FINAL REPORT

The influence of feeding various levels of wet and dry distillers grains to yearling steers on carcass characteristics, meat quality, fatty acid profile and retail case life of *longissimus* muscle

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Abstract

Due to increased production of ethanol, frequency of distillers grains is on the rise. The objective was to determine the effects of wet (WDG) or dry (DDG) distillers grains on final product quality. Yearling steers (n = 176) were assigned to one of five treatment groups: steam flaked corn (SFC), 10% DDG, 10% WDG, 20% WDG or 30% WDG. One inch steaks were cut from strip loins and identified for simulated retail display, Warner-Bratzler shear force (WBSF) analysis, sensory panel determination, and fatty acid composition. Treatment had no effect on adjusted fat thickness and USDA yield and quality grades. Steaks from cattle fed 10% WDG and 30% WDG had lower WBSF values than steaks from cattle fed 20% WDG. Trained sensory panelists found no differences in overall tenderness and off-flavors. No effects were found in total saturated and monounsaturated fatty acid composition among treatments, however, 20% WDG had a higher proportion of polyunsaturated and n-6 fatty acids. Data suggest that feeding WDG at higher levels (20% or 30%) does not affect eating quality, but shelf life of strip loin steaks were shorter. Further research needs to be conducted to evaluate methods that aid in increasing shelf life of steaks from cattle fed higher rates of WDG.

Introduction

The prevalence of distiller's grains (DG) seems to be on the rise due to the rapid increase in processing corn for ethanol production. Increased production in distiller's grains has led cattle feeders to consider DG as a feed source. Traditionally, distillers byproducts are dried; this drying process, however, tends to increase energy costs incurred by the ethanol plant and may produce changes that reduce its nutritional value. The majority of the research evaluating the use of DG in feedlot rations has been done with dry-rolled corn or high-moisture corn based diets. However, in the southern Great Plains (Oklahoma, Texas and Western Colorado) the majority of feedlots feed steam-flaked corn (SFC) based rations. Data evaluating the use of corn wet distiller's grains (WDGS) in SFC based feedlot rations is sparse. The highest inclusion level of corn WDGS evaluated in SFC diets that has been reported in recent literature is 10%.

Distillers grains have significant concentrations of vitamins, including B complex, A, D, and E; however, it is not known whether these characteristics of DG contribute to enhancing the value of beef (Roeber et al., 2005). Considerable effort must focus on the impact of feed rations and their influence on quality of red meats.

Color is an extremely critical component of the appearance of fresh beef sold through retail and has a substantial influence on purchasing decisions. In red meats, consumers relate a bright-red cherry color to freshness, but discriminate against meat that has turned brown (Morrissey et al., 1994). O'Sullivan et al. (2002 and 2003) showed that the feeding regimen of an animal can affect meat color and quality. Gray et al. (1994) also reported that feeding regimen can also affect flavor and lipid oxidation. Therefore, ration formulation may adversely affect meat quality, meat composition and ultimately shelf life. Dahlen et al. (2001) reported that steaks from steers fed a combination of condensed distiller's solubles and barley by-product were redder than steaks from steers fed corn gluten feed. Mancini and Hunt (2005) found that feeding practices on color were attributed to the relationship between lipid and pigment oxidation, particularly the instability of polyunsaturated fatty acids. Previous studies demonstrated that fatty acid composition of bovine tissues can be influenced by dietary regimes (Rule et al., 1994; Mandell et al. 1997).

The objectives of this experiment were to determine the effects of feeding higher levels of WDG, or DDG on carcass characteristics, meat quality, retail case life and fatty acid composition of *Longissimus* muscle.

Materials and Methods

One hundred seventy-nine yearling steers (avg. initial body weight = 750-800 lbs) were delivered to Oklahoma State University Research and Extension Center/ Oklahoma Panhandle State University feedlot near Goodwell, Oklahoma in early April, 2007. Upon arrival steers were individually weighed and ear tagged. Steers were blocked by initial weight and allocated into one of thirty pens with six head per pen. Treatments were deemed as: Steam flaked corn (SFC), 10% WDG, 10% DDG, 20% WDG, and 30% WDG. Based on visual appraisal, cattle were sent to a commercial harvest facility when the block was expected to have sufficient finish to grade 65% USDA Choice. Final individual body weights were recorded the morning of shipment.

Harvest and data collection

Steers were harvested at a commercial processing facility in Dodge City, KS. Trained Oklahoma State University (OSU) personnel completed tag transfer and obtained carcass measurements. Liver scores were also be recorded, using the scale 1 = condemned and 0 = not condemned. Measurements included hot carcass weight (HCW), ribeye area (REA), marbling score at the 12^{th} and 13^{th} rib interface, percentage of kidney, pelvic, and heart (KPH) fat, fat thickness, USDA yield grade, and USDA quality grade.

Strip loin collection and sample preparation

Following data collection, strip loins were tagged to maintain identity during fabrication. Carcasses were fabricated according to Institutional Meat Purchasing Specifications (IMPS; USDA, 1996). Strip loins were collected, vacuum packaged, and placed in ice chests for transit back to OSU Food and Agricultural Products Center. Strip loins were aged 14 d postmortem at 35.6° F.

After aging, the anterior end of the strip loin was faced and two samples from each strip face were vacuum packed and placed in a blast freezer (-4° F) for subsequent fatty acid profiling and pre-display thiobarbituric acid reactive substance (TBAR) analysis. A 1.0 inch thick steak was cut from the anterior end and labeled for simulated retail display. The remaining portion of the strip loin was vacuum packaged and frozen at - 4° F for shear force and taste panel analysis.

Simulated Retail Display

The steaks labeled for retail display were placed on a styrofoam tray with a soaker pad and were over-wrapped with a polyvinyl chloride film (PVC). Trays were placed into a coffin style display case which was maintained at 35.6° F ± 1° F, under constant light conditions (Phillips Delux Warm White Florescent lamps). The surface of the meat was exposed to (900 to 1365 lux) as recommended by AMSA (1991). Each steak was objectively and subjectively evaluated for color attributes at 12 h intervals during retail display for 7 d.

Objective Color Evaluation

Color of each steak was measured using a HunterLab Miniscan XE hand-held spectrophotometer equipped with a 6 mm aperture (Hunter Laboratory Associates, Inc., Reston, VA) to determine L* (brightness: 0 = black, 100 = white), a* (redness/greenness: positive values = red, negative values = green), and b* (yellowness/blueness: positive values = yellow, negative values = blue). Three readings were obtained for each steak, and were averaged to obtain the final L*, a*, b* values for each steak at each time of evaluation.

Subjective Color Evaluation

Subjective color was evaluated by a six-person, trained panel of OSU personnel. Panelists were trained using Munsell color tiles and were required to receive a passing score before being on a color panel. Panelists assigned scores to each steak for muscle color, surface discoloration, and overall appearance at every evaluation time as outlined by Hunt et al. (1991). Panelists characterized meat color (8 = extremely bright cherry red, to 1 = extremely dark red), surface discoloration (7 = no discoloration [0%], to 1 = total discoloration [100%]), and overall appearance (8 = extremely desirable, to 1 = extremely undesirable). As with objective evaluation, steaks were evaluated every 12 h for 7 d.

Thiobarbituric Acid Reactive Substance (TBAR)

Following retail display, a sample from each steak was taken and designated as post-thiobarbituric acid reactive substance, vacuum packaged, and frozen at -4°F for analysis. Lipid peroxidation was determined by the modified method of Buege and Aust (1978). A 10 g sample was placed in a waring blender and homogenized with 30 ml of deionized water, the slurry was transferred to a disposable tube and centrifuged for 10 min at 3000 rpm. Following centrifuging, 2 ml of the supernatant was placed in a disposable glass tube along with 4 ml of thiobarbituric acid/trichloroacetic acid (TCA/TBA) and 100 µl of butylated hydroxyanisol (BHA). The mixture was vortexed and incubated in a boiling water bath for 15 min to develop color. Samples were then placed in cold water for 10 min to allow samples to cool, and then centrifuged for 10 min at 3000 rpm. The absorbance of the resulting supernatant was determined at 531 nm against standards.

Fatty Acid Profiling

Steaks for fatty acid analysis were trimmed of all subcutaneous fat, cubed, froze in liquid nitrogen, and pulverized in a waring blender to a powder-like consistency. The samples remained in the freezer until analysis. In order to extract lipids from the tissues, it is necessary to find solvents which will not dissolve the lipids but will overcome the interactions between the lipids and the tissue matrix (Christie, 2003). Fatty acid methyl ester procedure was determined by gas chromatography as described by Bligh and Dyer (1959) with modifications (See Appendix A). Identification of the fatty acids were made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of a standard. Methyl ester peaks from samples were calculated as percentages of called fatty acids.

Tenderness Determination

A 1.0 inch steak was cut from each strip loin for Warner Bratzler shear force (WBSF) determination. Steaks were allowed to temper at 39.2°F for 24 h. Steaks were cooked on an impingement oven (model 1132-000-A; Lincoln Impinger, Fort Wayne, IN) at 356°F to an internal temperature of 158°F. Internal steak temperatures were monitored with copper constantan thermocouples (model OM-202; Omega Engineering, Inc., Stamford, CT). Individual steak weights were recorded prior to and following cooking to determine cooking loss percentage.

Following cooking, steaks are allowed to cool for 24 h before conducting shear force analysis. Six cores were taken from each steak; cores were 0.5 inches in diameter and removed parallel to muscle fiber orientation. Each core was sheared once with the Warner-Bratzler head on the Instron Universal Testing Machine (model 4502; Instron Corp., Canton, MA) at a crosshead speed of 200 mm/min. Peak force (lb) of cores was recorded by an IBM PS2 (Model 55 SX) using software provided by the Instron Corporation. Mean peak WBSF was then calculated by averaging the 6 cores.

Palatability Determination

Steaks were assigned a randomized 3 digit number for sensory sessions. Steaks were allowed to temper 24 h prior to each session and were then cooked as described above for WBSF analysis. After cooking, samples were uniformly cut into 1 x 1 inch cubes and placed in a cup with the corresponding randomized number. Cups were placed in a warmer (Food Warming Equipment, Model PS-1220-15, Crystal Lake, IL) until being served to the panelists.

The sensory panel consisted of eight trained OSU personnel. Panelists were trained on tenderness, juiciness, and three specific flavor attributes (Cross et al., 1978). Sensory sessions were conducted twice a day for two weeks and each session contained 10 samples. Samples were evaluated using a standard ballot from the American Meat Science Association (AMSA, 1995). The ballot consisted of a numerical, eight-point scale for initial and sustained juiciness (8 = extremely juicy, 1 = extremely dry), tenderness (8 = extremely tender, 1 = extremely tough), and connective tissue amount (8 = none, 1 = abundant). Three flavor attributes beef flavor, painty/fishy, and livery were evaluated. The flavor intensity of each attribute was scored on a three-point scale (1 = not detectable, 3 = strongly detectable).

During sessions, panelists were randomly seated in individual booths in a temperature and light controlled room. The 10 samples were served in a randomized order according to panelist. The panelists were provided distilled, deionized water and unsalted crackers in order to cleanse their palate.

Statistical Analysis

Data were analyzed using the mixed procedure of SAS. The analysis of variance of model for WBSF, sensory, TBAR, and fatty acid analysis included treatment as the fixed effect and identification number as the random effect. The analysis of variance model for color attributes were analyzed using a repeated measures model with time as the repeated measure, identification number as the subject and treatment as the fixed effect. When the model was significant ($\alpha = 0.05$), least-square means were calculated and separated using pre-planned contrasts (control vs. DG, 10% DDG vs. 10% WDG, 10% WDG vs. 20% WDG vs. 30% WDG, and WDG vs. DDG).

Results and Discussion

Carcass Data

The effects of dietary treatment on carcass characteristics are presented in Table 1. Carcasses from steers fed dry distillers grains had a higher ($P < 0.05$) marbling score ($Sm^{16} \pm 11.8$) than carcasses from steers fed wet distillers grains ($Sl^{86} \pm 6.7$). Koger (2004) found that cattle fed dried distillers grains at 20 and 40% had no effect on marbling when compared to cattle fed the control diet, which consisted of corn, soybean meal, and alfalfa hay. Vander Pol et al. (2004) also found no differences in marbling scores from steers fed DG at 0, 20, or 40%. Carcasses of steers fed 20% WDG tended ($P = 0.09$) to have a smaller ribeye area than 10% WDG (13.6 vs. 14.6 sq. in., respectively). No differences in treatment ($P > 0.05$) were found in adjusted fat thickness, and USDA yield grade.

Color Evaluation

The main effect of dietary treatment on L*, a*, and b* and subjective evaluation values at 48 h of simulated retail display (time at which 75% of steaks being evaluated were deemed moderately undesirable) are presented in Table 2 and Table 3. When comparing color scores from 10% WDG and 10% DDG, steaks from both treatment groups had a moderately dark cherry red color at 48 h. Furthermore, steaks from 10% DDG carcasses had a greater percentage of surface discoloration ($P < 0.05$), which resulted in those steaks being scored as very undesirable, while 10% WDG steaks were deemed as moderately undesirable ($P < 0.05$, Table 3). Steaks from cattle fed 10% and 20% WDG had higher ($P < 0.05$) b* values, which indicates more yellowness, than 30% WDG. On the other hand, L* and a* values were not significantly different (Table 2). Previous research indicated that steaks from control cattle fed SFC had lower L*, greater a*, and greater b* values than steaks from cattle diets containing DG (Gill et al., 2008).

Thiobarbituric Acid Reactive Substance Analysis (TBAR)

Dietary treatments did not have an effect on lipid oxidation as indicated by TBAR concentrations (Table 4). When comparing 10% WDG and 10% DDG, steaks from cattle fed 10% WDG had higher ($P < 0.05$) TBAR pre-display values indicating a higher amount of oxidation. Research conducted by Gill et al. (2008) found in one of two harvest groups, that cattle fed sorghum dried distillers grains with roughage had greater lipid oxidation ($P < 0.05$) occurrence in pre-display steaks than cattle fed sorghum dried distillers grains without roughage.

Tenderness and Sensory Attributes

Warner-Bratzler shear force values indicated that no differences among the control and distillers diets were observed (Table 5). Roeber et al. (2005) and Koger (2004) found that WBSF values did not differ when evaluating various inclusion levels of WDG and DDG in cattle rations. However, when comparing strip loin steaks from cattle fed various percentages of WDG, steaks from steers fed 30% WDG and 10% WDG had lower ($P < 0.05$) Warner-Bratzler shear force (8.44 ± 0.29 lbs and 9.10 ± 0.31 lbs) values than 20% WDG (9.55 ± 0.29 lbs). Overall tenderness determined by a trained sensory panel verified WBSF results as panelists found no differences among treatments. Dahlen et al. (2005) documented that neither flavor, juiciness, connective tissue, nor off-flavor intensity were influenced by dietary treatment when comparing steaks from cattle fed a combination of condensed distillers solubles and barley by-product with those fed wet corn gluten feed.

Fatty Acid Analysis

The chemical composition and percent fatty acids are presented in Table 6. No differences were found in total saturated fatty acids (SFA), or total monounsaturated fatty acids (MUFA), however, differences in individual fatty acids were detected. Cattle fed SFC had higher (P < 0.05) levels of margaric (17:0) than cattle fed DG. Those results disagree with Gill et al. (2008) who reported that margaric acid was higher in fresh steaks from steers fed DG than those fed SFC. Margaric acid concentrations are not of major health concerns because they do not aid in increasing human plasma cholesterol levels (Baghurst, 2004). Steaks from cattle fed DDG were significantly higher ($P < 0.05$) in myristic (14:0) levels, than steaks from cattle fed WDG. This is one of the primary SFA's responsible for increasing plasma low-density lipoprotein (LDL) and total cholesterol concentrations in the human body (Hegsted et al., 1965). Comparing steaks from cattle fed varying percentages of WDG demonstrated that the *longissimus* muscle (LM) from cattle fed 20% and 30% WDG were higher ($P < 0.05$) in polyunsaturated fatty acids (PUFA) than cattle fed 10% WDG. Conversely, Koger (2004) reported that higher levels of PUFA's were found in the LM from cattle fed 40% distillers grain as compared to cattle fed 20% distillers grains. The same result can be seen in the n-6 fatty acids (Table 6). Linoleic acid (18:2) tended to be higher ($P = 0.09$) in 20% and 30% WDG, which clarify the increased level of total PUFA in 20% and 30% WDG steaks.

Conclusion

Based on the results from this study feeding various levels of wet or dry distillers grains to cattle will not affect carcass characteristics, sensory attributes or eating quality. Cattle producers are able to save money by replace a percentage of steam flaked corn with distillers grain in feed rations without causing detrimental affects to product quality. Data demonstrated that adding distillers grains at 20% or 30% does not affect sensory attributes. Warner-Bratzler shear force values even indicated that steaks from cattle fed 30% WDG were more tender than steaks from cattle fed 10% and 20% WDG. Beef from cattle fed 20% or 30% WDG will tend to have higher proportions of polyunsaturated fatty acids and therefore be more susceptible to oxidation resulting in a shortened shelf life. Further research should be done to evaluate different processing techniques, injection of antioxidants, or adding Vitamin E, to aid in increasing the shelf life of steaks from cattle fed higher inclusion rates of WDG.

Table 1. Least squares means ± SEM and main contrasts for carcass data.

Treatments[1]	Adj. fat thickness, in.	Ribeye area, sq. in.	Marbling Score[2]	USDA Yield Grade
SFC	0.53 ± 0.30	14.42 ± 0.34	382.94 ± 11.63	3.1 ± 0.17
10% DDG	0.50 ± 0.30	14.05 ± 0.34	416.76 ± 11.63	3.1 ± 0.17
10% WDG	0.51 ± 0.29	14.62 ± 0.33	400.00 ± 11.30	2.9 ± 0.16
20% WDG	0.54 ± 0.29	13.59 ± 0.33	381.11 ± 11.30	3.3 ± 0.16
30% WDG	0.53 ± 0.30	13.71 ± 0.34	379.43 ± 11.46	3.3 ± 0.16
Main Contrasts[1]		*P*-values		
Wet vs Dry	0.40	0.84	0.03	0.88
SFC vs DG	0.74	0.40	0.40	0.88
10% W vs 10% D	0.78	0.27	0.40	0.35
% DG	0.54	0.10	0.06	0.23

[1] Treatments: SFC = steam flaked corn, D = dry, W = wet, DG = distillers grains

[2] Marbling: 100 = practically devoid^{00}, 200 = traces^{00}, 300 = slight^{00}, 400 = small^{00}, 500 = modest^{00}, 600 = moderate^{00}

Table 2. Least squares means ± SEM and main contrasts for instrumental color analysis of strip loin steaks under retail display.

Treatments[1]	L*[2]	a*[3]	b*[4]
SFC	39.53 ± 0.71	11.53 ± 0.56	13.49 ± 0.35
10% DDG	39.47 ± 0.72	10.82 ± 0.57	13.50 ± 0.36
10% WDG	39.37 ± 0.69	11.57 ± 0.55	13.67 ± 0.34
20% WDG	39.98 ± 0.70	10.74 ± 0.55	13.27 ± 0.35
30% WDG	38.20 ± 0.69	10.48 ± 0.55	12.52 ± .034
Main Contrasts[1]		*P*-values	
Wet vs Dry	0.74	0.86	0.41
SFC vs DG	0.72	0.32	0.53
10% W vs 10% D	0.90	0.29	0.70
% DG	0.22	0.50	0.04

[1] Treatments: SFC = steam flaked corn, D = dry, W = wet, DG = distillers grains
[2] L* = brightness (0 = black, 100 = white)
[3] a* = redness (positive values = red, negative values = green)
[4] b* = yellowness (positive values = yellow, negative values = blue)

Table 3. Least squares means ± SEM and main contrasts for visual color evaluation of strip loin steaks for muscle color, surface discoloration and overall appearance.

Treatments[1]	Muscle Color[2]	Surface Discoloration[3]	Overall Accppearance[4]
SFC	3.97 ± 0.20	3.60 ± 0.27	3.28 ± 0.22
10% DDG	3.63 ± 0.20	4.25 ± 0.27	2.59 ± 0.22
10% WDG	3.59 ± 0.19	3.54 ± 0.26	3.20 ± 0.21
20% WDG	3.81 ± 0.19	4.06 ± 0.27	2.85 ± 0.22
30% WDG	3.56 ± 0.19	4.23 ± 0.26	2.75 ± 0.21
Main Contrasts[1]		*P*-values[5]	
Wet vs Dry	0.51	0.33	0.18
SFC vs DG	0.30	0.17	0.08
10% W vs 10% D	0.17	0.04	0.03
% DG	0.56	0.84	0.56

[1] Treatments: SFC = steam flaked corn, D = dry, W = wet, DG = distillers grains
[2] Muscle Color: 1 = extremely dark red, 8 = extremely bright cherry red
[3] Surface Discoloration: 1 = no discoloration, 7 = total discoloration
[4] Overall Acceptability: 1 = extremely undesirable, 8 = extremely desirable

Table 4. Least squares means ± SEM and main contrasts of thiobarbituric acid reactive substances (TBAR; mg of malonaldehyde/kg of steak) measured pre- and post-retail color display on strip loin steaks.

Treatments[1]	Pre-Display	Post-Display
SFC	0.1466 ± 0.003	0.1772 ± 0.007
10% DDG	0.1423 ± 0.003	0.1852 ± 0.007
10% WDG	0.1505 ± 0.003	0.1977 ± 0.007
20% WDG	0.1451 ± 0.003	0.1837 ± 0.007
30% WDG	0.1484 ± 0.003	0.1893 ± 0.007
Main Contrasts[1]	***P*-values**	
Wet vs Dry	0.11	0.40
SFC vs DG	0.99	0.21
10% W vs 10% D	0.04	0.26
% DG	0.25	0.89

[1] Treatments: SFC = steam flaked corn, D = dry, W = wet, DG = distillers grains

Table 5. Least square means ± SEM, and main contrasts for Warner-Bratzler Shear (WBSF) force and sensory characteristics of strip loin steaks.

Treatments[1]	WBSF (lbs)	Overall Tenderness[2]	Livery Flavor[3]
SFC	8.84 ± 0.29	5.55 ± 0.08	1.12 ± 0.02
10% DDG	8.99 ± 0.31	5.53 ± 0.08	1.09 ± 0.02
10% WDG	9.10 ± 0.29	5.69 ± 0.08	1.09 ± 0.02
20% WDG	9.55 ± 0.29	5.74 ± 0.08	1.12 ± 0.02
30% WDG	8.44 ± 0.29	5.66 ± 0.08	1.15 ± 0.02
Main Contrasts[1]		*P*-values	
Wet vs Dry	0.90	0.08	0.34
SFC vs DG	0.58	0.25	0.77
10% W vs 10% D	0.84	0.22	0.88
% DG	0.04	0.45	0.09

[1] Treatments: SFC = steam flaked corn, D = dry, W = wet, DG = distillers grains
[2] Tenderness: 1 = extremely tough, 8 = extremely tender
[3] Flavor Intensity: 1 = not detectable, 3 = strongly detectable

Table 6. Least square means and main contrasts for individual fatty acids (% of called fatty acids), and total fatty acids concentrations found in *longissimus* muscle.

	Treatment[1]					Main Contrasts			
Fatty Acids	SFC	10DDG	10WDG	20WDG	30WDG	D vs. W	10% D vs. 10% W	% DG	SFC vs. DG
12:0, lauric	0.14	0.11	0.11	0.12	0.11	0.55	0.44	0.61	0.03
14:0, myristic	3.14[a]	3.16[a]	2.94[ab]	2.68[b]	2.58[b]	0.009	0.27	0.06	0.05
15:0, pentadecanoic	0.68[a]	0.63[a]	0.61[ab]	0.55[b]	0.56[b]	0.03	0.43	0.07	0.002
16:0, palmitic	25.44	25.38	25.24	24.48	24.37	0.12	0.80	0.08	0.17
17:0, margaric	1.82[a]	1.69[ab]	1.69[ab]	1.58[b]	1.57[b]	0.26	0.95	0.14	0.009
18:0, stearic	13.19[a]	12.87[ab]	12.99[b]	13.71[b]	14.37[b]	0.03	0.78	0.01	0.42
14:1, myristoleic	0.63[a]	0.72[ab]	0.65[abc]	0.55[bc]	0.49[c]	0.01	0.32	0.05	0.66
16:1, palmitoleic	3.06[a]	3.15[ab]	3.10[ab]	2.75[bc]	2.65[c]	0.04	0.78	0.02	0.32
17:1, heptadecenoic	1.32[a]	1.21[a]	1.24[ab]	1.07[b]	1.07[b]	0.16	0.70	0.009	0.004
18:1 9c, oleic	32.98	33.85	34.79	34.01	34.47	0.48	0.35	0.53	0.09
18:1 11c, *cis*-vaccenic	1.87	1.94	1.92	1.88	1.97	0.91	0.90	0.95	0.71
20:1, gadoleic	0.07	0.09	0.05	0.08	0.08	0.59	0.26	0.23	0.36
18:2, linoleic	6.36	6.04	5.99	7.10	6.69	0.27	0.93	0.09	0.85
18:3, α-linoleic	0.42	0.38	0.38	0.37	0.42	0.85	0.99	0.81	0.38
CLA 9,11	0.08	0.11	0.04	0.10	0.08	0.41	0.15	0.18	0.59
CLA 10,12	0.48	0.36	0.46	0.43	0.49	0.12	0.24	0.87	0.63
Total SFA[2]	48.46	48.81	48.02	50.60	48.19	0.96	0.82	0.64	0.87
Total MUFA[3]	45.06	46.15	46.59	45.53	45.40	0.69	0.65	0.18	0.26
Total PUFA[4]	9.73	9.15	8.94	11.53	9.99	0.23	0.83	0.04	0.83
Total CLA	0.56	0.48	0.51	0.54	0.57	0.30	0.69	0.44	0.51
Total n-3	1.67	1.46	1.57	1.77	1.68	0.24	0.63	0.41	0.75
Total n-6	7.97	7.57	7.33	9.65	8.23	0.25	0.78	0.03	0.75

[a,b,c] Least square means with the same letter, in the same row, are not different ($P < 0.05$)
[1] Treatments: SFC = steam flaked corn, D = dry, W = wet, DG = distillers grains
[2] SFA = calculated sum of fatty acids presented in this study that contain no double bonds
[3] MUFA = calculated sum of fatty acids presented in this study that contain 1 double bonds
[4] PUFA = calculated sum of fatty acids in this study that contain 2 or more double bonds

Appendix A

Fatty Acid Methyl Ester Preparation

*Note: Samples were run in triplicate.

Extraction

- Mix fresh 2:1 (v:v) MeOH:$CHCl_3$ solution
- Add 19:0, internal standard, (concentration = 10µg/mL), to fresh 2:1 solution
- Pipette 3 mL of 2:1 solution with internal standard into each homogenizer
    - Cap with aluminum foil to avoid evaporation
- Weigh out 64-66 mg of powdered sample and place into labeled homogenizer
    - Homogenize sample until it has gone into solution
- Transfer homogenate from each homogenizer into a pre labeled centrifuge tube
- Rinse homogenizer with 1 mL $CHCl_3$ and transfer to centrifuge tube
- Add 800 µl of distilled H_2O to centrifuge tube, vortex, and centrifuge samples for 5 min at 5000 rpm
- Collect lower phase and place into silicate vials
- Add 2 mL $CHCl_3$ to centrifuge tubes, vortex and centrifuge for 5 min at 5000 rpm
- Collect lower phase and place into silicate vial
- Repeat the previous 2 steps
- Dry silicate vials down completely under N_2, add 200 µl of BHT (0.05%) to each vial, and can place vials in freezer at this point

Sodium Sulfate Mini-columns

- Prepare mini-columns by adding Na_2SO_4 to disposable pasteur pipettes
- Add 200 µl $CHCl_3$ to vials, mix well, and transfer to mini-columns

- Rinse vial with 1 mL $CHCl_3$ and transfer to mini-column
- Wash each mini-column with an additional 5 mL $CHCl_3$
- Collect effluent into new silicate vials, dry down under N_2, leaving approx. 200 µl in vial, and can store in freezer

<u>Derivitizations</u>

- Finish drying down samples completely
- Add 200 µl toluene to re-suspend lipids, and then add 2 mL sodium methoxide
- Incubate samples in a heat block at 60°C for 30 min
- Cool samples to room temperature, add 1 mL boron trifluoride (BF_3), incubate again at 60°C for 30 min
- Cool samples to room temperature, add 2 mL $NaHCO_3$ saturated in H_2O, mix well

<u>Hexane Extraction</u>

- Add 2 mL hexane, mix by inverted vials 2-3 times, collect upper phase into new silicate vials (repeat twice more)
- Dry samples down under N_2 (can freezer here if 200 µl are left in vial)

<u>Biosil Mini-columns</u>

- Prepare min-columns by adding activated silica to disposable pasteur pipettes
- Place samples on to mini-columns, wash vials with 1 mL hexane and place onto mini-columns
- Wash mini-columns with an additional 3 mL hexane, collect into original vial
- Prepare a fresh mixture of 5% Ether/Hexane solution
- Wash same mini-column with 6 mL 5% ether/hexane solution into a hexane rinsed silicate vial
- Dry samples down completely under N_2
- Add 1 mL $CHCl_3$, and prepare GC vials for analysis

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