Variability of corn distillers solubles oil quality and carotenoid content bioavailability as determined by skin pigmentation in broiler chickens.

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Introduction

- Pigmentation of poultry products is important to consumers in many markets
- Carotenoids are generally the compounds added to the feed to increase yellowness
  - Synthetic and natural carotenoids increase yellowness of poultry products

Hernandez et al. (2001); Blanch and Hernandez (2000); Rajput et al. (2012)

Introduction

- Maternal carotenoids passed to chick in the egg yolk
- Pigmentation of the bird is indicative of its general health
- Synthetic and marigold flower petal carotenoid feed additives are used to simulate carotenoid profiles in corn

Olson and Owens (1998)
Saino et al. (2000)

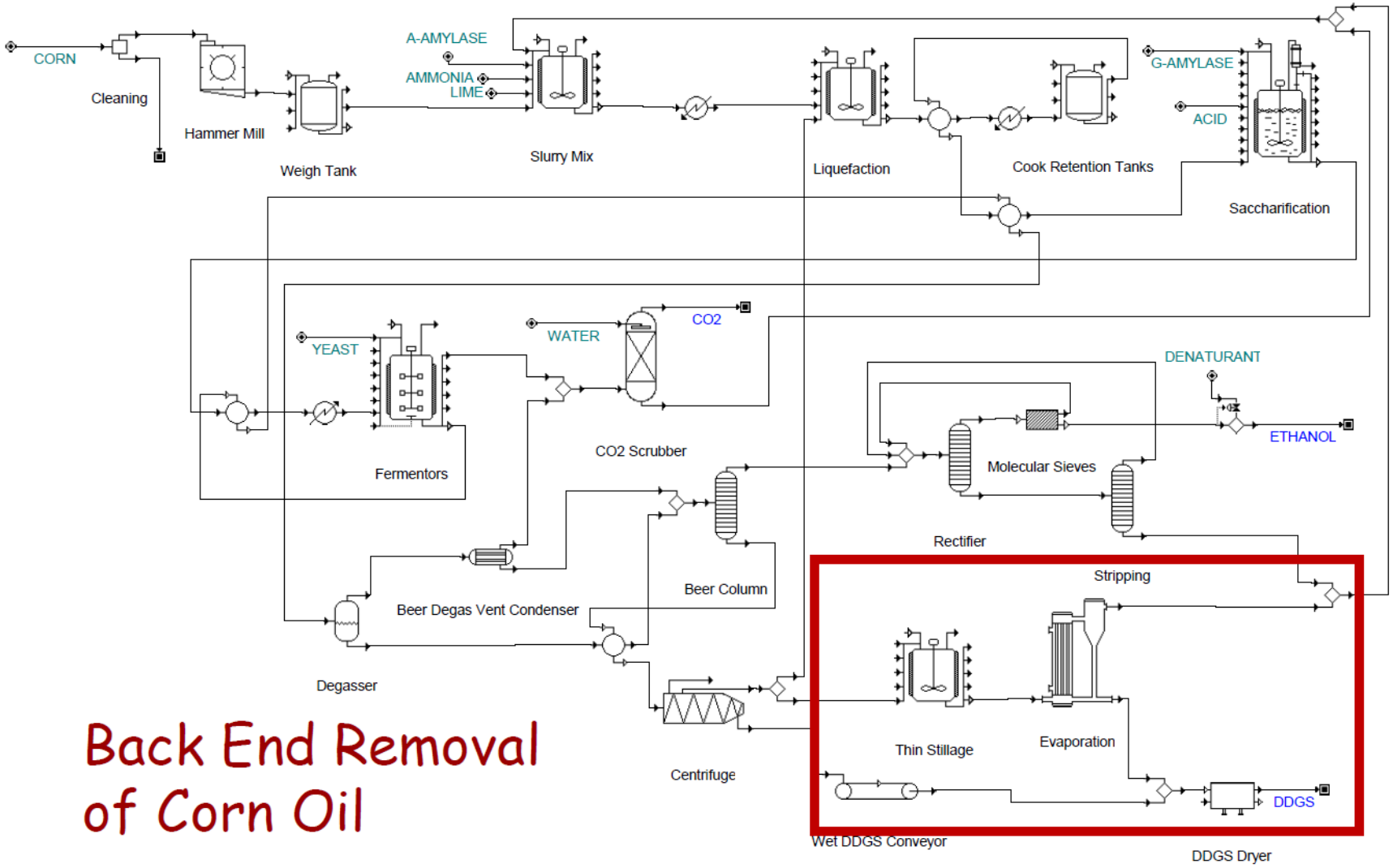
Demand for Biofuels is increasing Availability of Ethanol Co-Products for Feed

USA Mandate by 2017	Tons of Corn Required	Tons of DDGS
35 billion gallons	350 Million	120 Million

1 Ton of corn yields 100 gallons of ethanol and 666 lbs of DDGS and ~12 lbs CDS Oil

>2.5 billion pounds of CDS Oil Produced by US Ethanol Industry
(Renewable Fuels Association)

Dry Grind Process for Ethanol using Corn



Back End Removal of Corn Oil

Corn Distillers Solubles oil (CDSO)

A Sample of CDSO on left, and biodiesel produced by various technologies

- *Bob Modersohn*

- CDSO is a novel source of carotenoid
  - Co-product of ethanol production
  - Contains all the carotenoids present in corn
    - 100 times the concentration of corn oil
  - Natural, chemically intact

Moreau (2010)

Project Hypotheses

1. Carotenoid content and fat quality of CDS Oil is consistent among US Ethanol Plants
2. Carotenoid profile of CDS Oil is consistent among US Ethanol Plants
3. CDS oil Carotenoids are highly bioavailable as measured by broiler skin pigmentation.
4. CDS Oil Carotenoids has added value above it’s caloric value

Research Objectives Phase (Experiment) 1

Survey carotenoid content and fat quality of CDS oil produced by US Plants the use Dry Grind Ethanol Process.

1. Determine carotenoid content, variability, and profile characteristics
2. Assess fat quality and oxidative stability

Research Objectives Phase (Experiment 2)

Determine the effect of dietary level of CDS oil on the bioavailability of carotenoid for skin and meat pigmentation in broilers, relative to a commercial carotenoid pigment derived from marigold flower extract.

Research Objectives Phase 3

Estimate the potential economic added value of CDS Oil over it's current caloric value as a feed-grade fat.

Methods & Materials (Experiment 1)
Analytical Variability and Stability of CDS Oil

- CDS Oil Samples were collected from 11 different US Plants that use the Dry Grind Ethanol Process
  - 1 liter samples collected as produced within the same week of production and stored at 4 C until submitted for analysis (>3 weeks of production)
- CDS Oil sample preparation and purification (Tyczkowski and Hamilton, 1992), and carotenoid analysis by HPLC (Hamilton, 1992; Koutsos et al., 2003).
- Fat quality indicators as described by AOAC
  - Moisture and volitiles, insoluble, and unsuponifiables (MIU)
  - Initial peroxide value
  - P-anisidine content
  - 20 hr AOM

Methods & Materials (Experiment 2)

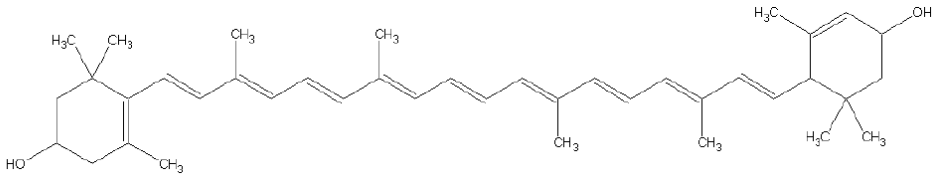
CDS Oil Carotenoid Bioavailability Assay

- Bioavailability of carotenoids was assessed from a 50 kg sample of CDS oil from a plant determined in Experiment 1 to be “average in carotenoid content and fat quality.
- 5 Experimental treatment oil premixes (added at 4% of the diet) was prepared as a blends of bleached soy oil:CDS Oil plus positive control

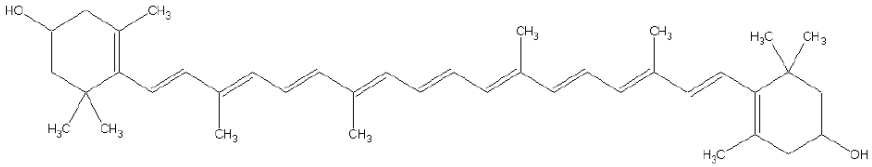
Experimental Positive Control

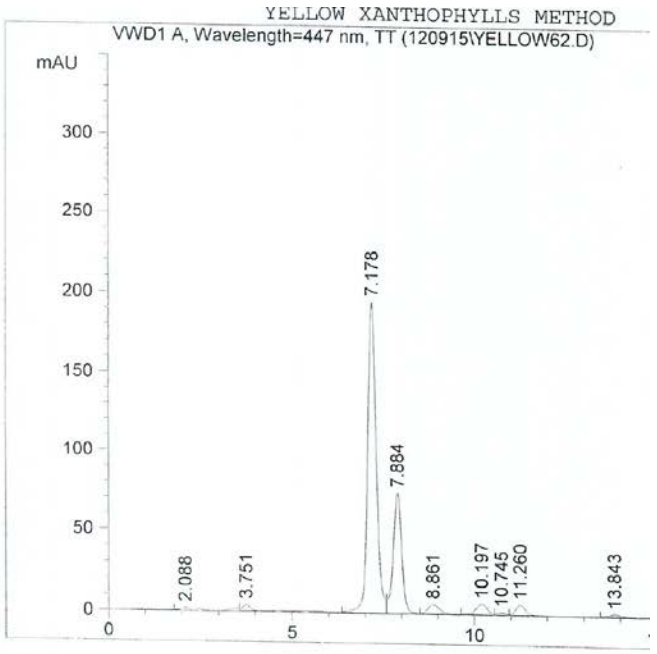
Yellow Pixafil Liquid-LZ (Alcosa Biotec, S.A., Apaseo El Grande, Mexico)

- Saponified xanthophylls extracted from marigold flower petals
- Total xanthophylls 15.30 g/Kg
  - 98.23% saponified
  - 64.78% trans-Lutein



  - 24.33% trans-Zeaxanthin





Experimental Design of Broiler Bioassay

- Day-old Ross 344x708 broiler chicks were randomly assigned to one of six diets
  - 10 chicks per cage with 7 reps per treatment
    - Alternative Design Brooder Cages 1-28 days
    - Alternative Design Grower Cages 29-51 day
- White corn/Soybean meal-based starter and grow-finish diets were formulated and treatment oil premix added post-pellet

Experimental Basal Diets

	Starter (1-28 d)	Grower (28-49 d)
Ingredient	%	%
Soybean Meal (48%)	36.38	28.08
White Corn	54.64	63.15
Soy Oil	0.57	0.50
Experimental Treatment Oil Premix	4.00	4.00
Dicalcium Phosphate 18.5% P	1.91	1.44
Limestone	1.02	1.43
Salt	0.29	0.27
Amino Acids	0.69	0.59
Vitamins and Minerals	0.51	0.53
	100	100
Calculated Nutrient Analysis		
Kcal ME/kg	3200	3253
Crude Protein, %	22.41	19
Dig Lys, %	1.2664	1.0893

Dietary Inclusion of Experimental Oil and Calculated and Determined Carotenoid Analysis

Treatment	% CDSO 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*	0	4*	9.2	

* 1.5% Yellow Pixifil Liquid-LZ in soybean oil

Mixed Mash Samples of Experimental Diets and Experimental Treatment Oil Premixes

Experimental Measurements

- Individual body weights and cage feed consumption at 14, 28, 35, 42, 49 and 54 d
- Yellowness (b*) of the shank skin measured by Minolta colorimeter at 14, 21, 28, 37, 44, 51 d
- 4 birds per replicate cage processed at 55 d
  - Yellowness (b*) of the breast skin
  - Yellowness (b*) of the breast muscle after overnight air chilling and carcass cut up

Minolta Chroma Meter

- Coloration measured with the Konica Minolta Chroma Meter CR-400/410
- Each value obtained was the average of 3 readings
- 3 measurements taken
  - L* lightness of the sample
  - a* redness of the sample
  - b* yellowness of the sample

Statistical Analysis

- All growth performance and skin pigmentation measurements were analyzed as a completely randomized block design
  - Cage of ~10 birds served as experimental unit
- Significance of dietary CDS oil level effects were determined by regression analysis ($P<.05$)
- Treatment mean comparisons with positive control by ANOVA using JMP Pro 12 (SAS, Cary NC)
- Bioavailability (% of positive control) was calculated by slope ratio method

Results

Experiment 1

- CDS Oil purity and stability Indicators
- CDS Oil Carotenoid Content Analysis
- CDS Oil Carotenoid Pigment Profile

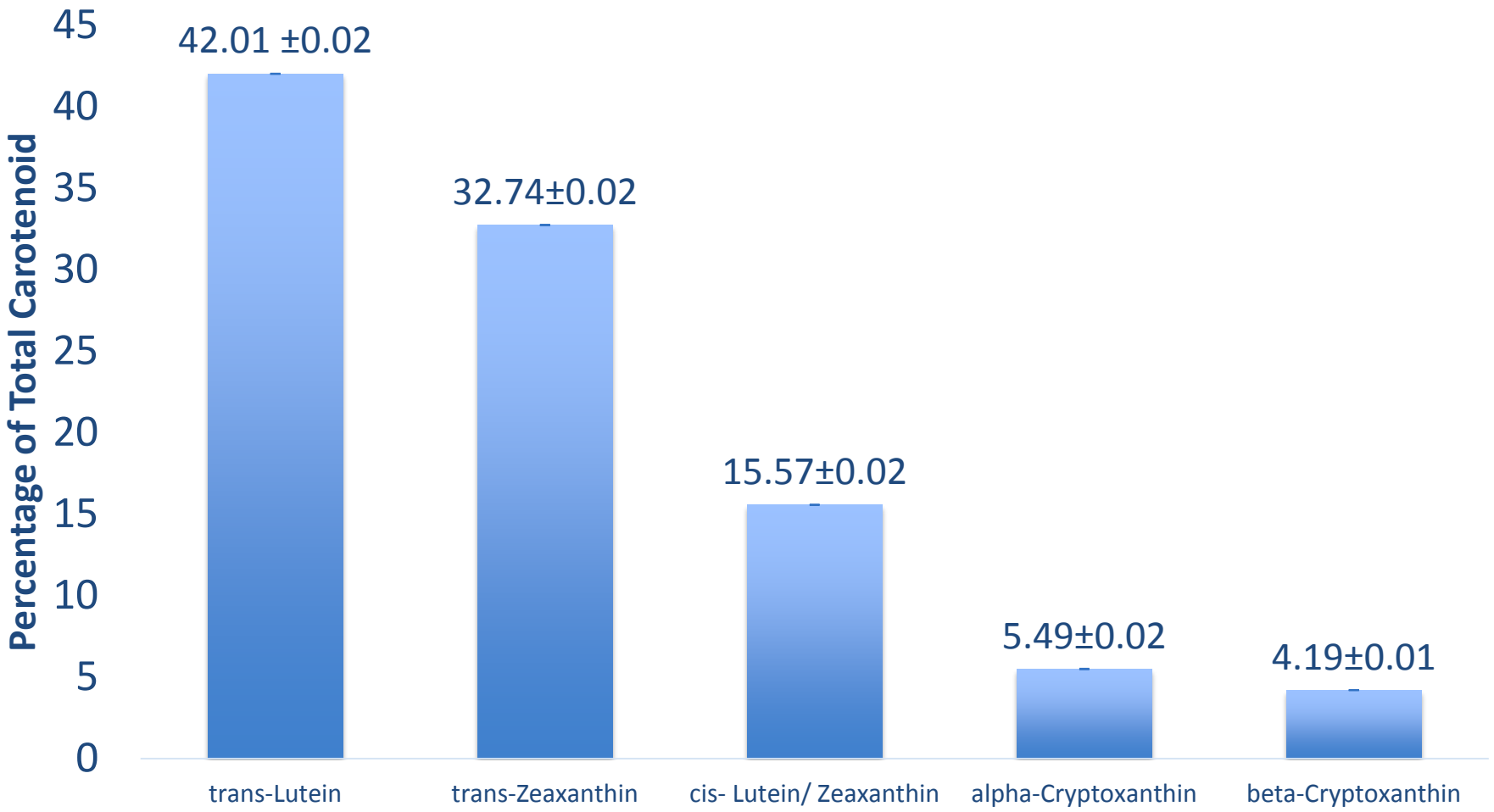
Quality Measurements of Corn Distillers Solubles Oil Collected from 11 Dry Grind Ethanol Plants in the USA

Quality Measurement	Mean Value ± Std.
Moisture and Volatiles (M), %	0.38 ± 0.41
Insolubles (I), %	0.03 ± 0.01
Unsaponifiable Matter (U), %	1.46 ± 0.20
Total M.I.U., %	1.88 ± 0.22
Initial Peroxide value, mEq/kg	0.20 ± 0
P-Anisidine Value	20.49 ± 2.63
AOM Stability @20 h, mEq/kg	49.64 ± 26.37

Carotenoid Analysis of Corn Distillers Solubles Oil Collected from 11 Dry Grind Ethanol Plants in the USA

	Mean Value ± Std	% CV
trans-Lutein	96.7 ± 21.10	21.8
trans- Zeaxanthin	75.5 ± 17.96	23.8
cis- Lutein/ Zeaxanthin	35.9 ± 8.96	25.0
alpha-Cryptoxanthin	12.1 ± 4.43	36.6
beta-Cryptoxanthin	9.4 ± 1.86	19.8
Total Carotenoids	229.7 ± 47.68	20.7

Distribution of Carotenoids in CDS Oil



Results

Experiment 2

- CDS Oil selected for use was chosen based on average carotenoid and AOM stability
- Growth Performance
- Shank Skin yellowness at 14, 21, 28, 37, 44, and 51 days of age
- Relative Bioavailability of Carotenoid for shank skin, breast skin, and breast meat

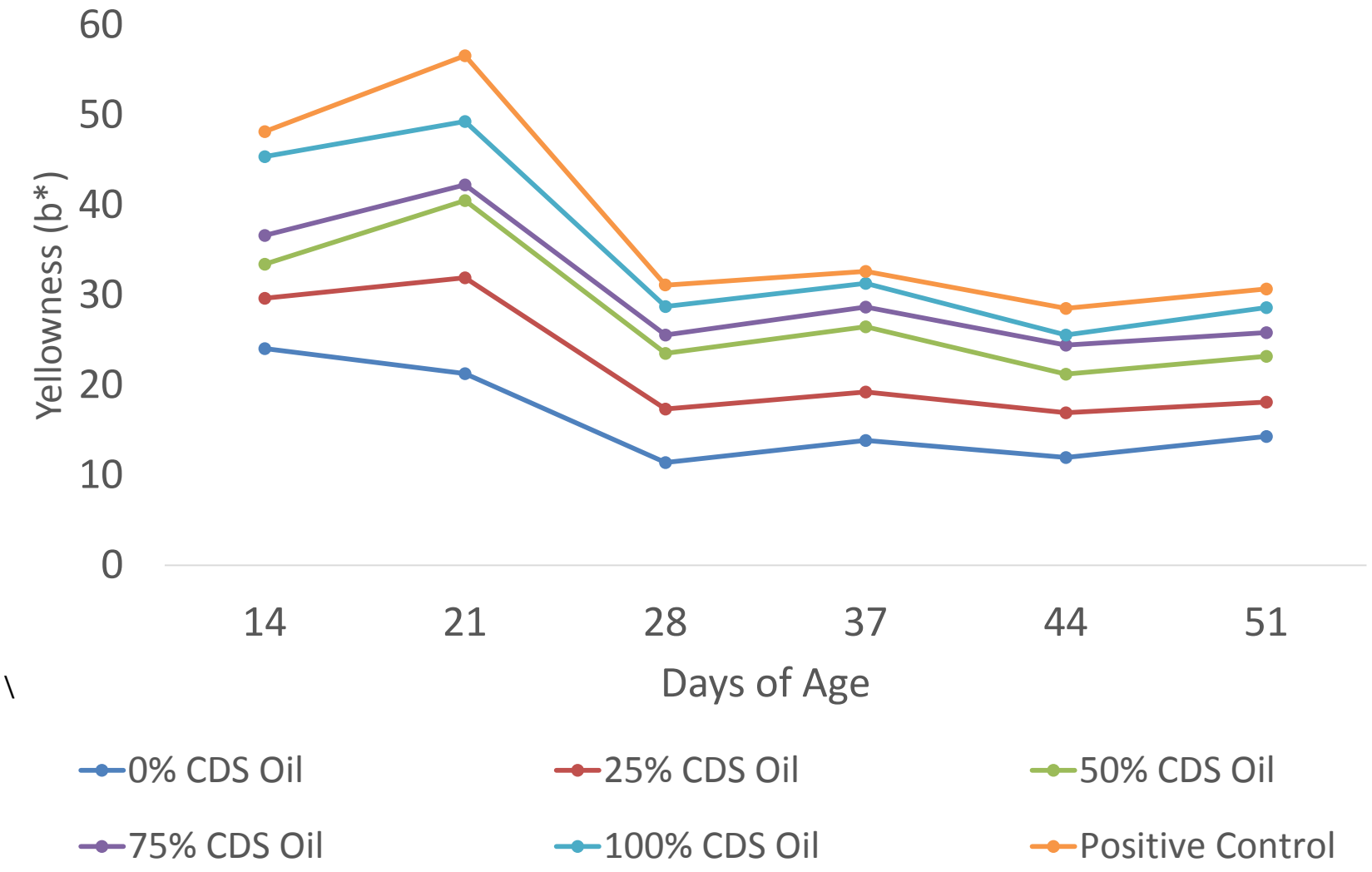
Effect of Dietary Level of Corn Distillers Oil (CDSO) Replacement of Soy Oil on Growth Performance of Broilers from 1 to 49 days of age

TREATMENT[1]	49 d Wt.	Feed intake	FCR
	---------- grams/bird ----------		g FI:g Gain
0 % CDSO	3265	6760	2.07
25 % CDSO	3218	6615	2.06
50 % CDSO	3139	6844	2.19
75 % CDSO	3203	7058	2.20
100 % CDSO	3299	7012	2.12
Positive Control[2]	3220	6738	2.09
Source of Variation	---------- P-Value ----------		
Treatment	0.28	0.44	0.34
SEM (27)	47.64	170.67	0.06

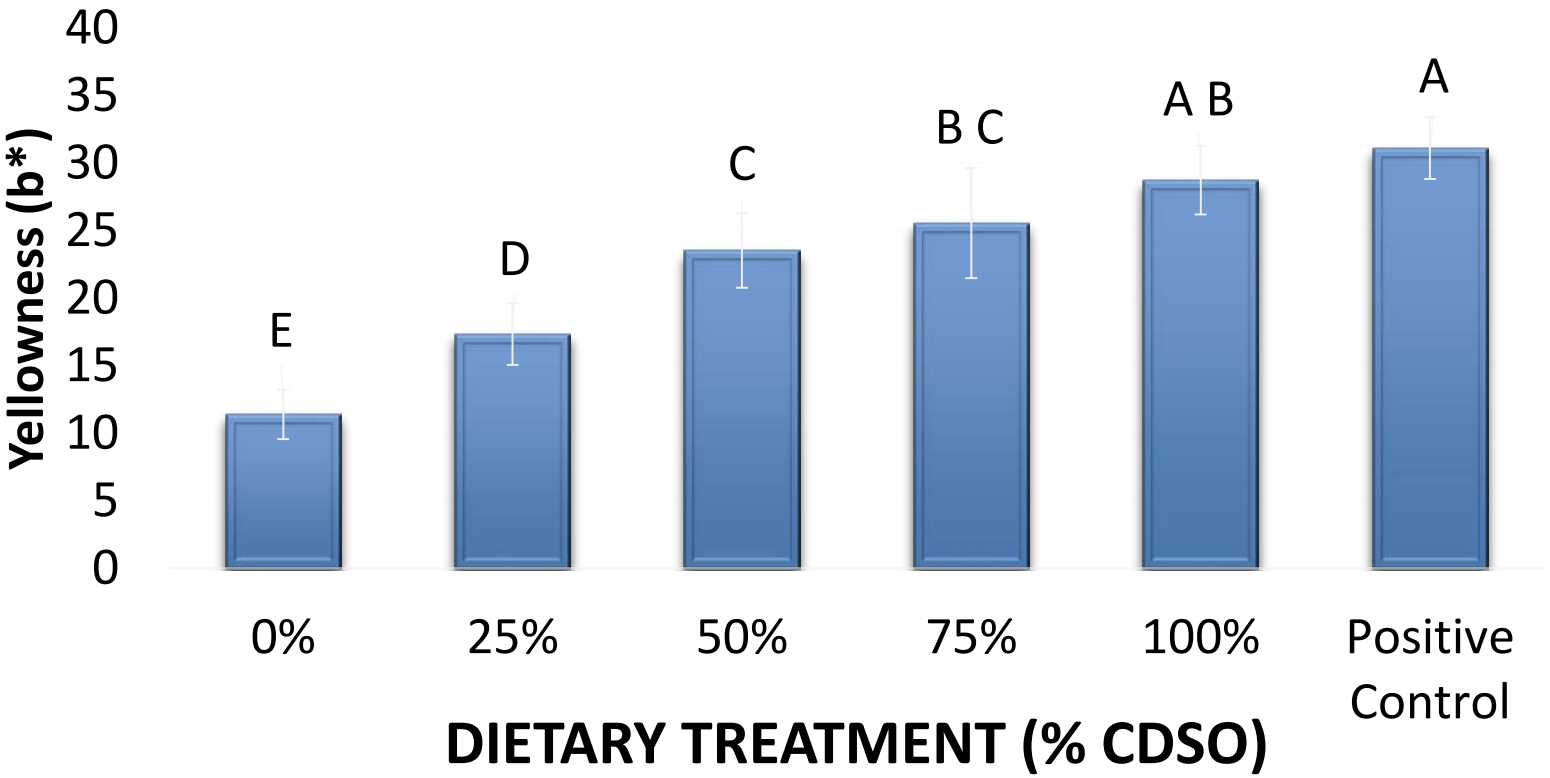
[1] All experimental diets received 4% added experimental treatment oil blend premix

[2]Positive Control contained total carotenoids similar to 100% CDSO, from Pixifil-LZ

Effect of Dietary Level of CDS Oil on Shank Skin Yellowness (b*) of Broilers at 14 to 51 d of age

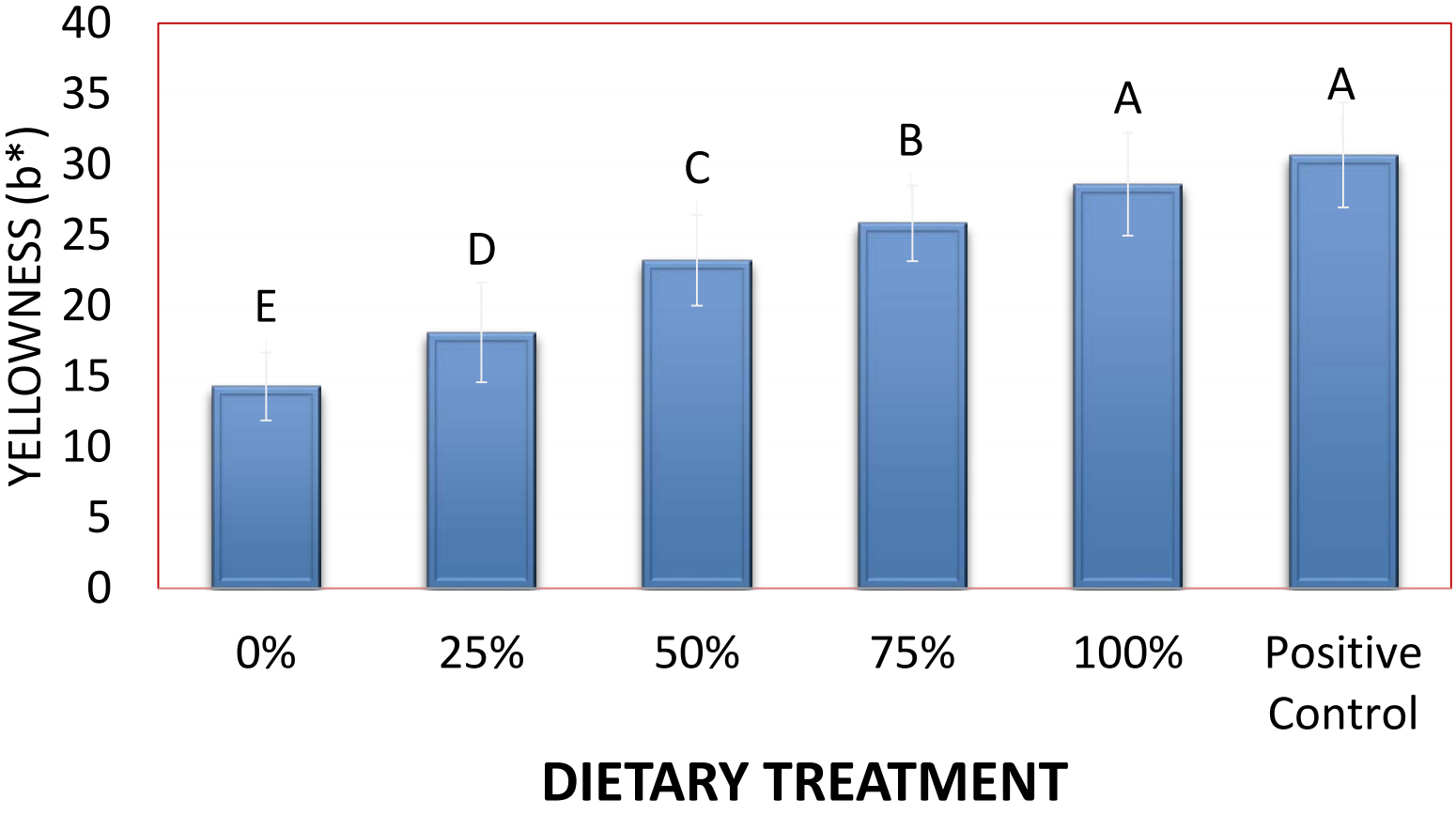


Effect of Dietary Level of Corn Distillers Oil (CDSO) Replacement of Soy Oil on Shank Skin Yellowness (b*) of Broilers at 28 d of Age



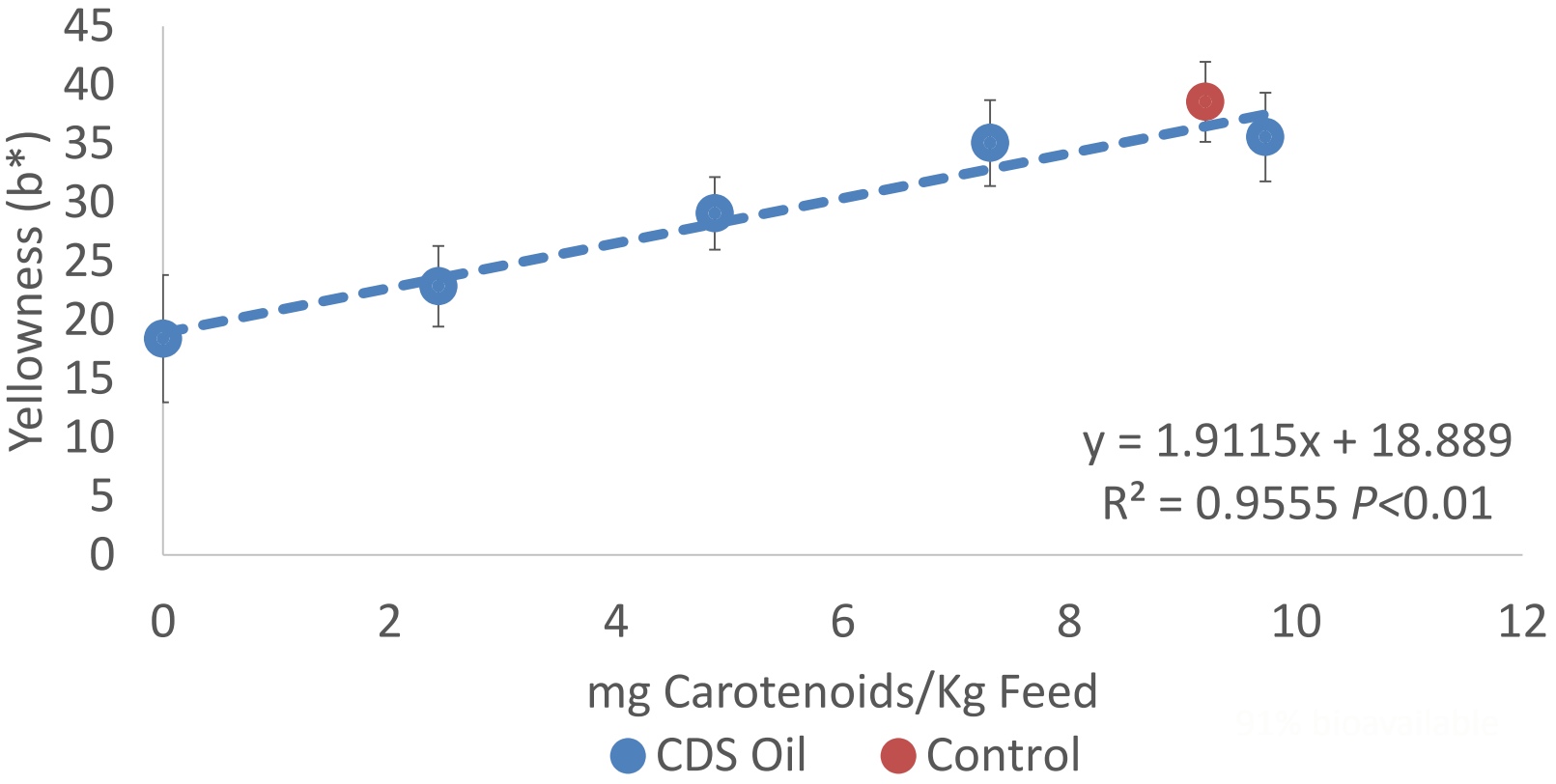
Different letters: p< 0.01

Effect of Dietary Level of Corn Distillers Oil (CDSO) Replacement of Soy Oil on Shank Skin Yellowness (b*) of Broilers at 51 d of Age



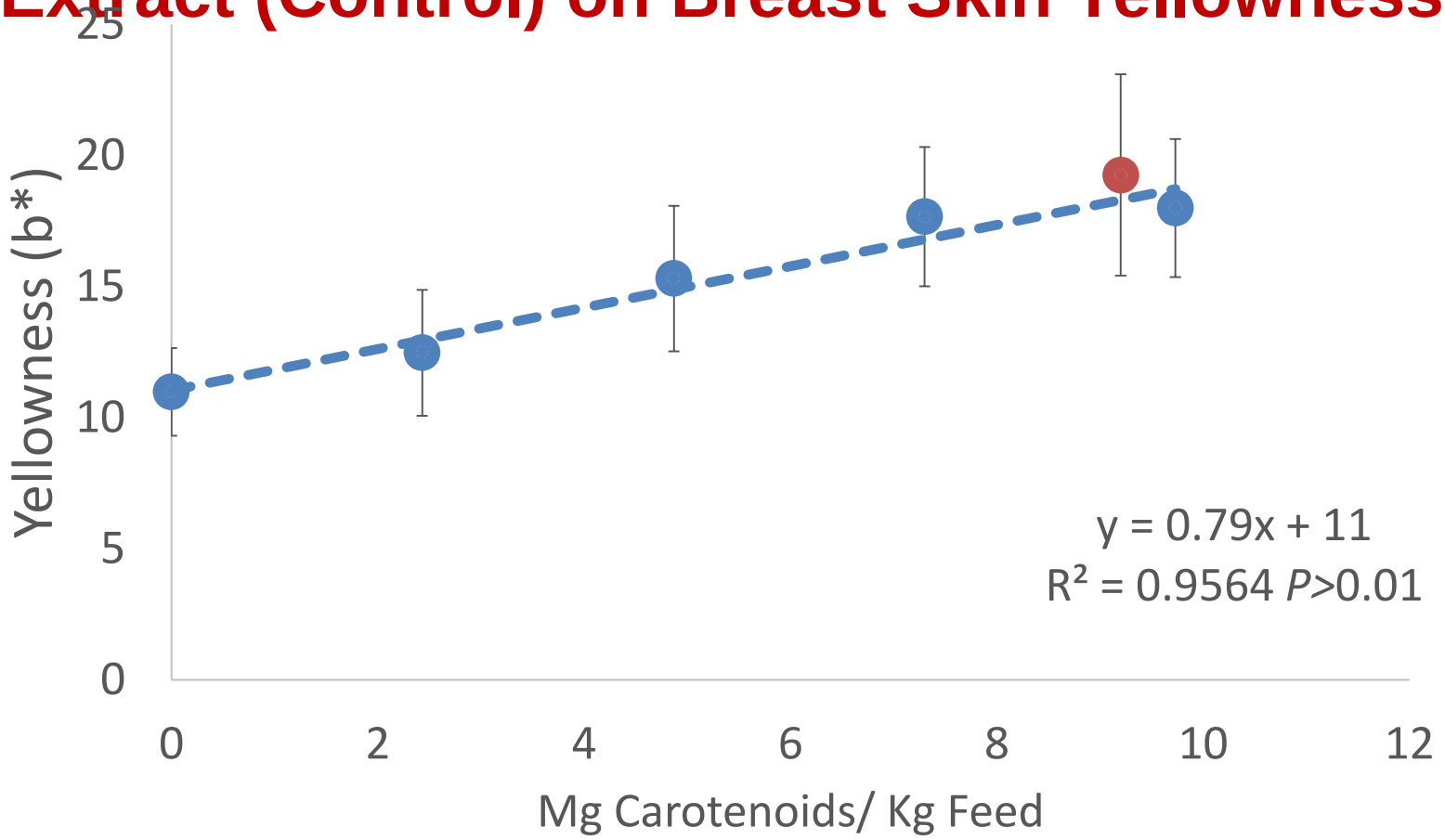
Different letters: p< 0.01

Shank Skin Yellowness (b*) Effect of Dietary Carotenoid Level from Corn Distillers Solubles Oil (CDSO) and Marigold Extract (Control) on Shank Skin Yellowness



Relative Bioavailability of Total Carotenoids in CDSO is 92.8%

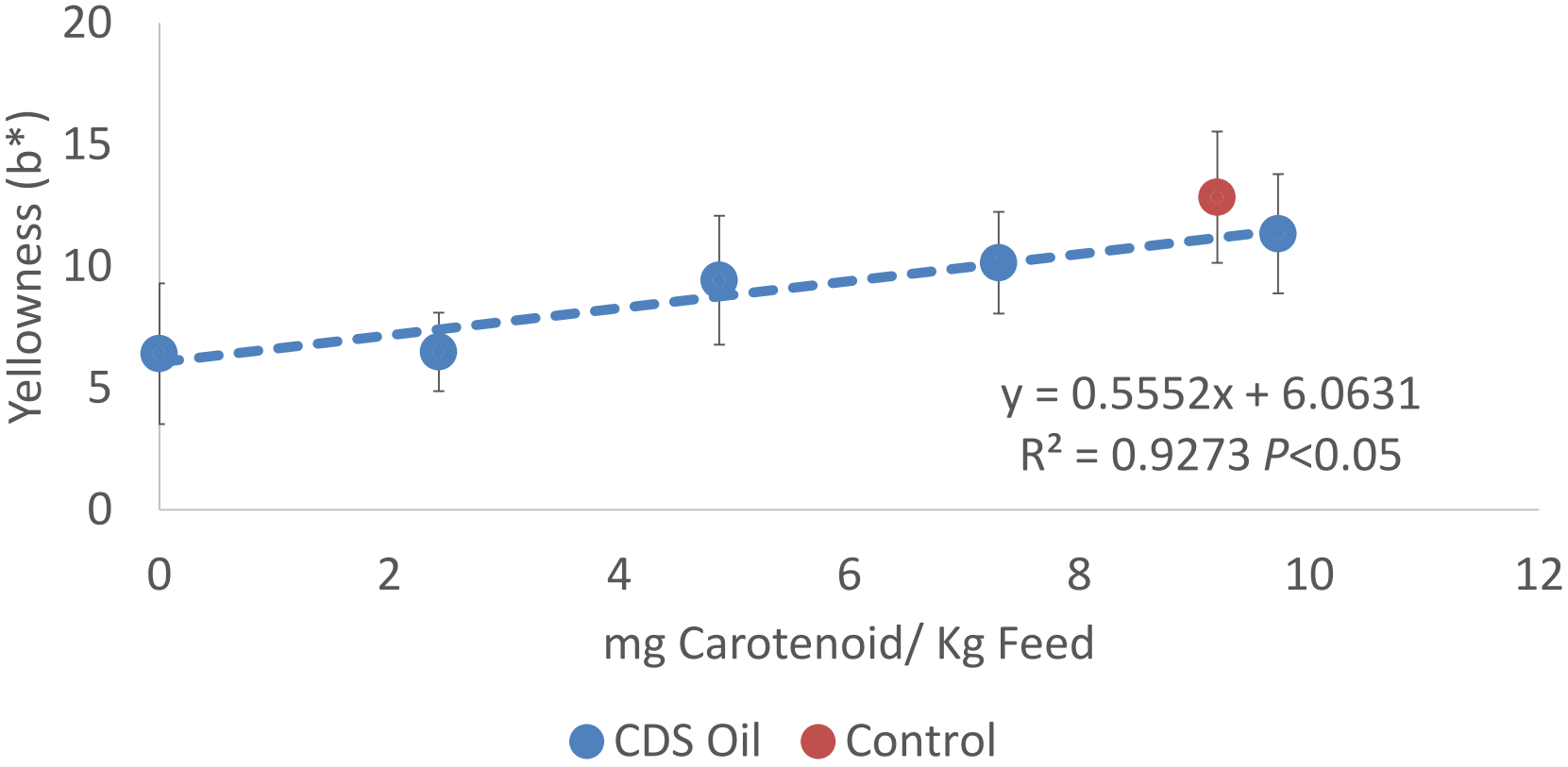
Effect of Dietary Carotenoid Level from Corn Distillers Solubles Oil (CDSO) and Marigold Extract (Control) on Breast Skin Yellowness



Relative Bioavailability of Total Carotenoids in CDSO is 95.3%

Effect of Dietary Carotenoid Level from Corn Distillers Solubles Oil (CDSO) and Marigold Extract (Control) on Breast Skin Yellowness

Effect of Dietary Carotenoid Level from Corn Distillers Solubles Oil (CDSO) and Marigold Extract (Control) on Breast Meat Yellowness



Relative Bioavailability of Total Carotenoids in CDS Oil is 87.2%

Economic Value of Total Carotenoids in Corn Distillers Solubles Oil

Assumptions:

- Yellow Pixafil Liquid-LZ (15 g Xanthophils/kg): $4/kg
  - Commercial value is $0.27/g of xanthophil

- If CDS Oil contains **230±48 g** total carotenoids/tonne
  - Added value of CDS Oil = $0.27/g X 230 g/tonne
    = $62.10/tonne CDS Oil

- Considering product variability and bioavailability of CDS Oil Caroteniods, the 95% confidence interval of CDS Oil added value ranges from **$32.80 - $78.97/tonne**

Hypotheses Revisited

1. Carotenoid content and fat quality of CDS Oil is consistent among US Ethanol Plants
2. Carotenoid profile of CDS Oil is consistent among US Ethanol Plants
3. CDS oil Carotenoids are highly bioavailable as measured by broiler skin pigmentation.
4. CDS Oil Carotenoids has added value above it's caloric value

Hypotheses Revisited

1. Carotenoid content and fat quality of CDS Oil is consistent among US Ethanol Plants **NO**
2. Carotenoid profile of CDS Oil is consistent among US Ethanol Plants **YES**
3. CDS oil Carotenoids are highly bioavailable as measured by broiler skin pigmentation. **YES**
4. CDS Oil Carotenoids has added value above it's caloric value **YES**

Conclusions
Experiment 1

- CDS Oil can be used as a dietary source of carotenoids for skin pigmentation of broiler chickens
- CDS oil contains **230±48 mg** total carotenoids/kg with 21% CV among ethanol plants.
- 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.

Conclusions
Experiment 2

- CDS Oil has a similar effect on pigmentation of broilers as an equal carotenoid level of Yellow Pixafil Liquid-LZ
- The total carotenoids in CDS Oil has a bioavailability of >90% relative to marigold extract for skin yellowness
  - Skin color of broilers fed CDS Oil maybe more appealing than commercial pigments because of a more “natural” corn carotenoid profile

Thank you Sponsors and Colleagues

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