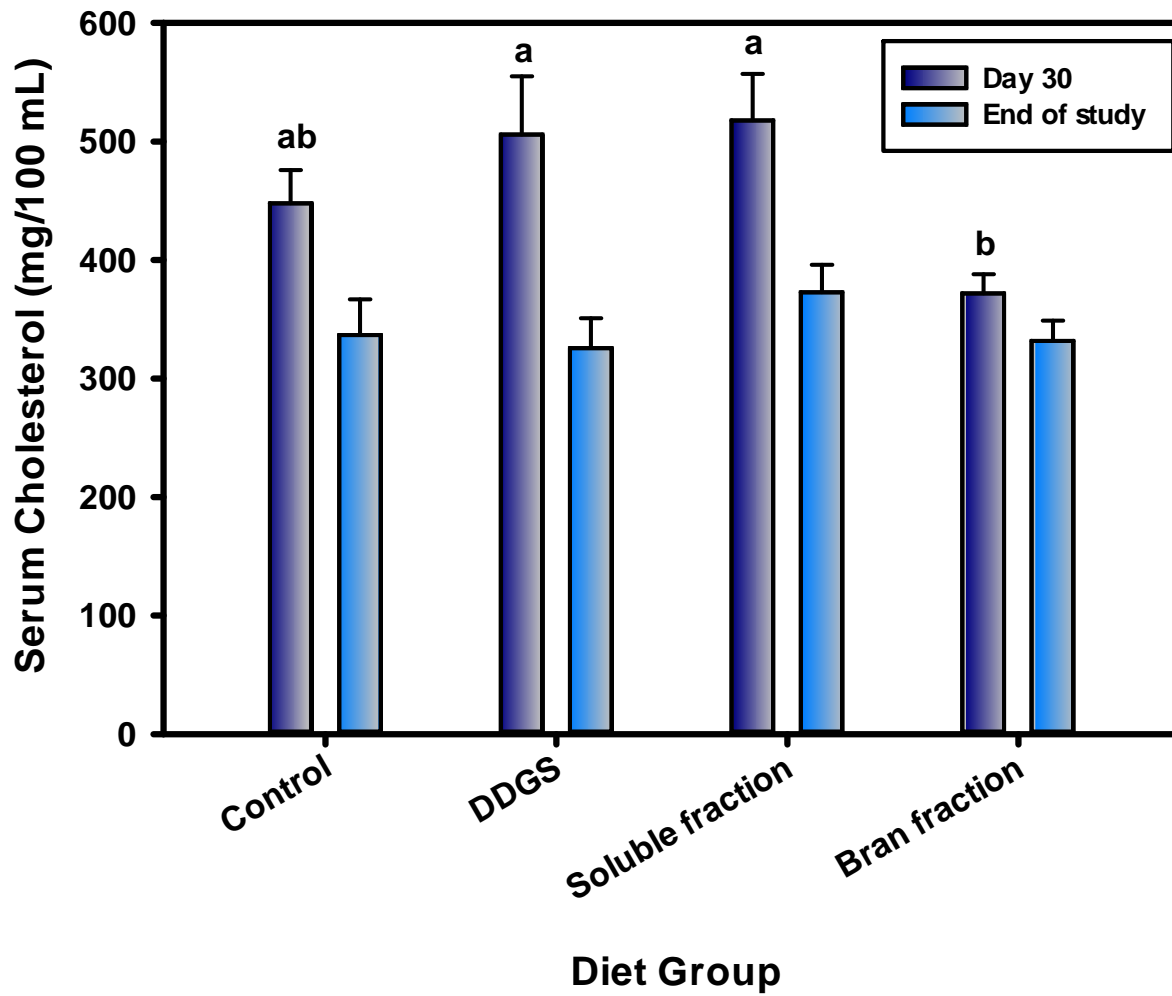


**Figure 6.** Hunter L\*a\*b\* color space coordinates of various DDGS samples from ethanol production plants across the United States and corn. L\*a\*b\* scores describe all the color visible to the human eye. L\* is a measure of lightness; a greater value indicates a lighter color. a\* and b\* are color-opponent dimensions. Roughly, the greater the positive a\*, the greater the redness, whereas the greater the b\*, the greater the yellow color.

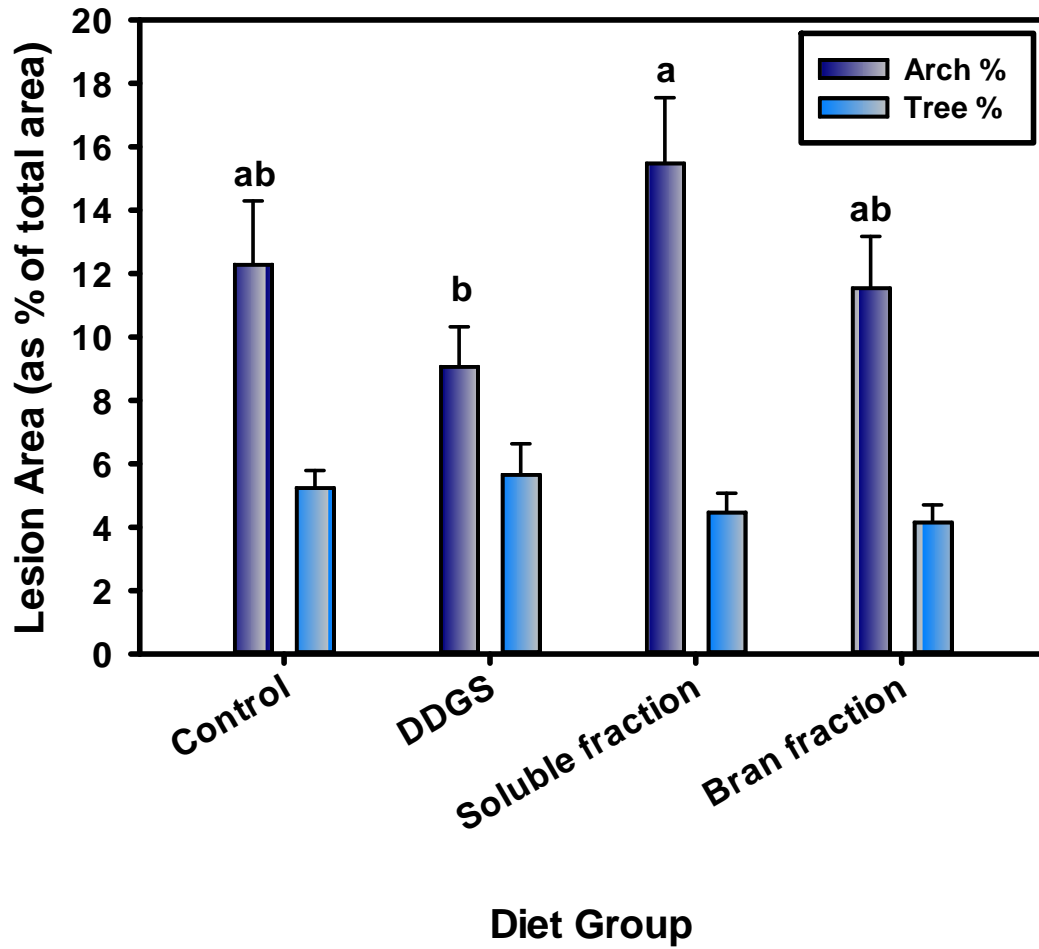




**Figure 7.** Body weight of mice fed a positive control diet and diets with soluble fraction, DDGS, and corn bran.



**Figure 8.** Serum cholesterol (mg/100 mL) of mice fed a positive control diet and diets with soluble fraction, DDGS, and corn bran.



**Figure 9.** Atherosclerosis (% lesions) in aortic arch and arterial tree of mice fed a positive control diet and diets with soluble fraction, DDGS, and corn bran.