INTRODUCTION

Reduction of feed cost has been one of the major research initiatives as feed costs currently account for 60-70% of total poultry production costs. Therefore, research is essential and different alternatives must be considered with the intention of mitigating feed costs. In many countries of the world, pigmentation of the chicken carcass plays an important role in the customer perception of quality and health of poultry products prior to purchase or consumption (Sunde, 1992; Castaneda et al., 2005; Rajput et al., 2012).

Carotenoids are the main compounds with coloring properties (Blanch and Hernandez, 200), and they have been used as antioxidants, immune modulators, and predecessors for the synthesis of vitamin A (Surai and Speake, 1998; Jung et al., 2012). Carotenoids are particularly important to avian species, as pigmentation quality is indicative of a bird's general health (Olson and Owens, 1998) and parental food allocation (Saino et al., 2000). Currently, synthetic and natural pigments (primarily lutein and canthaxanthin) are added to poultry feeds to increase the color of skin, meat and egg yolk in order to increase consumer acceptance of the final product (Hernandez et al. 2001).

More than 50% of the ethanol producing plants in the US extract oil from the co-products (DDGS) at the end of the ethanol production process to increase revenue from value added co-products. Currently, most of the extracted oil is used for biodiesel production, but a greater residual value may be recovered from the carotenoid pigments it contains. According to Moreau (2010), the oil obtained after the ethanol production contains all the yellow carotenoid pigments, which are chemically intact and therefore fully functional. We hypothesize that corn distillers solubles (CDS) oil, obtained as a co-product from the ethanol production, can be used as a replacement for the more expensive synthetic carotenoids or those extracted from marigold flowers that are currently used extensively in commercial poultry production. Therefore, the general objective of our project was to evaluate the ethanol bio-fuel co-product, corn distillers solubles oil extract as a source of dietary carotenoids for poultry.

To address the general objectives, our project was conducted in 3 phases, each to address one of the following specific objectives:

1. Determine the levels of naturally occurring carotenoids in DDGS extracted corn oil by chemical analysis and determination of oxidative stability.
2. Determine the potential economic value of carotenoid pigments in DDGS oil extract for various poultry applications relative to commercial sources of feed-grade pigments, using least-cost linear programming and parametric analysis.
3. Determine the biological value of carotenoid pigment in DDGS oil extract relative to a commercial source (i.e. Marigold extract, synthetic carotenoid pigments) using a chick bioassay.

METHODS AND MATERIALS

Phase 1; Specific Objective 1: Evaluation of Carotenoid Content and Stability of CDS Oil.

Samples of CDS oil (approx. 500 ml each) were obtained from 11 different ethanol production plants located in the Midwestern USA. All the samples were refrigerated at 4^{o} C upon receipt, and then submitted together after 3 weeks of cold storage for AOAC chemical analysis methods for fat stability (moisture and volitiles, insolubles, and unsapponifiables contents, initial peroxide value, p-anisidine, and 20 hr AOM) by Eurofins Laboratories (Des Moines, IA) and the levels of carotenoids determined by Craft Technologies, Inc. (Wilson, NC). Average carotenoid profile and the analytical variability of the CDS oil among ethanol plants were calculated. Based on the processing method, the carotenoid content may vary depending on the state of oxidation of the residual DDGS corn oil extract. Preparation and purification of carotenoids from the CDS oil was done as described by Tyczkowsky and Hamilton (1992), and carotenoid profile of the prepared samples were determined using an HPLC method of Hamilton (1992) and modified by Koutsos et al. (2003).

Phase 2; Specific Objective 2: Evaluation of the potential economic value of CDS oil extract carotenoid pigments for use in poultry feeds.

Once the carotenoid profile of the corn DDGS extract oil from different processes were determined, the potential economic value was calculated relative to the market price of a commercially available carotenoid pigment extract from marigold (Yellow Pixafil Liquid-LZ, Alcosa Biotec, S.A., Apaseo El Grande, GTO, Mexico). The potential opportunity price of the CDS oil will depend on the prices of competing commercial pigments, feed ingredient formulation, and the carotenoid formulation constraints for the different poultry applications. For the sake of simplicity, the added value of the CDS oil due to its carotenoid pigment analysis value was determined as a function of the commercial value the carotenoid unit costs from the commercial marigold pigment source. Assuming Yellow Pixafil Liquid-LZ, containing 15 g of total Xanthophils/kg of product, has a market price of $4 USD/kg, the total xanthophil in CDS oil would have a commercial value of about $0.27 USD/g of xanthophil.

Phase 3; Specific Objective 3: Evaluation of the biological value of CDS oil carotenoids for broilers

An experiment was conducted to determine the bioavailability of CDS oil carotenoids in broilers raised to 56 days of age in cages. Ross 344x708 broiler chicks were randomly assigned among six dietary treatment groups, each treatment was assigned to 8 replicate cages containing 10 chicks. The birds were raised until 28 days of age in 36 brooder battery cages and then transferred to 36 grower battery cages (Alternative Design Manufacturing and Supply, Inc., http://www.altdesign.com) and raised until 55 days of age. The birds were raised in thermal comfort conditions with 23 hours light per day until 7 days of age, and then 14 hours light per day thereafter until the termination of the experiment. All the birds were given ad libitum access to feed and water throughout the experiment. This experiment was approved by the North Carolina State University Institutional Animal Care and Use Committee (Protocol No. 15-061A).

All the birds were fed common starter (1-28 days) and grower (29-55 days) basal diets (Table 1). The diets were formulated utilizing white corn and soybean meal to minimize carotenoids in the basal diet. The experimental diets were produced by supplementing the basal diet with 0, 25, 50, 75 or 100% of added CDS oil in place of 4% soybean oil, and as a positive control, an equivalent level of total xanthophils as in the CDS oil was added by Yellow Pixifil Liquid-LZ (Alcosa Biotec, S.A., Apaseo El Grande, Mexico) in the negative control soy oil (1.5% Yellow Pixifil Liquid-LZ in soybean oil, w/w). The CDS oil acquired from plant G was used to supplement the diet because it had good stability and a fairly average carotenoid content. The carotenoid level of the CDS oil, the negative control (0%), and 100% inclusion were analyzed for carotenoid concentration, and the carotenoid level was calculated for the remaining diets (Table 2). Figure 1 illustrates the color differences of sampled starter feed among the dietary treatments. Note the increase in yellow color as the level of CDS oil replacement of soybean oil increased and the comparison with the positive control sample, which contained soybean oil with 1.5% Yellow Pixifil Liquid-LZ.

Group body weights were measured on placement at 1 day of age, and individual body weights and cage feed consumption were determined at 14, 28, 35, 42, 49 and 54 days of age. Mortality rate was recorded, by week as it occurred and the weight of mortality was recorded to adjust cage feed conversion data.

Bioavailability of the dietary carotenoid pigment supplementation level was determined by the degree of yellowness in shank skin, breast skin, and breast muscle using a Konica Minolta chromometer (Model CR400). Yellowness of shank skin of individual birds was measured at 14, 21, 28, 37, 44, and 51 days. At 55 days, 4 birds per cage were humanely processed at the NCSU Chicken Education Unit, and the thymus, bursa, spleen and liver were removed and weighed. Yellowness of the breast skin of each hot processed broiler was measured using the chromometer. The eviscerated carcasses were then air-chilled to 4 C overnight, cut up to commercial parts, and weighed to determine carcass parts yield. Yellowness color of the breast muscle filet of each carcass was measured with the chromometer.

Table 1. Basal diet formulation for the starter and grow-finisher feed fed to broilers in the bioassay trial

	Starter feed (1-28 d)	Grow-Finisher Feed (28-49 d)
Ingredient	**%**	**%**
Soybean Meal 48%)	36.38	28.08
White Corn	54.64	63.15
Soya Oil	0.57	0.50
Experimental Treatment Oil Premix[1]	4.00	4.00
Dicalcium Phosphate 18.5% P	1.91	1.44
Limestone	1.02	1.43
Salt	0.29	0.27
DL-Methionine	0.37	0.27
L-Lysine HCL	0.21	0.26
Sodium Bicarbonate	0.16	0.18
L-Threonine	0.11	0.06
Choline Chloride (60%)	0.10	0.10
Sodium Selenite Premix[2]	0.10	0.10
NCSU Trace Mineral Premix[3]	0.10	0.10
NCSU Vitamin Premix[4]	0.05	0.05
	100	**100**
Calculated Nutrient Analysis		
Kcal ME/kg	3200	3253
Crude Protein, %	22.41	19
Crude Fat, %	6.71	6.80
Calcium, %	1.00	0.80
Total P, %	.735	.614
Av. Phosphorus, %	.480	.380
Na, %	.180	.180
K, %	1.111	.923
Cl, %	.250	.250
Arginine, %	1.52	1.2609
Dig Arg, %	1.4056	1.1679
Lysine, %	1.4	1.2
Dig Lys, %	1.2664	1.0893
Met, %	.7167	.5809
Dig Met, %	.6825	.5505
Met+Cys, %	1.08	.9000
Dig Met+Cys, %	.9068	.7488
Threonine, %	.97	.7800
Dig Threonine, %	.8736	.6974
Tryptophan, %	.2609	.2134
Dig. Tryptophan, %	.2274	.1851
Leucine, %	1.9266	1.7092
Isoleucine, $%	.9474	.7872
Valine, %	1.1675	.9896
Choline mg/kg	1756.9510	1600.5090

[1]Experimental treatment oil premix comprised of a blend for each treatment comprising a replacement of 0, 25, 50, 75, or 100% of the soy oil with CDS oil, or the inclusion of Yellow Pixifil Liquid-LZ (Alcosa Biotec, S.A., Apaseo El Grande, Mexico) that provides the equivalent of the 100% CDS oil treatment.

[2]$NaSeO_3$ premix provided 0.3 mg Se/kg of complete feed.

[3]Each kilogram of mineral premix (.1% inclusion) supplied the following per kg of complete feed: 60 mg Zn as $ZnSO_4 \cdot H_2O$; 60 mg Mn as $MnSO_4 \cdot H_2O$; 40 mg Fe as $FeSO_4 \cdot H_2O$; 5 mg Cu as $CuSO_4$; 1.25 mg I as $Ca(IO_3)_2$; 1 mg Co as $CoSO_4$.

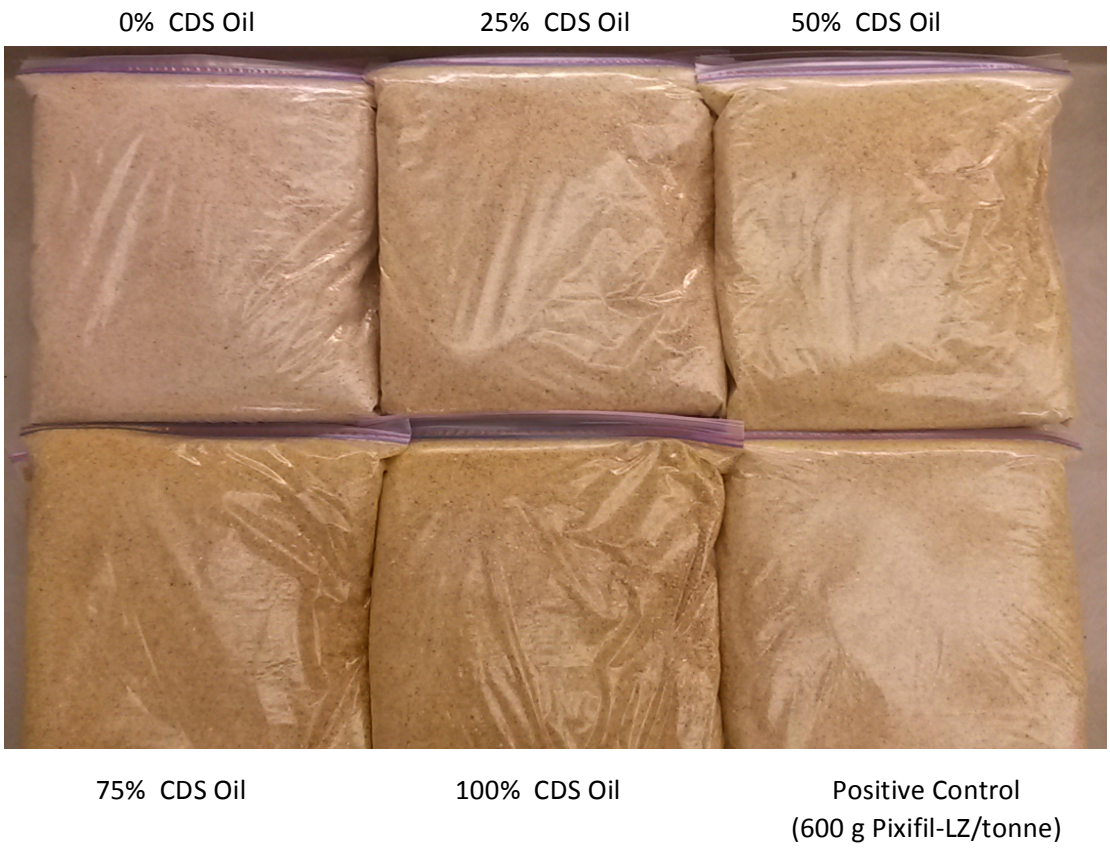
[4]Each kilogram of vitamin premix (.1% inclusion) supplied the following per kg of complete feed: vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; alpha-tocopherol, 66 IU; niacin, 110 mg; pantothenic acid, 22 mg; riboflavin, 13.2 mg; pyridoxine, 8 mg; menadione, 4 mg; folic acid, 2.2 mg; thiamin, 4 mg; biotin, 0.253 mg; vitamin B_{12}, 0.04 mg; ethoxyquin, 100 mg.

Table 2: Dietary treatments for the broiler growth trial to determine the bioavailability (%BV) of carotenoids in corn distillers solubles (CDS) oil.

Treatment[1]	% CDS oil in diet	% Soy oil in diet	mg carotenoids/ kg diet (Calculated)	mg carotenoids/ kg diet (Determined)
0%	0	4	0	0.75
25%	1	3	2.43	ND
50%	2	2	4.87	ND
75%	3	1	7.3	ND
100%	4	0	9.73	9.78
Positive Control[2]	0	4*		9.2

[1]Treatment is described as % replacement of soy oil for Corn Distillers Solubles oil.
[2]Positive control included 1.5% Yellow Pixifil Liquid-LZ in soybean oil* (w/w).

Figure 1. Samples of the dietary treatment feeds fed to the broilers in the bioassay trial. The samples are identified by treatment as described in table 2.



RESULTS AND DISCUSSION

Phase 1; Specific Objective 1: Evaluation of Carotenoid Content and Stability of CDS Oil. Although all the ethanol plants follow a similar general procedure of extracting the oil from the corn distillers solubles fraction Corn oil by centrifugation, process differences that do exist evidently resulted in qualitative variation in fat quality and carotenoid content. Table 3 summarizes the fat quality indicators for samples submitted from each of the 11 plants (identified as A to L). The % Coefficient of variation (%C.V.) appear to be high for some fat quality variables (i.e. 20 hr AOM) and low for others (i.e. Initial Peroxide Value), but this is a function of their low levels relative to the analytical detection limits. The results from this fat quality survey indicate that all of the samples are of good quality relative to the expectations for corn oil. The content of moisture, insoluble, and unsaponifiables (Total % MIU) are well below the 3% maximum % MIU typically expected for feed grade corn oil. Initial peroxide value is also below the expected detection limit of 0.5 mEq/kg fat. To further confirm that this low peroxide value is not due to excessive oxidation, the p-Anisidine value (a relative measure of aldehydes in excessively rancid fat) is also low. All of the samples had relatively good oxidative stability as indicated by the 20 h AOM stability test, although a sample from one plant was appeared to have a much higher value than the others. As a point of comparison, corn oil subjected to no, slow (72 h at 95 C with 12 L compressed air flow/min), and rapid (7 h at 185 C with 12 L compressed air flow/min) peroxidation conditions resulted in p-Anisidine values of <1, 61.4 and 142.9 mEq/kg, respectively, and 20 h AOM values of 103, 575, and 528 mEq/kg, respectively (Shurson et al., 2015).

Table 3: Fat quality indicators of samples of CDS oil sourced from 11 different ethanol plants (A to L).

Ethanol Plant	Moisture and volatiles, %	Insoluble, %	Unsapon-ifiables, %	Total MIU, %	Initial Peroxide value, mEq/kg	p-Anisidine	20 h AOM Stability, mEq/kg
A	0.40	0.03	1.39	1.82	0.2	17.8	38
B	0.56	0.04	1.37	1.97	0.2	20.8	110
C	0.39	0.02	1.43	1.84	0.2	18.6	24
D	0.45	0.03	1.19	1.67	0.2	18	40
E	0.36	0.02	1.34	1.72	0.2	21	38
F	0.56	0.03	1.82	2.41	0.2	16.1	28
G[1]	**0.38**	**0.02**	**1.35**	**1.75**	**0.2**	**20.4**	**29**
H	0.47	0.03	1.41	1.91	0.2	22.7	40
I	0.18	0.06	1.4	1.64	0.2	24.2	53
K	0.24	0.03	1.81	2.08	0.2	21.8	83
L	0.17	0.05	1.6	1.82	0.2	24	63
Mean ± std	0.38 ± 0.14	0.03± 0.01	1.46 ± 0.20	1.88 ± 0.22	0.20 ± 0	20.49 ±2.63	49.64 ± 26.37
% C.V.	36.8	33.3	13.7	11.7	0	12.8	53.1

[1]CDS oil from ethanol plant G was used in the carotenoid bioassay trail (Phase 3).

Table 4 is a summary of the carotenoid composition of the corn distillers solubles oil collected from the 11 ethanol plants surveyed. To our knowledge, this is the first study that analyzed the carotenoid content of CDS oil and the variability among ethanol plants. Total carotenoid pigment averaged about 230 mg/kg with a coefficient of variation of 21%. The carotenoids contained in CDS oil ranged from highest to lowest as follows: trans-lutein (97 mg/kg); trans-zeaxanthan (75 mg/kg); cis-Lutein/Zeaxanthan (36 mg/kg); α-Cryptoxanthin (12 mg/kg); and β-Cryptoxanthin (9 mg/kg). The most variable carotenoid was α-Cryptoxanthin with a CV > 36%. This degree variability in carotenoid content of CDS oil among plants is expected, since yellow corn itself varies in carotenoid levels. Weber (1987) found samples ranged between 15.8 and 73.6 micrograms per gram. Even though the carotenoid content varied among ethanaol plants (Figure 2), the profile content of the different carotenoids

relative to total carotenoids was impressively consistent to contain 42% trans-lutein, 33% trans-zeaxanthan, 25% cis-lutein/zeaxanthin, 5.5% α-Cryptoxanthin, and 4% β-Cryptoxanthin (Figure 3). Evidently, CDS oil is a concentrated source of carotenoids from corn, as the process of extracting the oil from CDS during corn ethanol processing concentrates the carotenoids by more than 500%.

Table 4: Carotenoid content (mg/kg) of Corn Distillers Soluble oil sampled from 11 Ethanol Plants.

Ethanol Plant	Trans-Lutein	Trans-Zeaxanthin	Cis- Lutein/ Zeaxanthin	α-Cryptoxanthin	β-Cryptoxanthin	Total Carotenoids
	---------- mg/kg CDS oil ----------					
A	98.36	77.39	33.14	15.24	11.23	235.37
B	49.63	39.53	16.09	13.83	8.98	128.06
C	95.07	87.11	43.83	4.93	9.04	239.98
D	75.31	57.4	26.44	6.37	5.53	171.04
E	118.87	86.53	31.45	17.48	10.09	264.78
F	107.15	102.52	42.48	7.6	11.56	271.3
G[1]	**106.71**	**77.4**	**39.65**	**8.93**	**10.12**	**242.82**
H	121.75	91.29	45.98	16.94	11.58	287.55
I	81.39	57.12	33.99	11.89	7.15	191.54
K	98.27	74.46	38.56	14.77	9.01	235.08
L	111.1	80.04	43.71	15.15	8.98	258.99
Mean ± std	96.69 ± 21.10	75.53±17.96	35.94±8.96	12.10±4.43	9.39±1.86	229.68±47.68
% C.V.	21.8	23.8	24.9	36.6	19.8	20.7
% of total Carotenoids						
Average (%)	42.01± 0.02	32.74 ±0.02	15.57±0.02	5.49± 0.02	4.19 ±0.01	

[1]CDS oil from ethanol plant G was used in the carotenoid bioassay trail (Phase 3).

Figure 2: Carotenoid profile of CDS oil from 11 ethanol plants

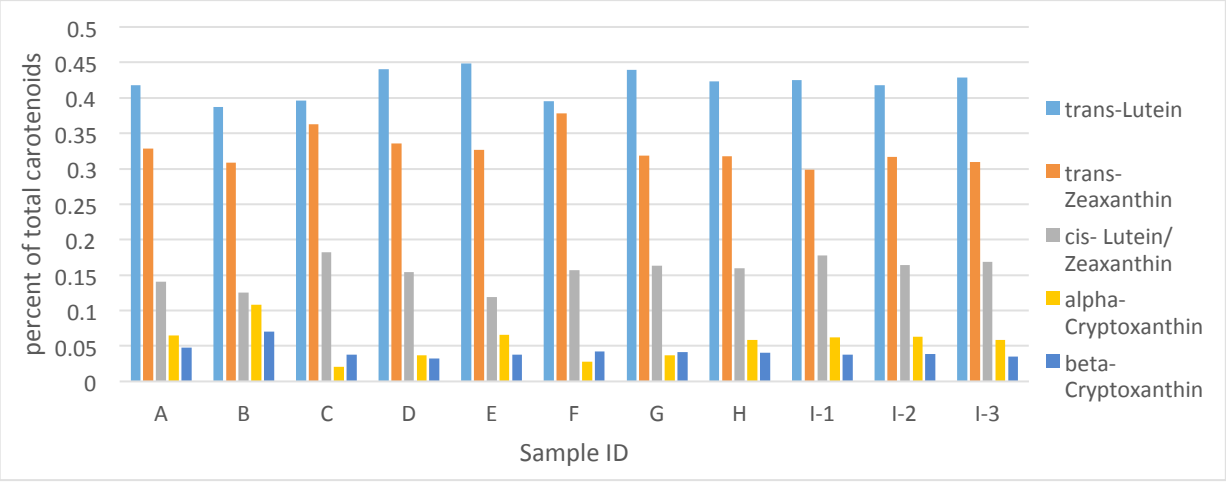
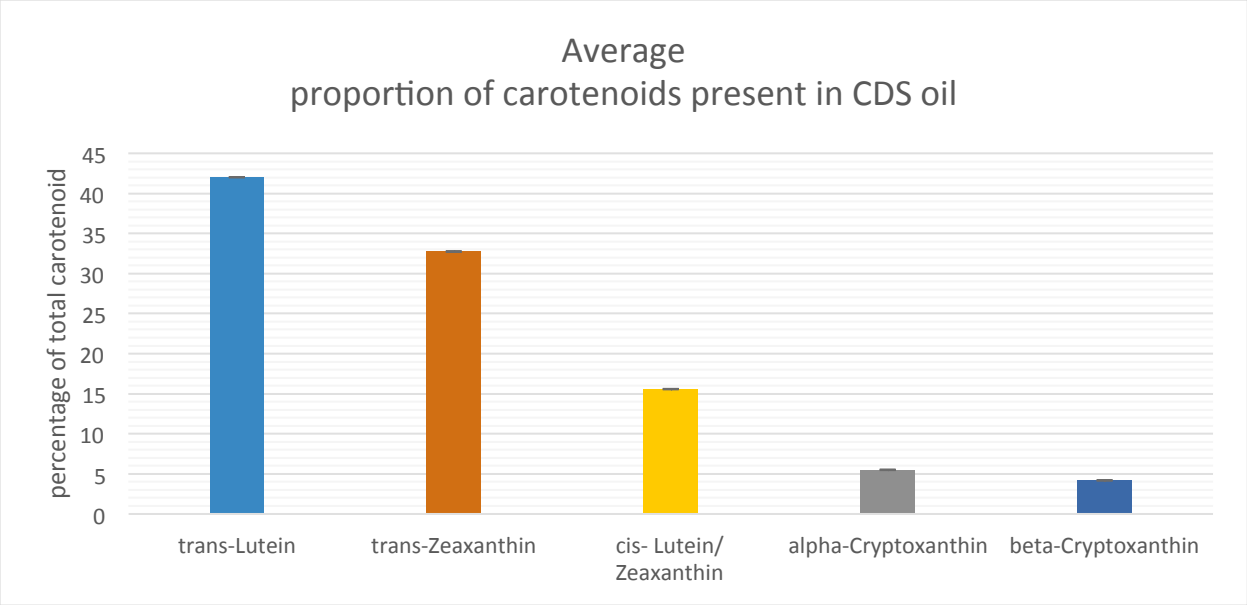


Figure 3: Average Carotenoid content of CDS oil, expressed as a percentage of total carotenoids. Total carotenoid content is ~230 ± 48 mg/kg of CDS oil (n= 11 ethanol plants).



Phase 2; Specific Objective 2: Evaluation of the potential economic value of CDS oil extract carotenoid pigments for use in poultry feeds.

The economic value of carotenoid pigment supplementation of commercial broiler feed is dependent upon the carotenoid content and relative market value of the ingredients in the feed formulation. Using least-cost linear programming software, the "shadow price" of either a constrained ingredient or nutrient can be determined, but it will differ for each formulation scenario. So, the economic value of the carotenoids CDS oil as calculated by least-cost feed formulation software would be different for each feed formulation scenario. In order to avoid the complications of differences in feed formulation objectives, the added value of the CDS oil due to its carotenoid pigment analysis value was determined as a function of the commercial value the carotenoid unit costs from the commercial marigold pigment source. Assuming Yellow Pixafil Liquid-LZ, containing 15 g of total Xanthophils/kg of product, has a market price of $4 USD/kg, the total xanthophil in CDS oil would have a commercial value of about $0.27 USD/g of xanthophil. The results of our ethanol plant survey revealed that the analyzed total carotenoid content of CDS oil is 230 ± 47.7 mg/kg (table 4), which indicates a 95% confidence interval ranging from 135 to 325 mg total carotenoid/kg of CDS oil. Assuming 1% CDS oil, containing 230 mg total carotenoid/kg) with a bioavailability of ~90% (results from phase 3) is used in the broiler formula (i.e. 10 kg CDS oil/tonne of feed), then 2.1 g (2.3 g X 90% BV) of total carotenoid is added per tonne of feed for each 1% added CDS oil, having a value of $0.56/tonne of feed (2.3 g carotenoid/tonne X $0.27/g carotenoid). Assuming a typical broiler diet includes about 3% supplemental fat as CDS oil, then it would replace about $1.68/tonne of feed worth of carotenoids from a commercial pigment additive, such as Yellow Pixafil Liquid-LZ. As another perspective, the carotenoids contained in CDS oil would justify a premium of about $0.05/kg over it's value as a dietary energy source. Assuming the current price for feed-grade corn oil is $0.50/kg, the CDS oil should have a value of $0.55/kg if the replacement value of supplemental carotenoid pigment is considered.

If we consider the potential variability of carotenoids in CDS oil from different ethanol plants to range within the 95% confidence interval of 135 to 315 mg/kg, then each 1% inclusion level CDS oil would contribute a value between $0.36 and $0.88 of supplemental carotenoid pigment/tonne of feed, and it would justify a market premium between $0.04 and $0.09 per kg of CDS oil over it's market value as an energy source.

Phase 3; Specific Objective 3: Evaluation of the biological value of CDS oil carotenoids for broilers

The broilers were raised in stainless steel cages so as to eliminate the influence of liter consumption. There were no significant treatment effects on growth performance of broilers throughout the trial. Table 6 illustrates the average body weight and feed intake per bird, and pen feed conversion ratio (FCR) from 1 to 49 days of age. The 49 day body weights of these female Ross 708 broilers was 3224 grams, which is well over the 3030 g growth performance objectives for 49 day broilers published by Aviagen (2014). The feed intake and FCR observed in this study were higher than these published performance objectives because of some feed spillage that normally occurs for broilers raised in experimental cages. Evidently, the replacement of soybean oil for the different levels of CDS oil had no significant effect on growth performance.

Table 6. Effect of dietary level of inclusion of Corn Distillers solubles (CDS) oil in place of soy oil on growth performance of broilers from 1 to 49 days of age.[1]

TREATMENT[2]	49 d Body Wt., g	FEED INTAKE/BIRD, g	FCR
0 % CDS oil	3265	6760	2.07
25 % CDS oil	3218	6615	2.06
50 % CDS oil	3139	6844	2.19
75 % CDS oil	3203	7058	2.20
100 % CDS oil	3299	7012	2.12
Positive Control[3]	3220	6738	2.09
P value	0.28	0.44	0.34
SEM (27)[4]	47.64	170.67	0.06

[1]Values are means of 6 replicate cages containing 10 broilers per cage.
[2]Treatment is described as % replacement of soy oil with Corn Distillers Solubles (CDS) oil.
[3]Positive control included 1.5% Yellow Pixifil Liquid-LZ in soybean oil* (w/w).
[4]SEM(27) = Standard Error of the Mean with 27 degrees of freedom

Bioavailability of the dietary carotenoid pigment supplementation level was assessed by the degree of yellowness (b* parameter) in shank skin, breast skin, and breast muscle using a Konica Minolta chromometer (Model CR400). Yellowness of shank skin of individual birds within each cage at 14, 21, 28, 37, 44, and 51 days of age are reported in Table 6. Due to residual maternal carotenoid from egg yolk, shank skin yellowness did not reach a basal level in the negative control treatment (0% CDS Oil) until day 28 of the trial, and no significant changes occurred subsequently until day 51. Therefore, shank skin yellowness determined after day 28 is assumed to be due to dietary carotenoid source and level. Shank skin yellowness measured on days 28, 37, 44, and 51 increased linearly as the dietary level of CDS oil increased in place of the unpigmented soybean oil. Figure 4 illustrates the shank skin yellowness values among the treatment groups at 51 days of age. There was a significant increase in yellowness value with each incremental increase in dietary CDS oil supplementation until all the 4% dietary inclusion of soybean oil was replaced with CDS oil. It is noteworthy that there was no significant difference in shank skin yellowness observed between the 100% CDS oil treatment and the positive control treatment (Yellow Pixifil Liquid-LZ), which included a similar dietary level of carotenoid pigment. This comparative difference confirms a bioavailability of carotenoid pigments from CDS oil is similar to commercial carotenoid pigments extracted from marigold.

Table 6: Effect of dietary inclusion of corn distillers solubles oil in place of soybean oil in a while corn-soybean diet on shank skin yellowness color of broilers at 14, 21, 28, 37, 44, and 51 days of age.

Treatment	14 d	21 d	28 d	37 d	44 d	51 d
	---------- b* (Yellowness) value ----------					
0% CDS Oil	24.08	21.28	11.41	13.87	11.97	14.29
25% CDS Oil	29.65	31.93	17.36	19.24	16.94	18.11
50% CDS Oil	33.44	40.50	23.53	26.50	21.22	23.22
75% CDS Oil	36.62	42.24	25.56	28.69	24.44	25.84
100% CDS Oil	45.39	49.30	28.74	31.32	25.58	28.61
Positive Control	48.18	56.61	31.10	32.64	28.52	30.68

Figure 4: Effect of dietary level of corn distillers solubles oil replacement of soybean oil (4% of diet) on shank skin yellowness (b*) value of in broilers at 51 days of age. Treatment responses with different letter are statistically different (P<.05).

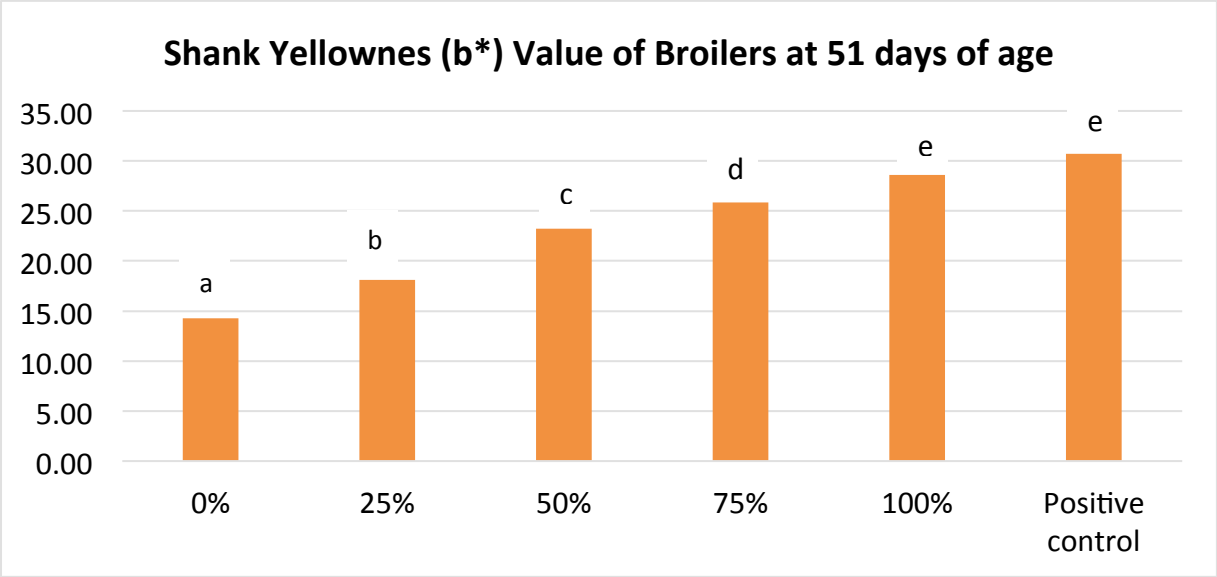


Figure 5: Effect of dietary level of carotenoid pigment supplemented from corn distillers solubles oil (blue dots and line) and commercial marigold carotenoids (orange star) on shank skin yellowness (b*) in broilers at 51 days of age. Bioavailability of carotenoids in CDS oil determined to be 93% of the carotenoids supplied by a commercial marigold extract (Yellow Pixifil Liquid-LZ, Alcosa Biotec, S.A., Apaseo El Grande, GTO, Mexico).

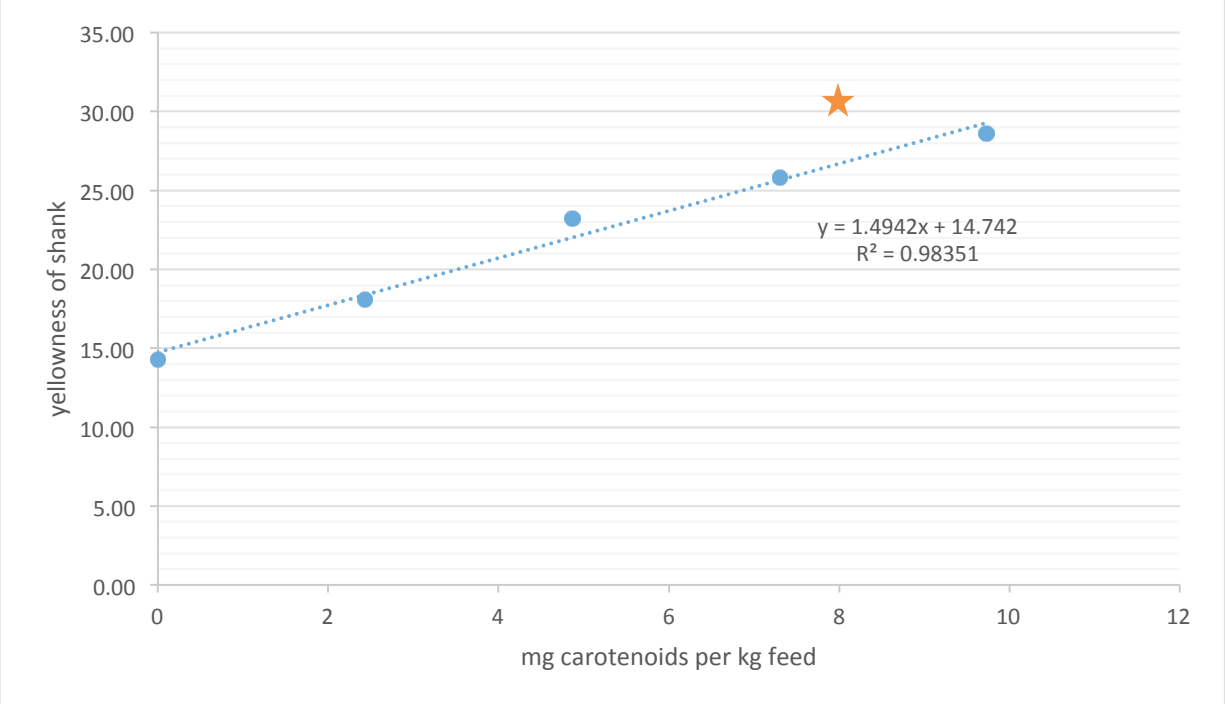


Figure 5 illustrates the effect of dietary level of carotenoid pigment supplemented from corn distillers solubles oil (blue dots and line) and commercial marigold carotenoids (orange star) on shank skill yellowness (b*) in broilers at 51 days of age. Figure 5 illustrates the response of the analyzed dietary carotenoids levels supplied from the difference sources so that relative bioavailability can be determined. Based on the shank skin color response, the bioavailability of carotenoids in CDS oil was determined to be 93% of the carotenoids supplied by a commercial marigold extract.

Table 7 summarizes the response of dietary inclusion of CDS oil and the positive control on blood plasma carotenoid content of sampled from 5 broilers per treatment. Whereas skin and meat pigmentation is an accumulative effect of dietary pigment bioavailability, the plasma carotenoid level measurement is a more acute effect of diet carotenoid pigment absorption at the time the blood sampling. These data clearly show a highly significant (P<.0001) linear effect of dietary CDS oil inclusion level (and thus carotenoid pigment intake) on plasma carotenoid concentration at all ages of measure. There was no significant difference in plasma carotenoid concentration between the 100% CDS oil and positive control (containing the commercial marigold pigment) in broilers sampled at 28 days of age, but the positive control treatment resulted in significantly greater plasma carotenoid concentration when the birds were sampled at older ages. Although plasma carotenoid level may be a good measure of absorption of dietary carotenoid pigments, it's greater variability and acute response makes it a less reliable measure of cumulative bioavailability than skin pigmentation response.

Table 7. Effect of dietary inclusion of corn distillers solubles oil in place of soybean oil in a while corn-soybean diet on plasma carotenoid content (mg/l) of broilers at 28, 37, 44, and 51 days of age.[1]

TREATMENT[2]	mg Carotenoid/kg Diet	28 d	38 d	42 d	51 d
		--------- (mg carotenoid / L of Blood Plasma) ----------			
0 % CDS Oil	0	0.57^{c}	1.31^{f}	0.85^{d}	1.13^{e}
25 % CDS Oil	2.43	1.13^{c}	2.07^{e}	1.59^{cd}	1.97^{d}
50 % CDS Oil	4.87	2.25^{b}	2.80^{d}	2.24^{c}	2.59^{c}
75 % CDS Oil	7.30	2.62^{ab}	3.63^{c}	4.36^{a}	3.47^{b}
100 % CDS Oil	9.73	3.64^{a}	4.21^{b}	3.24^{b}	3.05^{bc}
Positive Control[3]	9.20	3.04^{ab}	5.44^{a}	4.44^{a}	5.79^{a}
	P value	<0.0001	<0.0001	<0.0001	<0.0001
	SEM(25)[4]	0.25	0.11	0.23	0.16

[1]Values are means of 5 replicate birds per treatment.
[2]Treatment is described as % replacement of soy oil with Corn Distillers Solubles (CDS) oil.
[3]Positive control included 1.5% Yellow Pixifil Liquid-LZ in soybean oil* (w/w).
[4]SEM(27) = Standard Error of the Mean with 27 degrees of freedom

The effect of dietary level of carotenoid pigment supplementation from CDS oil and a commercial marigold extract product on breast skin yellowness (b*) as measured by the Konica Minolta chromometer and representative photos are illustrated in figures 6 and 7, respectively. As observed with shank skin color, breast skin color of carcasses of 51 d broilers increased linearly as the dietary carotenoids supplied by either the CDS oil or commercial marigold extract (Yellow Pixifil Liquid-LZ). Based on the breast skin color response, the bioavailability of carotenoids in CDS oil was determined to be 95% of the carotenoids supplied by a commercial marigold extract, closely confirming the response observed with shank skin color. Visual comparison of the carcasses reveal that the 100% CDS oil treatment may produce a more intense yellow skin color and an equal amount of total carotenoids from the marigold extract product. Breast meat color, although not as intensely responsive to dietary carotenoids because of lower fat deposition than breast skin, also showed a linear increase in yellowness as the dietary level of carotenoids increased by CDS oil or commercial pigment supplementation (Figure 8). Based on the breast meat color response, the bioavailability of carotenoids in CDS oil was determined to be 87% of the carotenoids supplied by a commercial marigold extract.

Figure 6: Effect of dietary level of carotenoid pigment supplemented from corn distillers solubles oil (blue dots and line) and commercial marigold carotenoids (orange star) on breast skin yellowness (b*) in broilers at 51 days of age. Bioavailability of carotenoids in CDS oil determined to be 95% of the carotenoids supplied by a commercial marigold extract (Yellow Pixifil Liquid-LZ, Alcosa Biotec, S.A., Apaseo El Grande, GTO, Mexico).

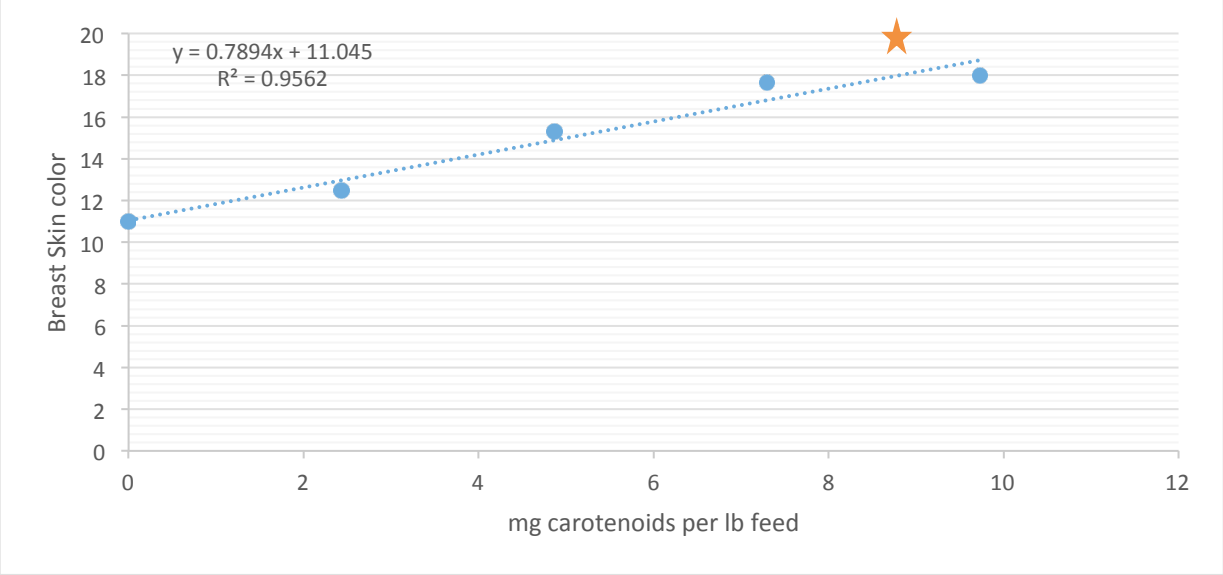
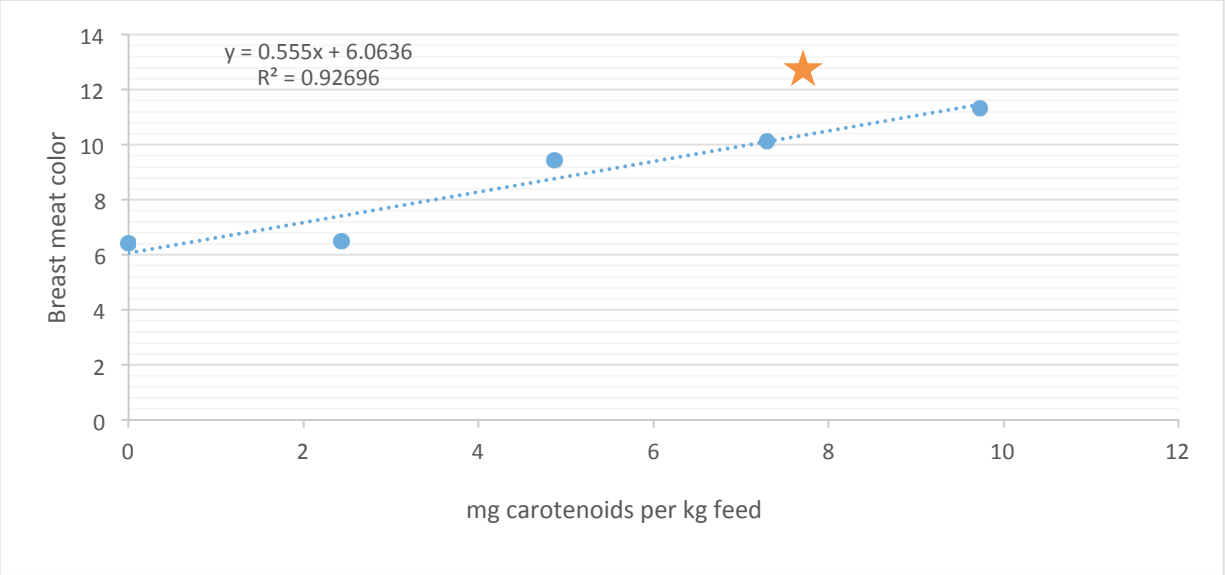


Figure 7: Effect of dietary level of carotenoid pigment supplemented from corn distillers solubles oil and commercial marigold carotenoids on breast skin yellowness color in broilers at 51 days of age (Upper panel). Carcass comparisons of the negative control (0% CDS Oil), 100% CDS oil, and the positive control (Marigold pigment) treatments, using a chrome lens filter.

Figure 8: Effect of dietary level of carotenoid pigment supplemented from corn distillers solubles oil (blue dots and line) and commercial marigold carotenoids (orange star) on breast meat yellowness (b*) in broilers at 51 days of age. Bioavailability of carotenoids in CDS oil determined to be 87% of the carotenoids supplied by a commercial marigold extract (Yellow Pixifil Liquid-LZ, Alcosa Biotec, S.A., Apaseo El Grande, GTO, Mexico).



CONCLUSIONS

The results of this research project clearly demonstrate that corn distillers solubles (CDS) oil can be utilized as a concentrated source of xanthophil carotenoids to enhance skin color in broilers. Depending on the dietary inclusion of CDS oil, significant cost savings can be realized as a replacement (or sparing) of supplemental marigold extract carotenoid pigment products. When CDS oil was added to corn-soybean meal based diets at similar total carotenoid levels, the chicks responded to the replacement of the added soybean oil with 100% CDS oil in a similar manner to the addition of the commercial color enhancer Yellow Pixafil Liquid-LZ. There was no difference in any growth parameter, feed intake, feed conversion ratio or body weight gain. Relative to an equal dietary supplementation of total carotenoid from marigold extract, the CDS oil enhanced yellowness of the shank, skin and meat as measured by the Minolta chromometer yellowness value (b*), with a bioavailability value of 93%, 95% and 87%, respectively). Although visually, the carotenoids from CDS oil may result in a more intense yellow color of the breast skin. The following are the conclusion of our project objectives:

1. CDS oil contains about **230 ± 48 mg** total carotenoids/kg with a coefficient of variation of 21% among ethanol plants. However, the carotenoid profile was very consistent among ethanol plants: 42% trans-lutein, 33% trans-zeaxanthan, 25% cis-lutein/zeaxanthin, 5.5% α-Cryptoxanthin, and 4% β-Cryptoxanthin.
2. Assuming a commercial marigold extract containing 15 g of total Xanthophils/kg of product, has a market price of $4 USD/kg, the total xanthophil in CDS oil would have a commercial value of about $0.27 USD/g of xanthophil. Assuming a typical broiler diet includes about 3% supplemental fat as CDS oil, then it would replace about $1.68/tonne of feed worth of carotenoids from a commercial pigment additive. Therefore, the carotenoids contained in CDS oil would justify a premium of about $0.05/kg over it's value as a dietary energy source.
3. Based on the bioassay of shank and breast skin pigmentation, dietary carotenoids supplied by corn distillers oil has a bioavailability of 93 to 95%, respectively, relative to an equal amount of carotenoid supplied by a commercial marigold extract. However, due to the natural variability in response, this difference in bioavailability is not statistically significant ($p<.05$).

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