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## Original Research Article

## Antioxidant capacity and phytochemical content of 16 sources of corn distillers dried grains with solubles (DDGS)

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## ABSTRACT

Corn distillers dried grains with solubles (DDGS) is a co-product of the fuel ethanol industry that is an excellent source of energy, digestible amino acids, and digestible phosphorus. However, the antioxidant capacity and phytochemical content of DDGS and how it is influenced by production parameters is unknown. Therefore, 16 DDGS samples obtained from different ethanol production plants in the U.S. were characterized for antioxidant capacity, vitamin E, xanthophylls, and ferulic acid content and compared with corn. The antioxidant capacity of DDGS samples, measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, varied almost 2 folds among samples, but in all cases was more than 3 times that of corn. All DDGS samples had a considerably greater concentration of tocopherols and tocotrienols (lipid-soluble antioxidants) than corn. However, the relative concentrations among the tocopherols and tocotrienols tended to be similar to corn. The xanthophyll lutein was present in all DDGS samples and in all cases exceeded the content in corn. Zeaxanthin was detected in most samples. The total ferulic acid content of the DDGS samples was almost 3-fold greater than corn, but, as in corn, almost all was present in a bound form. However, the concentration of unbound (free) ferulic acid was more than 3 times the concentration found in the corn sample. Thus, DDGS is a rich source of phytochemical compounds and may provide antioxidant and health benefits beyond its macronutrient composition. However, differences in processing can greatly influence the phytochemical content and quality of DDGS. In particular, thermal abuse due to excessive heat used in drying may lead to lipid oxidation products that may have deleterious effects when incorporated into feeds.

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## 1. Introduction

Corn distillers dried grains with solubles (DDGS) is a co-product of U.S. ethanol production, and is used as a source of digestible energy, protein and amino acids, and phosphorus in ruminant,

swine, poultry, and aquaculture diets in many countries around the world. The non-starch chemical composition of DDGS is increased by about 3 folds compared with the concentrations in corn because starch is converted to ethanol during the fermentation process. However, there is considerable variation in energy and nutrient content among DDGS sources due to differences in the nutrient content of the feedstock used, processing methods, and the amount of condensed distillers solubles combined with the wet grains to produce DDGS in various ethanol plants (Liu, 2011; NRC, 2012). Despite numerous recent publications documenting the variation in energy and nutrient composition of DDGS (Liu, 2011), there is very little published information on the variability in antioxidant capacity and phytochemical content among corn DDGS sources.

Knowledge of the antioxidant capacity in feed ingredients with relatively high concentrations of lipid (e.g. DDGS) is important because it may affect the peroxidation potential and storage stability, as well as the need for adding synthetic antioxidants (Hanson

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et al., 2015a). Song and Shurson (2013) showed that the peroxide value and thiobarbituric acid reactive substances (TBARS) values ranged from 4.2 to 84.1 milliequivalents (meq)/kg oil and 1.0 to 5.2 ng malondialdehyde equivalents (equiv.)/mg oil, respectively, among 31 corn DDGS sources. Under extreme peroxidation conditions, the extent of peroxidation in some DDGS sources can be 25 times more than found in corn grain (Song and Shurson, 2013). These results suggest that antioxidant capacity and lipid soluble phytochemicals may vary substantially among DDGS sources. Furthermore, the knowledge of the antioxidant capacity of feed ingredients may be important for minimizing the risk of negative nutritional and physiological effects associated with metabolic oxidative stress (Hanson et al., 2015b; Kerr et al., 2015).

Corn is an excellent source of bioactive phytochemicals including vitamin E (NRC, 2012), ferulic acid (Mattila et al., 2005), and carotenoids (Bacchetti et al., 2013), which contribute to its antioxidant capacity and potential health benefits. Estimates of the vitamin E content of corn have been reported to be in the range from 11.65 mg/kg (NRC, 2012) to 22 mg/kg (NRC, 1994). Ferulic acid is the predominant phenolic acid in corn and other cereals, and has significant antioxidant activity (Itagaki et al., 2009). Corn also contains about 20 mg/kg of xanthophylls (Leeson and Summers, 2005), especially lutein and zeaxanthin (Sommerburg et al., 1998), which are important components in poultry diets. Xanthophylls increase yellow-red pigmentation in skin and adipose tissue of broilers, and egg yolks from layers, which are desirable characteristics for consumers in many global markets (Perez-Vendrell et al., 2001; Leeson and Caston, 2004). In addition, carotenoids are responsible for fish and shellfish color (Shahidi and Brown, 1998). An orange-red color is desired in flesh of many seafoods and the amount of pigmentation is used to establish grade and price (Sacton, 1986). However, in catfish, minimizing xanthophyll content in catfish diets to less than 7 to 11 mg/kg is necessary to avoid accumulation of yellow pigments in fillets, which reduces consumer acceptance (Hu, 2012). Therefore, data on the variability of antioxidant capacity and concentrations of these phytochemicals in DDGS are needed to make informed feed formulation and management decisions for various DDGS feeding applications.

The objective of this study was to examine the variability in antioxidant capacity, vitamin E, and phytochemical content of DDGS samples obtained from 16 different fuel ethanol production plants, which varied in their processing methods.

## 2. Materials and methods

Sixteen DDGS samples (2 kg) were obtained from 16 different ethanol plants in the Midwestern United States from June to August, 2010. Processing methods, including drying times and temperatures, varied considerably among the different plants, but only 8 of the 16 sample providers agreed to provide details of the production conditions used. A yellow dent corn sample representing a common commercial variety, was obtained in August 2010 from the feed mill storage bin located at the University of Minnesota Southern Research and Outreach Center (Waseca, MN), and was used for comparison with DDGS samples. All samples were finely ground in a commercial coffee mill (Model Tipo 203, Krups, New York, NY) and were placed in plastic tubes, the headspace flushed with N<sub>2</sub> gas, and stored at –20 °C until analyzed.

### 2.1. Antioxidant capacity by DPPH

Antioxidant capacity was determined by monitoring the reaction of antioxidants with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH; Miller et al., 2000). Between 20 and 50 mg (exact amount was determined) of these samples was added 50 mL

of 101 µmol/L DPPH in 50% aqueous methanol. The flask was capped and incubated at 37 °C for 4 h. After incubation, the mixture was filtered through Whatman #1 filter paper and absorbance measured at 515 nm, using a Varian Cary 50 UV-Vis spectrophotometer (Varian Instruments, Walnut Creek, CA). A DPPH solution with no added sample was used as a blank solution. Trolox was used as a standard for antioxidant capacity. Antioxidant capacity was expressed as mmol tocopherol equiv./kg of sample.

### 2.2. Lipid peroxidation level by peroxide value and thiobarbituric acid reactive substances (TBARS)

Peroxide value (PV) of lipid extracts from the DDGS samples and corn sample was determined by an Association of Official Analytical Chemistry official method (AOAC, 2012; Method 965.33), as described by Song and Shurson (2013). Thiobarbituric acid reactive substances were measured in lipid extracts from the DDGS samples and corn sample by the method of Buege and Aust (1978), as described by Song and Shurson (2013).

### 2.3. Determination of tocopherols and tocotrienols

A direct solvent extraction method was used for extracting vitamin E from DDGS samples (Lee et al., 1998). One gram of ground sample was accurately weighed into a 125 mL round-bottom flask to which 4 mL of 80 °C deionized water was added. The contents were mixed with a stainless steel spatula and then sonicated for 1 min to facilitate solubilization and extraction. Ten mL of isopropanol containing 0.01% (wt/vol) butylated hydroxytoluene (BHT), 5 g of anhydrous MgSO<sub>4</sub> powder, and 25 mL of extracting solvent (ethyl acetate/hexanes, 10:90, vol/vol) containing 0.01% (wt/vol) BHT, were added and mixed with the spatula. The mixture was vortexed for 90 s and filtered through #1 Whatman filter paper. The filter cake was transferred back to the 125 mL round-bottom flask, and 5 mL of isopropanol containing 0.01% (wt/vol) BHT along with 25 mL of the extracting solvent were added, homogenized, and filtered. The pooled filtrates were transferred to a 100 mL volumetric flask and diluted to the mark with the extracting solvent. The solution was passed through a 0.45 µm nylon membrane filter (GE Osmonics Labstore, Minnetonka, MN, USA). A 1.0 mL aliquot of the extract was evaporated under a stream of nitrogen. The residue was re-dissolved in 1 mL of the mobile phase prior to injection into the HPLC.

The vitamin E extracts were injected into an HPLC system consisting of a Gilson 305 pump equipped with a fluorescence detector (Gilson Inc., Middleton, WI, USA), a normal-phase column (4 mm × 250 mm, 5 µm particle size; LiChrosorb Silica, ES Industries, West Berlin, NJ), and an isocratic mobile phase of 0.85% (vol/vol) isopropanol in hexane at a flow rate of 1.0 mL/min. The excitation and emission wavelengths for the fluorescent determination of tocopherol isomers were 290 and 330 nm, respectively. Twenty µL of sample extract or tocopherol standard solution were injected. Identification and quantification of tocopherol and tocotrienol isomers were based on the retention time and peak area of authentic standards.

### 2.4. Determination of xanthophylls

Xanthophylls were extracted using a slightly modified method (Nidhi and Baskaran, 2011). One gram of ground sample was accurately weighed into a 250-mL flask and extracted with ice-cold acetone until the acetone added to the sample was colorless. The acetone extract was filtered through Whatman #1 filter paper containing anhydrous sodium sulfate powder. The extract was saponified with methanolic KOH (30%, wt/vol) at room temperature

for 3 h in the dark to prevent photo-oxidation. After saponification, xanthophylls were extracted with 10 mL of hexane 3 times. The pooled hexane extract was dried under nitrogen. The residue was re-dissolved in 2 mL of hexane for quantification by HPLC.

The xanthophyll extracts were injected into an HPLC system consisting of an Agilent 1260 pump equipped with an UV/Vis detector (Agilent Technologies, Santa Clara, CA, USA), a Discovery C18 column (4.6 mm × 250 mm, 5 µm particle size; Supelco Company, Bellefonte, PA, USA), and an isocratic mobile phase consisting of acetonitrile:methanol:dichloromethane (60:20:20, vol/vol/vol) and containing 0.1% ammonium acetate, at a flow rate of 1.0 mL/min. The xanthophylls were measured at 450 nm. Twenty µL of sample extract were injected. Identification and quantification of xanthophylls (lutein and zeaxanthin) were based on the retention time and peak area of authentic standards.

### 2.5. Determination of ferulic acid

Total and free (unbound) ferulic acid were extracted as previously described (Vaidyanathan and Bunzel, 2012). For determination of free ferulic acid, 5 mL of distilled water was added to 100 mg of ground sample, the mixture was vortexed, and concentrated HCl was added until the pH was less than 2. The sample was then extracted with 20 mL of diethyl ether 3 times. The organic phase was evaporated with nitrogen gas and the residue was reconstituted in 0.5 mL methanol:water mixture (50:50, vol/vol).

For determination of total ferulic acid, 100 mg of the ground sample was added to 5 mL of 2 mol/L NaOH solution and the mixture was vortexed. The samples were purged with nitrogen gas then stored in the dark for 18 h. Subsequently, concentrated HCl was added to the samples until the pH was below 2. The samples were then extracted with 20 mL of diethyl ether 3 times. The organic phase was evaporated to dryness with nitrogen gas. After evaporation, the residues were reconstituted in 0.5 mL methanol:water mixture (50:50, vol/vol).

Ferulic acid in the extracted DDGS samples was measured using a Luna phenyl-hexyl column (250 mm × 4.6 mm i.d., 5 µm particle size, Phenomenex Inc., Torrance, CA, USA) using a ternary gradient system at a flow rate of 1.0 mL/min. The mobile phases used were as follows: eluent A, 1 mmol/L aqueous trifluoroacetic acid (TFA); eluent B, acetonitrile (ACN):1 mmol/L aqueous TFA (90:10, vol/vol); eluent C, MeOH:1 mmol/L aqueous TFA (90:10, vol/vol). The initial conditions were 87% A, 13% B, and 0% C, which were held for 10 min, followed by a linear change over 10 min to 77% A, 20% B, and 3% C, linear over 5 min to 70% A, 25% B, and 5% C, linear over 5 min to 25% A, 50% B, and 25% C, following a 10 min re-equilibration step. Twenty µL were analyzed per run, and the separation was performed at 45 °C. Ferulic acid was detected at 280 nm.

### 2.6. Statistical analysis

Individual values and standard deviations in Tables 1 to 4 are means of duplicate determinations. Associations between different parameters were determined by calculation of Person correlation coefficients.

## 3. Results

### 3.1. Antioxidant capacity by DPPH

Antioxidant capacity in DDGS samples, measured by the DPPH assay, is shown in Table 1. Antioxidant capacity of the DDGS samples ranged from 29.04 to 65.20 mmol tocopherol equiv./kg, indicating a 2-fold difference in the apparent ability among DDGS sources to resist peroxidation. The corn sample had an antioxidant

capacity of 8.09 mmol tocopherol equiv./kg, which was approximately one-fifth of the average value for the DDGS samples, indicating a high degree of antioxidant capacity in DDGS produced from corn.

### 3.2. Lipid peroxidation by peroxide value and thiobarbituric acid reactive substances (TBARS)

Peroxide value (PV) and TBARS are shown in Table 1, for the 16 DDGS samples. Peroxide values varied considerably among the DDGS samples, from a low of 4.84 meq/kg oil to a high of 84.13 meq/kg oil in sample 3. Excluding sample 3, which had an extremely high PV, there was still a 4-fold range of PV among DDGS samples. Values for TBARS ranged from 1.07 to 5.23 ng malondialdehyde (MDA) equiv./mg oil. Excluding sample 3, there was over a 3-fold difference among DDGS samples.

Peroxide value and TBARS content were highly correlated ( $r = 0.857$ ,  $P < 0.001$ ), and DPPH values were correlated with both PV ( $r = 0.758$ ,  $P = 0.0007$ ) and TBARS ( $r = 0.604$ ,  $P = 0.013$ ). However, the significant correlation of DPPH with PV and TBARS were caused by the high values from DDGS sample 13. When sample 13 was removed from the analysis, DPPH values were no longer significantly correlated with PV or TBARS ( $r = -0.003$ ,  $P = 0.99$  and  $r = 0.040$ ,  $P = 0.88$ , respectively). However, with sample 13 removed, PV and TBARS still remained significantly correlated ( $r = 0.670$ ,  $P = 0.006$ ).

### 3.3. Tocopherol and tocotrienol contents

The profile of concentrations of tocopherols and tocotrienols in the DDGS samples and corn sample are shown in Table 2. In DDGS,  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherols and  $\alpha$ - and  $\gamma$ -tocotrienols were identified. The predominant tocopherol was  $\gamma$ -tocopherol, and  $\gamma$ -tocotrienol was the predominant tocotrienol in all DDGS samples and in the corn sample. However, the concentrations of tocopherols and tocotrienols in the DDGS samples were more than double that in the corn sample, indicating that these lipid-soluble antioxidants are highly concentrated in DDGS. There were also notable differences among the DDGS samples in the total concentration of tocopherols and tocotrienols. For example, sample 3, which had the highest antioxidant capacity, had one of the lowest concentrations of tocopherols and tocotrienols. This suggests that the high antioxidant capacity of sample 3 is due to antioxidants other than tocopherols and tocotrienols. There was no statistically significant correlation between the antioxidant capacity of the DDGS samples and the concentration of any individual tocopherol or tocotrienol, although there was a trend for an inverse correlation between antioxidant capacity and  $\delta$ -tocopherol concentration ( $r = -0.49$ ,  $P = 0.057$ ). Furthermore, there was a trend for an inverse correlation between antioxidant capacity and total tocopherol concentration ( $r = -0.47$ ,  $P = 0.064$ ).

### 3.4. Xanthophyll content

Lutein and zeaxanthin were the two xanthophylls identified in the DDGS samples (Table 3). Lutein was found in all DDGS samples and ranged from 447 to 1,343 µg/kg, whereas zeaxanthin ranged from being not detected in 4 samples to 243 µg/kg. In cases where both xanthophylls were detected, lutein was always the predominant compound and was generally found in concentrations from 4.4 to 9.3 times higher than that of zeaxanthin. There was more than a 3-fold variation (447 to 1,586 µg/kg) in total xanthophyll concentration among DDGS samples. The average lutein concentration of all DDGS samples ( $627 \pm 218$  µg/kg) was considerably greater than that of the corn sample (385 µg/kg), but average

**Table 1**

Antioxidant capacity, peroxide value, and TBARS of DDGS samples obtained from various fuel ethanol production plants across the United States compared with a sample of yellow dent corn.

Item	Antioxidant capacity, mmol tocopherol equiv./kg	Peroxide value, meq/kg oil	TBARS, ng MDA equiv./mg oil
DDGS sample			
1	33.36	22.27	3.47
2	33.07	5.98	1.07
3	65.20	84.13	5.23
4	52.96	11.90	2.58
5	38.20	11.34	2.24
6	29.04	12.00	1.70
7	30.91	12.78	1.71
8	35.99	8.40	1.74
9	45.73	14.76	1.26
10	33.95	12.73	2.23
11	39.75	4.84	1.02
12	35.62	11.65	1.16
13	37.51	9.02	1.12
14	33.39	11.42	2.50
15	33.94	10.00	1.73
16	30.55	6.94	2.09
Mean ± SD	38.07 ± 93.91	15.64 ± 18.70	2.05 ± 1.08
Corn sample	8.09	ND	ND

TBARS = thiobarbituric acid reactive substances; DDGS = distillers dried grains with solubles; meq = milliequivalents; equiv. = equivalents; MDA = malondialdehyde; ND = not determined.

Values are the average of duplicate determinations.

zeaxanthin ( $95 \pm 50 \mu\text{g}/\text{kg}$ ) and total xanthophylls ( $697 \pm 257 \mu\text{g}/\text{kg}$ ) concentrations were similar to those in corn (63 and 448  $\mu\text{g}/\text{kg}$ , respectively).

### 3.5. Ferulic acid content

Free and total ferulic acid content of the DDGS samples and corn sample is shown in Table 4. The average total ferulic acid concentration ( $7.455 \pm 0.675 \text{ mg}/\text{g}$ ) was approximately 3-fold greater in the DDGS samples compared with the corn sample ( $2.503 \text{ mg}/\text{g}$ ). Only a small proportion of the average total ferulic acid was present in the free form in the DDGS samples ( $0.042 \pm 0.016 \text{ mg}/\text{g}$ ), as was the case with the corn sample ( $0.011 \text{ mg}/\text{g}$ ), although this fraction varied

**Table 2**

Tocopherols (T) and tocotrienols (T3) in DDGS samples obtained from various fuel ethanol production plants across the United States compared with a sample of yellow dent corn, (mg/kg dry matter).

Item	$\alpha$ -T	$\alpha$ -T3	$\gamma$ -T	$\gamma$ -T3	$\delta$ -T	Total-T
DDGS sample						
1	10.1	9.9	79.7	12.0	14.4	126.1
2	6.4	7.5	70.0	13.9	18.2	116.0
3	4.1	5.4	64.0	8.7	10.0	92.2
4	5.3	7.2	54.4	11.6	14.4	92.9
5	12.2	8.9	65.5	14.8	17.6	119.0
6	8.3	8.7	71.6	15.1	17.9	121.6
7	9.6	5.7	52.7	7.6	15.2	90.8
8	10.0	8.5	69.5	13.9	19.1	121.0
9	19.6	10.6	61.4	13.1	22.0	126.7
10	10.7	10.7	78.5	17.0	24.3	141.2
11	9.3	10.4	80.7	17.5	19.6	137.5
12	12.7	9.7	67.2	14.4	19.2	123.2
13	9.5	12.2	81.4	17.1	20.9	141.1
14	16.3	12.8	73.3	16.6	19.7	138.7
15	19.7	11.9	65.8	17.0	22.5	136.9
16	9.7	9.0	68.1	13.3	16.1	116.2
Mean ± SD	10.8 ± 4.5	9.3 ± 2.2	69.0 ± 8.6	14.0 ± 2.9	18.2 ± 3.6	121.3 ± 16.9
Corn sample	3.2	2.4	32.7	8.6	10.1	57.0

DDGS = distillers dried grains with solubles.

**Table 3**

Xanthophylls in DDGS samples obtained from various fuel ethanol production plants across the United States compared with a sample of yellow dent corn.

Item	Xanthophylls, $\mu\text{g}/\text{kg}$ dry matter		Total
	Lutein	Zeaxanthin	
DDGS sample			
1	873	ND	873
2	727	ND	727
3	565	ND	565
4	544	58	602
5	492	93	585
6	507	72	579
7	628	97	725
8	555	75	620
9	633	106	739
10	552	86	638
11	1,343	243	1,586
12	597	64	661
13	489	53	542
14	447	ND	447
15	609	99	708
16	474	88	562
Mean ± SD	627 ± 218	95 ± 50	697 ± 257
Corn sample	385	63	448

DDGS = distillers dried grains with solubles; ND = not detected.

considerably among the DDGS samples (0.018 to 0.087  $\text{mg}/\text{g}$ ). Overall, the proportion of free to total ferulic acid was similar between the DDGS samples and the corn sample (average of 0.57% vs. 0.42%). Total ferulic acid concentration did not correlate with antioxidant capacity ( $r = 0.068$ ,  $P = 0.80$ ), but there was a significant positive correlation between antioxidant capacity and free ferulic acid concentration ( $r = 0.533$ ,  $P = 0.03$ ).

## 4. Discussion

Corn DDGS is a highly nutritious feed ingredient that is a concentrated source of energy, protein, lipids, fiber, and phosphorus (Speihs et al., 2002). However, there is considerable variation in the concentration of these nutrients among sources of DDGS, due to differences in the feedstock used, processing methods, and the amount of condensed distiller solubles mixed with the wet distillers grains to create the DDGS (Liu, 2011). As a

**Table 4**

Ferulic acid contents of DDGS samples, obtained from various fuel ethanol production plants across the United States compared with a sample of yellow dent corn.

Item	Ferulic acid, $\text{mg}/\text{g}$ dry matter	
	Free ferulic acid	Total ferulic acid
DDGS sample		
1	0.063	9.511
2	0.036	8.037
3	0.087	7.406
4	0.044	7.824
5	0.033	6.907
6	0.036	6.976
7	0.035	7.301
8	0.034	7.606
9	0.036	7.546
10	0.038	6.801
11	0.033	7.071
12	0.041	7.410
13	0.018	7.395
14	0.061	7.263
15	0.042	6.774
16	0.040	7.049
Mean ± SD	0.042 ± 0.016	7.455 ± 0.675
Corn sample	0.011	2.503

DDGS = distillers dried grains with solubles.



result, DDGS would also be expected to be a variable but concentrated source of micronutrients and bioactive phytochemicals present in corn. However, few studies have examined this.

Antioxidant capacity among the DDGS samples evaluated in this study varied considerably, although all samples had substantially greater antioxidant capacity than found in the corn sample. Differences in antioxidant capacity among the DDGS samples are likely due to both the antioxidant capacity of the corn sources used in the fermentation process of ethanol and production, as well as the drying conditions employed when producing DDGS. High drying temperatures used in the processing of protein-containing foods can produce colored Maillard reaction products that have high antioxidant capacity (Anese et al., 1999). Therefore, in some circumstances, a high antioxidant capacity can actually be an indicator of excessive thermal treatment. Color has been used as a general indicator of the extent of excessive thermal treatment and protein damage in DDGS (Cromwell et al., 1993; Fastinger and Mahan, 2006). Song and Shurson (2013) showed that  $L^*$  (lightness or darkness of color) and  $b^*$  (yellowness of color) were moderately and negatively correlated with the extent of lipid peroxidation in DDGS samples based on peroxide value and TBARS measures. Furthermore, the extent of lipid damage is highly variable among DDGS sources, which has been attributed largely to thermal processing conditions (Song and Shurson, 2013), and is likely a contributor to the considerable variation in antioxidant capacity among DDGS samples observed in the current study.

Lipid peroxidation of the DDGS samples also varied considerably, which was likely due to differences in thermal processing of the samples because thermal abuse is well known to increase PV and TBARS of lipids. Antioxidant capacity (measured as DPPH) and lipid peroxidation (measured as PV and TBARS) may be expected to vary inversely because of the greater antioxidant capacity to protect the lipids from peroxidation. However, when all DDGS samples were considered, there was a positive correlation between DPPH and PV or TBARS. When sample 13 (which had extreme values for DPPH, PV, and TBARS) was removed from the correlation analysis, DPPH showed no correlation with either PV or TBARS. This suggests that the greater antioxidant capacity produced in the DDGS by thermal processing, which presumably are Maillard reaction products, are not capable of limiting lipid peroxidation. Zhang et al. (2015) reported that for a large number of phenolic antioxidants, there was little correlation between antioxidant capacity, measured as DPPH activity, and anti-lipid peroxidation ability. However, few, if any, studies have examined the antioxidant capacity of Maillard reaction products, such as those measured by the DPPH assay, along with measures of lipid peroxidation in the same lipid-rich food or feed ingredient source. Thus, our findings suggest that a high antioxidant activity resulting from the formation of Maillard reaction products does not necessarily protect that food or feed ingredient from lipid peroxidation. Given the relatively high prevalence of Maillard reaction products in feed ingredients and human foods, this area warrants further investigation.

Vitamin E is a lipid soluble chain-breaking antioxidant that quenches free radicals in cell membranes. It has 8 identified naturally occurring forms:  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocotrienols. Because DDGS has a higher lipid content than corn (90 to 120 g/kg for DDGS vs. 35 g/kg for corn; NRC, 2012), it would be expected that the vitamin E concentration in DDGS would also be greater than corn. Estimates of the vitamin E content of corn have been reported to be in the range from 11.65 mg/kg (NRC, 2012) to 22 mg/kg (NRC, 1994).

In the present study, the average concentration of total tocopherols in DDGS was approximately double (121.3 mg/kg) than

that found in the corn sample (57 mg/kg). The NRC (2012) provides no values for vitamin E in DDGS with >10% oil, > 6% and <9% oil, or <4% oil. In contrast, NRC (1994) indicates that DDGS contains 40 mg/kg vitamin E (presumably as  $\alpha$ -tocopherol). The average  $\alpha$ -tocopherol content in DDGS samples in the present study was 10.8 mg/kg, and ranged from 4.1 to 19.7 mg/kg, which was greater than the average of 6.8 mg/kg of 6 corn DDGS samples evaluated by Jung et al. (2013). For corn, the concentration of total tocopherols was 57.0 mg/kg, which was greater than the vitamin E value (11.65 mg/kg) provided in NRC (2012) and 22 mg/kg reported in NRC (1994). The predominant tocopherol isomer in the DDGS samples was  $\gamma$ -tocopherol, as has been reported by others (Winkler et al., 2007; Moreau et al., 2011). Although there was no statistically significant correlation between total tocopherols or any tocopherol isomer and antioxidant capacity, as measured by the DPPH assay, interestingly both  $\delta$ -tocopherol and total tocopherols showed a trend toward an inverse correlation with antioxidant capacity, which, however, was primarily driven by 2 samples with very high antioxidant capacity and very low total tocopherol concentration. This would be consistent with the formation of Maillard reaction products due to thermal abuse in these samples, and the destruction of tocopherols in the process. Regardless, the antioxidant capacity of the DDGS sources, as measured by DPPH, is clearly due to the presence of antioxidant compounds other than tocopherols.

Xanthophylls are oxygenated derivatives of carotenoids found mainly in cereals and vegetables. Due to their conjugated double bond system, they are strong scavengers of singlet oxygen and peroxy radicals *in vitro* (Yeum et al., 2009; Kaulmann and Bohn, 2014). In addition to their antioxidant capacity, xanthophylls are important dietary components for poultry (Perez-Vendrell et al., 2001; Leeson and Caston, 2004) to increase pigmentation of skin and adipose tissue of broilers, and egg yolk color of layers, which are desired characteristics by consumers in many markets around the world. Similarly, providing adequate xanthophylls in many aquaculture diets is important for achieving optimum market value and consumer acceptance of fish and shellfish products (Shahidi and Brown, 1998), but in some species like catfish, minimizing xanthophyll content in the diet is necessary to avoid accumulation of yellow pigment in filets, which reduces consumer acceptance (Hu, 2012).

In the present study, the total xanthophyll concentration of the DDGS samples varied considerably (447 to 1,586  $\mu$ g/kg), but in all cases, it equaled or exceeded that of corn (448  $\mu$ g/kg). Lutein content was found in the greatest concentrations compared with zeaxanthin, but both were highly variable among sources. Bacchetti et al. (2013) reported that lutein content ranged from 231 to 496  $\mu$ g/kg, and zeaxanthin content ranged from 1,762 to 2,183  $\mu$ g/kg among 5 corn (*Zea mays* L.) sources, which were considerably higher than found in the corn source analyzed in the present study, where the concentration of lutein was greater than zeaxanthin, and zeaxanthin was not detected in 4 of the DDGS samples. Salim et al. (2010) reported that the xanthophyll content in DDGS ranged from 23.26 to 54.40 mg/kg. Roberson et al. (2005) reported a large difference (29.75 vs. 3.48 mg/kg) in xanthophyll content in 2 samples of corn DDGS, and showed that egg yolk pigmentation increased when 10% DDGS was added to a laying hen diet. However, Lumpkins et al. (2005) reported no effects of feeding 15% corn DDGS on egg yolk color. Zaripheh and Erdman (2002) reviewed factors that affect the bioavailability of xanthophylls and concluded that lutein is more bioavailable from food sources than carotene, but not much is known about the bioavailability of other xanthophylls. Therefore, it is not surprising that pigmentation responses in poultry and aquaculture studies vary due to highly variable xanthophyll content among DDGS sources, and no information about the bioavailability of various xanthophylls.

Ferulic acid acts as an antioxidant due to its phenolic nucleus and extended side chain (Kanski et al., 2002). Our values for total ferulic acid in DDGS are somewhat greater than those reported by Luthria et al. (2012), who showed more than a 2-fold variation in concentration among 3 different sources, which is a much greater variation than found in the present study. In corn, ferulic acid exists almost entirely in the cell wall of the bran, bound to arabinoxylans (Kato and Nevins, 1985). Although the fermentation of starch in corn to produce DDGS was expected to increase the concentration of total ferulic acid in the DDGS samples relative to corn, it did not result in an increase in the proportion of free to total ferulic acid. This suggests that fermentation either did not release bound ferulic acid, or that released ferulic acid was solubilized in the aqueous phase and removed. However, the greater concentration of free ferulic acid in DDGS may be of benefit as a feed preservative, because free ferulic acid has been shown to inhibit the growth of spoilage yeasts (Stead, 1995) and to have antifungal activity (Lattanzio et al., 1994). Furthermore, the positive correlation between free ferulic acid and antioxidant capacity suggests that the free ferulic acid may have contributed to the antioxidant capacity of DDGS.

## 5. Conclusions

The DDGS samples evaluated in this study showed a greater concentration of the antioxidant capacity and phytochemical components compared with those present in ground corn, specifically, tocopherols, xanthophylls and ferulic acid. These findings suggest that DDGS has antioxidant capacity and properties that may provide health benefits to animals and consumers of animal products derived from animals fed DDGS diets. Furthermore, the antioxidants present in DDGS may serve as preservatives and prolong shelf-life in feeds. However, further studies are required to demonstrate that the greater antioxidant capacity results in a reduction in lipid peroxidation. It is also noteworthy that excessive thermal treatment during DDGS production may reduce tocopherol concentrations and produce undesirable Maillard reaction products.

Our findings indicate that there is considerable variation among DDGS samples in their antioxidant capacity and phytochemical content, and a few samples show large differences from the average, which most likely was due to excessive heating during the production process.

## Conflict of interest

None.

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