Effect of Feeding Ethanol By-Products on Performance and Marbling Deposition in Steers Fed High-Concentrate or High-Forage Diets

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Jon Schoonmaker, post-doctorate; Allen Trenkle, distinguished professor of animal science; Donald Beitz, distinguished professor of animal science and biochemistry

Summary

Research on the effect of dietary ethanol by-products on beef quality has been limited. Some Universities have reported a decrease in marbling due to distillers grains inclusion, while others have not. It is unclear why marbling deposition may be decreased when increasing amounts of distillers grains are fed; however, decreased starch availability, increased vitamin A and D, and the high oil content in ethanol by-products may contribute. In contrast, distillers grains can increase unsaturated fatty acid content of beef, thus increasing healthfulness. Our objective was to measure the effect of wet distillers grains (0, 20, or 40 % of the diet) on growth, feed intake, and marbling deposition and to determine what may be responsible for decreased marbling. Average daily gain and feed intake did not differ between wet distillers grains treatments, but cattle fed distillers grains were more efficient. Marbling score decreased in high-concentrate-fed steers as WDG concentration was increased, but increased in high-foragefed steers from the 0 to 20% WDG inclusion rate and then decreased from the 20 to 40% WDG inclusion rate. Backfat thickness decreased in high-concentrate-fed steers as WDG concentration increased but increased in high-forage-fed steers from the 0 to 20% WDG inclusion rate and then decreased from the 20 to 40% WDG inclusion rate. Cattle fed distillers grains had lower plasma total vitamin A and plasma vitamin D. Retinol, however, was positively related to marbling and vitamin D was negatively related to marbling. Polyunsaturated fatty acids, which can enhance the healthfulness of beef, were increased by feeding wet distillers grains, but were related to decreased marbling.

Introduction

Ethanol industry by-products are an excellent feed resource for feedlot cattle. Distillers grains are rich in protein and energy. Extensive research dating back to the 1980s has been performed determining the effectiveness of distillers grains in diets on growth and performance of beef cattle. Optimal dietary inclusion rates for individual farms depend largely on price of distillers grains and distance from an ethanol plant. With regard to performance, reports from the University of Nebraska indicate that daily gain is maximized at the 30% inclusion rate and feed efficiency is maximized at the 50% inclusion rate.

Research on the effect of dietary ethanol by-products on beef quality has been limited. Researchers from Saskatchewan observed that steers fed a diet consisting of wheat-based distillers grain had more seam fat but less backfat than did those fed wet brewers grains or barley and that steaks from steers fed wheat-based distillers grains were similar in sensory traits and tenderness to those from steers fed brewers grains or barley. Reports from the University of Nebraska have observed no effect of distillers grain inclusion on marbling scores. In contrast, researchers at Kansas State University summarized 13 studies that included wet or dry distillers grains at varying amounts and reported that marbling decreased with increasing inclusion of distillers grains. Researchers at Iowa State University have reported no differences in marbling score related to distillers grains inclusion in the diet but have indicated that as multiple trials are analyzed together a general trend exists for lowered marbling content as distillers grains concentration in the diet increases.

It is unclear why marbling deposition may be decreased when increasing amounts of distillers grains are fed; however, decreased starch availability in ethanol byproducts is believed to contribute. Thus, we hypothesize that as the amount of starch or grain in the diet increases, intramuscular fat deposition will increase. Although feeding ethanol by-products lower the starch content of the diet, we hypothesize that the ethanol by-products will enhance beef healthfulness and will maintain USDA quality grade in beef from feedlot cattle. Our objective is to evaluate effects of feeding ethanol by-products on cattle performance and on intramuscular and subcutaneous fat deposition in feedlot cattle.

Materials and Methods

One hundred thirty-seven Angus cross yearling steers (859.5 \pm 2.1 lb.) were allotted by body weight to a 3 × 2 factorial arrangement of 6 treatments (4 pens per treatment) to determine the effect of wet distillers grains concentration (WDG; 0, 20, 40 % diet dry matter) in high concentrate and high forage diets on growth performance and marbling content. Upon arrival at the Iowa State Beef Center, steers were vaccinated against IBR, BVD, PI₃, BRSV, and clostridia (7-way), treated with IVOMEC[®] for internal and external parasites, and implanted with Component TE-S[®] (provided courtesy of VetLife, Overland Park, KS). Initial weight was calculated as an average of weights on 2 consecutive days.

Steers fed the high concentrate diets received, on a DM basis, 12% brome-grass hay, 0, 20, or 40% wet distillers grain, and 67.5, 57.0, or 45.0% dry-rolled corn (Table 1). Steers fed the high forage diets received, on a DM basis, 50% brome-grass hay, 0, 20, or 40% WDG, and 30.4, 22.2, and 8.4% dry-rolled corn. All diets were balanced to provide 17.6 \pm 0.2% crude protein. Soybean meal was used as an alternative protein source, and soybean oil was used to balance diets for oil content. Feed was offered once daily at 0800, and feed refusals were recorded daily for each pen. Diet compositions are shown in Table 1. Feed samples were taken every other week and were composited for analysis of DM, CP, ADF, and NDF.

On d 98, blood samples were collected by coccygeal venipuncture and rumen samples were collected by stomach tube from 36 steers (6 steers per treatment). Steers were selected on the basis of body weight to represent the average body weight for each specific treatment group. Feed had been removed 16 hours prior to sample collection. Blood samples were collected into heparinized tubes and centrifuged at 1500 x g for 15 minutes. Plasma was harvested, separated into 3 aliquots, and frozen at -20°C until later analysis. Rumen samples were snap frozen in liquid nitrogen and stored at -20°C until later analysis. Thawed plasma was analyzed for glucose concentration by colorimetric determination using the glucose oxidase procedure (Sigma Diagnostics, St. Louis, MO.). Insulin concentrations were determined using a heterologous radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Volatile fatty acids (VFA) in the rumen samples were quantified on a Varian 3900 gas chromatograph (Varian Inc, CA) as butyl esters. 25-hydroxyvitamin D_3 and vitamin A (retinol and β carotene) were analyzed by HPLC using UV detection. Briefly, plasma was homogenized in phosphate-buffered saline with a polytron homogenizer. Vitamin D and vitamin A metabolites were removed from the homogenate via hexane and chloroform/methanol extraction, and separated by solid phase extraction with a Varian LRC 500 mg silica cartridge (Varian, Harbor City, CA).

Cattle were slaughtered, at 3 different time-points (3 to 4 weeks apart), when the average body weight for a pen was estimated to be 1275 lbs. The 36 steers that had previously been selected for rumen and blood sampling were slaughtered at O'Neill Packing (Omaha, NE). Muscle samples were collected from these steers immediately after slaughter, snap frozen in liquid nitrogen, and stored at -80°C until later analysis of fatty acid and transcription factor content. The remaining steers were slaughtered at Tyson Foods (Denison, IA). Hot carcass weight, backfat depth, percentage kidney, pelvic and heart fat, rib-eye area, and USDA quality and yield grades were determined for all cattle by qualified Iowa State University personnel 48 h after slaughter.

Lipids were extracted from beef using a chloroform/methanol extraction procedure and fatty acids

were derivatized as methyl esters by a CH₃COCl-catalyzed methanolysis derivitization procedure. Briefly, a 0.5 inch steak that represents an entire cross section of the longissimus muscle was trimmed of all external fat and ground to a very fine consistency in an Oskar® food processor. Ground beef samples are vortexed for 1 hour in chloroform/methanol/water (28:56:16), and the chloroform laver was removed following addition of chloroform and a 0.37 % KCl solution, centrifugation at 1700 rpm, and filtration through a 4.25 cm glass filter. Five-hundred uL of sample (representing approximately 10 mg of lipid) was trans-esterified by addition of 1 mL of methanol, 100 uL of acetyl chloride, heating at 80°C, addition of 5 mL of 4% potassium carbonate, and centrifugation at 1700 rpm. A portion of the chloroform layer was removed for analysis by gas chromatography. The methyl esters of fatty acids from beef were quantified on a Varian 3800 gas chromatograph equipped with an automatic injector (Varian Inc, CA) to allow for 24-hour operation. A 100-meter SP-2560 column (Supelco Inc., Bellefonte, PA) was used so that all fatty acids, including conjugated 18:2, were resolved. Fatty acid percentage was calculated by dividing individual fatty acid peak areas by the total of all fatty acid peak areas. An atherogenic index (AI), as described by Ulbricht and Southgate (1991), was calculated for each beef sample. The AI described by these authors ranks mixtures of fatty acids according to their propensity to cause atherogenesis, as predicted from concentrations of individual fatty acids in the lipid. The AI is calculated as: $(12:0 + 4(14:0) + 16:0) \div$ $(\Sigma MUFA + \Sigma PUFA).$

Data were analyzed using the MIXED procedures of SAS (Version 8.0, SAS Inst. Inc., Cary, NC) with compound symmetry as the co-variance structure. Treatments were arranged as a 2 x 3 factorial. The model included effects due to amount of concentrate and distillers grain inclusion. Means were separated using LS means, residual mean square was used as the error term, and pen was the experimental unit.

Results and Discussion

Weight gain and feed intake

Final weight was similar among treatments and averaged 1273 lb (Table 2). Concentrate-fed steers gained faster (P < 0.01) than did forage-fed steers; amount of WDG fed did not affect (P > 0.25) daily gain. Concentrate-fed steers were on feed for fewer days (P < 0.01) tended to consume less DM on a daily basis (P < 0.14), consumed less total DM (P < 0.01), and were more efficient (P < 0.01) than forage-fed steers. Steers fed 40% WDG consumed less DM on a daily basis (P < 0.01) compared with steers fed 0 or 20 % WDG (22.3 vs. 23.7 and 23.7, respectively). An interaction between concentration of grain and concentration of WDG in the diet occurred (P < 0.04). In concentrate-fed cattle, daily DM intake decreased as the amount of distillers grains increased, but in forage-fed cattle, daily DM intake increased from the 0 to 20% WDG inclusion rate and then decreased from the 20 to 40% WDG inclusion rate. Steers fed no WDG were less efficient (P < 0.04) compared with steers fed 20 or 40% WDG (8.2 vs. 7.4 and 7.3 lb of DM per lb of gain; respectively).

Carcass characteristics

Carcass characteristics are presented in Table 3. Hot carcass weight (785.5 vs 745.0 lb), dressing percentage (61.6 vs 58.5 %), fat thickness (0.44 vs 0.35 in.), % KPH (2.3 vs 2.1), marbling score (298.7 vs 264.5), and vield grade (3.0 vs 2.7) were greater (P < 0.01) for concentratefed than for forage-fed steers, respectively. Rib-eye area tended (P < 0.08) to be greater for concentrate-fed than for forage-fed steers (12.7 vs 12.4 in²). Steers fed 40% WDG tended (P < 0.08) to have a higher dressing percentage (60.5 vs 59.7 and 59.8 %), and produced carcasses with the least (P < 0.05) amount of backfat (0.37 vs 0.41 and 0.40 in.), the lowest (P < 0.01) marbling score (263.1 vs 287.4 and 294.4), and the lowest (P < 0.01) percentage that graded choice or better (10.9 vs 34.3 and 33.8 %) compared with steers fed 0 or 20% WDG, respectively. Steers fed no WDG produced carcasses with the smallest (P < 0.02) rib-eye area $(12.2 \text{ vs } 12.7 \text{ and } 12.9 \text{ in}^2)$ and the highest (P < 0.03) yield grade (3.0 vs 2.8 and 2.7) compared with steers fed 20 or 40% WDG, respectively. An interaction between the concentration of grain in the diet and concentration of WDG in the diet tended to occur for fat thickness (P < 0.08) and did occur for marbling score and for percentage of carcasses grading choice or better (P < 0.01). In concentrate-fed steers, fat thickness tended to decrease as distillers grain concentration increased, but, in forage-fed steers, fat thickness tended to increase from the 0 to 20% inclusion rate, and then tended to decrease from the 20 to 40%inclusion rate. Similarly, in concentrate-fed steers, marbling score decreased as distillers grain concentration increased (325, 306, 265), but, in forage-fed steers, marbling score increased from the 0 to 20% inclusion rate and then decreased from the 20 to 40% inclusion rate (249, 282, 262).

Effect of starch fermentation on ruminal propionate and plasma glucose and insulin

Because WDG is abundant in Iowa and competition for corn for production of ethanol has increased the cost of high corn diets, it is imperative that the reason for decreased intramuscular fat deposition in cattle fed high WDG diets be discovered. Finding the responsible factor could lead to development of prevention strategies that producers can use when cattle are fed large amounts of WDG. Researchers at Texas A&M demonstrated that glucose provides 50 to 75% of the acetyl units for in vitro lipogenesis in the intramuscular fat depot. Therefore, a starch-rich fermentation, resulting in elevated blood glucose and insulin, may be a key component in triggering intramuscular adipocyte development in cattle fed a high-grain diet. Previous research at The Ohio State University has demonstrated that cattle fed diets low in concentrate produce less propionate when digested in the rumen, which results in less circulating insulin compared with cattle fed higher amounts of concentrate. Circulating glucose did not differ between cattle fed diets differing in starch concentration. Elevated serum insulin, however, may have led to an increased uptake of glucose by peripheral tissues, and an increased marbling score at 218 d of age for earlyweaned steers fed a high concentrate diet ad libitum. It has been postulated that decreased starch availability in WDG may lead to less ruminal propionate production and less circulating insulin when fed to cattle.

In the present study, we have demonstrated that, although the molar percentage of ruminal propionate is higher (P < 0.01) for concentrate-fed than for forage-fed cattle, ruminal propionate does not differ (P > 0.71) among the amount of WDG that was included in the diet (Table 4, Figure 1). Concentration of plasma glucose did not differ between concentrate-fed and forage-fed steers and did not differ among cattle that were fed different amounts of WDG (Figure 2). Circulating insulin, however, tended to respond differently (interaction, P < 0.08) in concentrate-fed and forage-fed cattle as the amount of WDG increased (Figure 3), indicating that something in addition to the amount of dietary concentrate is affecting circulating insulin concentrations.

Vitamins A and D

Decreasing dietary vitamin A has had some success improving intramuscular fat deposition. The Ohio State University researchers reported a 4.3% drop in marbling score when 2700 IU of vitamin A /kg DM was added to the diet. The ability of bovine-derived adipocytes to deposit lipid has been reported by Japanese researchers to be negatively correlated (r = -0.73) with serum retinol concentration, and serum retinol and liver vitamin A concentration have been demonstrated to be inversely related with the Japanese beef marbling index. Japanese steers consuming diets not supplemented with vitamin A exhibited less serum retinol 4 to 6 mo before slaughter and produced greater marbling scores compared with steers fed diets supplemented with vitamin A. Additionally, Japanese researchers observed serum retinol concentrations that were 63% lower 6 mo prior to slaughter in cattle that produced higher marbled carcasses.

In contrast, other reports have demonstrated no significant difference in carcass grade between supplemented and non-supplemented vitamin A dietary groups. Interestingly, research in Japan has demonstrated a dose response to dietary vitamin A. When high amounts of vitamin A (5 to 50 μ mol) are fed, vitamin A has had a predominantly negative effect on marbling; however, when small amounts of vitamin A (1 pmol to 10 nmol) are fed, marbling can actually be enhanced. Vitamin D is thought to have a predominantly negative effect on marbling, but

again, when large doses are fed; when small doses are fed, vitamin D may enhance marbling.

Vitamin A is present in feeds and forages as provitamin A, or β -carotene. Upon entry into the small intestine, β -carotene is cleaved into 2 molecules of retinal (a vitamin A metabolite) by intestinal cells and subsequently reduced to retinol. Forages are much higher in β -carotene concentration than are grains (NRC, 1996). It is not clear if grain-finishing enhances marbling because of higher energy and starch concentration or because of vitamin A status. Additionally, β -carotene is concentrated in distillers grains, which may explain why some have reported decreased quality grades in cattle fed these byproducts.

Vitamin data for the present study is in Table 5. Total plasma vitamin A (β-carotene + retinol) was lower in grainfed compared with forage-fed steers (783.3 vs. 980.6 ng/mL; P < 0.01) and was affected by WDG inclusion rate (P < 0.06). Steers fed 20 % WDG had lower plasma total vitamin A compared with steers fed 0 and 40 % WDG (930.2, 796.6, 919.2 ng/mL for 0, 20, and 40% WDG, respectively). Total vitamin A did not correlate with marbling (P > 0.98). When vitamin A was analyzed separately as either retinol or β -carotene, a different picture arises. Plasma retinol was elevated in the higher marbled grain-fed cattle (554.9 vs. 471.3 ng/mL; P < 0.01) and had a weak positive correlation with marbling content ($r^2 = 0.14$, P < 0.03). Plasma retinol did not differ among distillers grains treatments (P > 0.21). Plasma β -carotene was elevated for forage-fed compared with concentrate-fed cattle (509.3 vs 228.4 ng/mL; P < 0.01), did not correlate with marbling (P > 0.17), and did not differ among distillers grains treatments (P > 0.12). Interestingly, the ratio of retinol:β-carotene, followed the same trend as marbling (Figure 4), and had a weak positive correlation with marbling content ($r^2 = 0.10$, P < 0.08), indicating that increasing the conversion of β -carotene to retinol may improve marbling.

Plasma 25-hydroxyvitamin D₃ was lower for concentrate fed compared with forage-fed steers (23.4 vs 42.2 ng/mL, P < 0.01) and seems to decrease (P < 0.10) linearly in response to dietary WDG inclusion (38.5, 31.3, and 28.7 ng/mL for 0, 20, and 40 % WDG, respectively). Plasma 25-hydroxyvitamin D₃ was negatively correlated ($r^2 = -0.09$, P < 0.09) with marbling content indicating that dietary vitamin D may inhibit marbling.

Fatty acid content of beef

Saturated fatty acid content of the human diet continues to be of concern for health-conscious consumers, especially with regard to plasma cholesterol concentration. The reason for concern is that cardiovascular disease is the leading cause of death in the United States, killing nearly one million people per year from cardiovascular disease or stroke (American Heart Association). Animal products provide collectively 56% of total fat, 74% of saturated fatty acids, and 100% of the cholesterol consumed by humans. Beef is a major source of animal saturated fatty acids. Decreasing the consumption of dietary fats is commonly recommended by human nutritionists as the best dietary means for decreasing risk of coronary disease. Oftentimes, beef is removed from the human diet. Recommendations by healthcare providers have been a major factor in the decline in total red meat consumption from 1960 through 1987.

Replacing dietary saturated fatty acids with monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA), however, can have positive long-term effects on human health. In a 14-year study of more than 80,000 women, reduction of total fat intake had little effect on long-term coronary health, whereas replacement of saturated fatty acid with MUFA decreased relative risk of coronary disease significantly. By developing animal products with greater percentages of MUFA and PUFA, coronary disease may be decreased significantly. Decreasing the saturated fatty acid composition of beef by replacing these "less healthy" fatty acids with MUFA may lead to an improvement in healthfulness for the human diet and may prevent further declines in beef consumption. PUFA have been reported by researchers at the University of Missouri to be higher in the Longissimus dorsi of cattle fed dry distillers grains, thus feeding dry or wet distillers grains may be a way to improve healthfulness of beef.

Fatty acid composition of the *Longissimus dorsi* of cattle in the current study is presented in Table 6. Saturated fatty acid content was lower in concentrate-fed compared with forage-fed steers (46.11 vs 47.7 g/100g; P < 0.04), but the saturated fatty acid thought to be most responsible for atherogenic plaque build-up in arteries, C14:0, was greater (P < 0.01) in concentrate than in forage-fed steers (2.73 vs 2.17 g/100g). *Cis*-9, *trans*-11 (0.12 vs 0.40 g/100 g; P < 0.01) and *trans*-10, *cis*-12 (0.01 vs 0.05 g/100g; P < 0.01) conjugated linoleic acid (CLA) was lower for concentrate-fed than for forage-fed steers. PUFA (Figure 5) content of the *Longissimus dorsi* muscle of concentrate-fed steers was greater than that of forage-fed steers (8.9 vs 7.4 g/100 g; P < 0.01).

Saturated fatty acid, C14:0, and CLA (*cis-9*, *trans-*11 and *trans-*10, *cis-*12) content of the *Longissimus dorsi* of cattle fed distillers grains did not differ (P > 0.11); however, PUFA content was greatest (P < 0.03) in the *Longissimus dorsi* of cattle fed 40% WDG (9.2 g/100 g) compared with the *Longissimus dorsi* of cattle fed 40% WDG (9.2 g/100 g) compared with the *Longissimus dorsi* of cattle fed 0 or 20% WDG (7.7 and 7.6 g/100 g). An interaction for PUFA (P < 0.01) content occurred. In concentrate-fed steers, PUFA content increased as WDG grain concentration increased in the diet, whereas in forage-fed steers, PUFA content decreased from the 0 to 20% WDG inclusion rate and then increased from the 20 to 40% WDG inclusion rate.

Despite healthfulness implications, PUFA content of the Longissimus dorsi muscle was negatively correlated with marbling ($r^2 = 0.19$; P < 0.01), and C14:0 content was positively correlated with marbling ($r^2 = 0.13$; P < 0.03). A negative effect of PUFA on intramuscular adipose is not

unprecedented. Smith et al. (1996) demonstrated that PUFA can decrease adipocyte size and number in porcine adipocytes cultured in vitro. It may be that PUFAs, although enhancing beef's healthfulness, are inhibiting adipocyte growth through insulin in the present study.

In conclusion, distillers grains did not affect daily gains, improved feed conversion, increased rib-eye area, but decreased marbling score in steers fed high concentrate diets; marbling score was not decreased in high forage diets. Decreased circulating insulin may be responsible, but not through an increase in propionate production. Another factor is altering insulin concentrations and marbling score in distillers grain-fed cattle; perhaps poly-unsaturated fatty acids and/or vitamin A metabolites.

Table 1. Diet composition.

		High concentra	te			
	0^{a}	20^{a}	40^{a}	0^{a}	20 ^a	40^{a}
Ingredient						
Corn, rolled	67.53	57.03	45.03	30.38	22.18	8.36
Bromegrass hay	12.00	12.00	12.00	50.00	50.00	50.00
Wet distillers grain		20.00	40.00		20.00	40.00
Soybean meal	14.00	6.50		14.00	4.00	
Soybean oil	2.90	1.40		2.80	1.30	
Limestone	1.65	1.65	1.65	1.10	1.10	1.10
Urea	1.50	1.00	0.20	1.30	1.00	0.00
Molasses	0.00	0.00	0.70	0.00	0.00	0.12
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin A ^b	0.08	0.08	0.08	0.08	0.08	0.08
Trace mineral ^c	0.025	0.025	0.025	0.025	0.025	0.025
Rumensin 80 [®]	0.018	0.018	0.018	0.018	0.018	0.018
Nutrient composition						
Crude protein	17.51	17.79	17.62	17.60	17.48	17.72
Calcium	0.72	0.71	0.71	0.71	0.70	0.70
Phosphorus	0.43	0.44	0.46	0.33	0.34	0.37
Potassium	0.76	0.92	1.13	1.26	1.39	1.62
NEm, Mcal/cwt	937	954	973	779	795	818
NEg, Mcal/cwt	610	609	609	446	445	448

^aPercentage of wet distillers grains in the diet

^bVitamin A premix contained 5 million IU/kg.

^cTrace mineral mix contained: Ca 13.2%, Co 0.10%, Cu 1.5%, Fe (ferrous) 10.0%, Fe (ferric)

0.44%, I (as EDDI) 0.20%, Mn 8.0%, and Zn 12%.

	High Concentrate			High Forage				P-value			
	0^{b}	20 ^b	40^{b}	0^{b}	20 ^b	40 ^b	SE	Diet	WDG ^a	Diet x WDG ^a	
Initial weight, lb	861.0	861.5	859.7	858.4	859.0	857.3	2.1	0.17	0.70	0.99	
Final weight, lb	1269.2	1284.2	1269.2	1256.1	1283.4	1280.2	14.5	0.93	0.36	0.71	
Average daily gain, lb/d	3.47	3.69	3.57	2.71	2.98	2.82	0.14	0.01	0.25	0.98	
Days on feed	120	115	115	158	149	158	7.9	0.01	0.67	0.84	
Daily dry matter intake, lb/d	23.3 ^{cd}	23.1 ^c	22.6 ^{cf}	24.1 ^d	24.3 ^e	22.0^{f}	0.4	0.14	0.01	0.04	
Total dry matter intake, lb.	2814.3	2678.8	2621.3	3806.5	3630.9	3460	179.8	0.01	0.35	0.91	
Feed conversion, lb/lb	6.9	6.3	6.4	9.6	8.5	8.2	0.4	0.01	0.04	0.51	

Table 2. Effect of WDG^a concentration in high concentrate or high forage diets on cattle growth performance.

 $^{a}WDG =$ wet distillers grains. $^{b}Percentage of WDG in the diet.$ $^{cdef}Means within the same row with different superscripts differ (P < 0.04; interaction).$

	High Concentrate				High Forage	e	P-value			
	0^{b}	20 ^b	40 ^b	0^{b}	20 ^b	40 ^b	SE	Diet	WDG ^a	Diet x WDG ^a
Hot carcass weight, lb.	784.0	786.9	785.6	728.1	749.6	757.2	7.9	0.01	0.15	0.23
Dressing percentage	61.6	61.3	61.9	57.9	58.4	59.1	0.4	0.01	0.08	0.31
Fat thickness, in.	0.48 ^g	0.42 ^h	0.42 ^h	0.35 ^{ij}	0.38^{hi}	0.32^{j}	0.02	0.01	0.05	0.08
Kidney, pelvic, heart fat, %	2.3	2.3	2.3	2.1	2.1	2.1	0.1	0.01	0.81	0.77
Rib-eye area, in ²	12.5	12.9	12.9	11.9	12.5	12.8	0.2	0.08	0.02	0.46
Marbling score	325.4 ^c	306.3 ^d	264.5 ^{ef}	249.3 ^e	282.4^{f}	261.7 ^e	6.5	0.01	0.01	0.01
Carcass quality grade										
Standard, %	0.0	0.0	0.0	23.3	0.0	4.3	2.2	0.01	0.01	0.01
Select ⁻ , %	5.0	8.5	21.5	4.3	9.3	19.0	5.2	0.85	0.02	0.95
Select ⁺ , %	31.5	47.5	56.5	67.5	67.5	73.0	8.3	0.01	0.22	0.48
Choice and greater, %	63.5 ^c	44.0 ^d	17.5 ^{ef}	5.0 ^e	23.5 ^f	4.3 ^e	6.3	0.01	0.01	0.01
Choice, %	53.5 ^g	40.0^{gh}	17.5 ⁱ	5.0 ⁱ	23.5 ^{hi}	4.3 ⁱ	7.5	0.01	0.03	0.06
Choice ^o , %	10.0	4.3	0.0	0.0	0.0	0.0	2.9	0.07	0.26	0.26
Yield grade	3.1	2.9	2.9	2.8	2.7	2.5	0.1	0.01	0.03	0.47

Table 3. Effect of WDG^a concentration in high concentrate or high forage diets on carcass characteristics.

^aWDG = wet distillers grains. ^bPercentage of WDG in the diet. ^{cdef}Means within the same row with different superscripts differ (P < 0.01; interaction). ^{ghi}Means within the same row with different superscripts tended to differ (P < 0.06; interaction).

	High Concentrate				High Forag	ge				
	0 ^b	20 ^b	40 ^b	0 ^b	20 ^b	40 ^b	SE	Diet	WDG ^a	Diet x WDG ^a
Acetate	55.22	54.36	54.70	62.48	62.96	60.12	1.55	0.01	0.59	0.58
Propionate	22.42	21.69	21.78	16.88	15.13	18.13	1.99	0.01	0.71	0.75
Butyrate	10.75	9.09	9.94	10.46	9.14	9.01	0.82	0.57	0.19	0.83
Isobutyrate	2.52	2.94	2.17	2.06	2.57	2.53	0.27	0.49	0.20	0.26
Valerate	1.83	2.17	2.36	1.51	1.68	1.71	0.27	0.04	0.40	0.83
Isovalerate	2.25	2.84	2.22	1.94	2.39	2.48	0.33	0.53	0.30	0.51
Caproate	1.89	2.95	2.75	1.78	2.61	2.32	0.37	0.33	0.05	0.91
Isocaproate	1.22	1.99	1.61	0.86	1.29	1.40	0.27	0.07	0.09	0.65
Formate	0.67	0.99	0.87	0.61	0.36	0.85	0.21	0.17	0.52	0.27
Fumarate	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.75	0.98	0.13
Lactate	0.32	0.16	0.47	0.57	0.94	0.46	0.31	0.19	0.94	0.43
Oxalate	0.09	0.18	0.15	0.12	0.08	0.14	0.05	0.52	0.75	0.45
Succinate	0.80	0.63	0.97	0.72	0.83	0.85	0.13	0.99	0.35	0.43

Table 4. Effect of WDG^a concentration in high concentrate or high forage diets on ruminal volatile fatty acid concentrations (µmol/mL).

^aWDG = wet distillers grains. ^bPercentage of WDG in the diet.

	High Concentrate				High Forage	e	P-value			
	0 ^b	20 ^b	40 ^b	0 ^b	20 ^b	40 ^b	SE	Diet	WDG ^a	Diet x WDG ^a
25-hydroxyvitamin D ₃ , ng/mL	29.3	23.3	17.7	47.7	39.3	39.7	4.7	0.01	0.10	0.80
α-tocopherol, ng/mL	1590.0	2360.0	3883.3	2078.3	2996.7	4337.0	515.9	0.09	0.01	0.97
Retinol, ng/mL	661.8 ^c	439.9 ^{df}	563.1 ^e	394.7 ^f	517.7 ^{de}	501.5 ^{de}	46.1	0.01	0.21	0.01
β-carotene, ng/mL	226.3	198.1	260.8	577.5	437.4	513.0	61.7	0.01	0.12	0.36
Total vitamin A, ng/mL	888.1	638.0	823.9	972.2	955.1	1014.5	85.5	0.01	0.06	0.15
Retinol:β-carotene	2.97 ^c	2.32 ^d	2.21 ^d	0.70 ^e	1.27^{f}	1.00 ^{ef}	0.27	0.01	0.43	0.01

Table 5. Effect of WDG^a concentration in high concentrate or high forage diets on plasma vitamin concentrations (ng/mL).

^aWDG = wet distillers grains. ^bPercentage of WDG in the diet. ^{cdef}Means within the same row with different superscripts differ (P < 0.01; interaction).

	H		High Forage				P-value			
	0 ^b	20 ^b	40 ^b	0 ^b	20 ^b	40 ^b	SE	Diet	WDG ^a	Diet x WDG ^a
C10:0	0.06 ^c	0.06 ^c	0.05 ^c	0.03 ^d	0.05 ^c	0.04 ^{cd}	0.01	0.01	0.25	0.05
C12:0	0.07	0.06	0.05	0.03	0.06	0.04	0.01	0.08	0.43	0.11
C14:0	2.85 ^c	2.62 ^c	2.72 ^c	1.85 ^d	2.58 ^c	2.08 ^d	0.16	0.01	0.28	0.03
C14:1	0.34	0.36	0.36	0.31	0.34	0.32	0.02	0.10	0.47	0.85
C15:0	1.22 ^c	1.63 ^d	1.49 ^c	1.66 ^d	1.02 ^c	1.18 ^c	0.17	0.29	0.77	0.02
C15:1	0.06	0.08	0.07	0.08	0.03	0.05	0.02	0.32	0.77	0.22
C16:0	25.92	25.90	25.37	22.99	25.41	24.32	0.60	0.01	0.14	0.12
C16:1	3.35	3.21	2.85	2.41	2.92	2.46	0.18	0.01	0.10	0.18
C17:0	0.99	1.03	0.97	0.73	0.77	0.78	0.05	0.01	0.74	0.78
C17:1	0.69	0.68	0.58	0.36	0.40	0.39	0.04	0.01	0.32	0.16
C18:0	14.27	15.12	14.47	19.77	18.13	18.13	0.76	0.01	0.64	0.26
C18:1 cis-9	36.91°	34.56 ^{cd}	32.03 ^e	31.90 ^e	32.90 ^{de}	32.27 ^{de}	1.04	0.02	0.10	0.06
C18:1 cis-11	0.26	0.27	0.44	0.31	0.29	0.32	0.05	0.63	0.07	0.14
C18:1 cis-12	0.32	0.24	0.24	0.13	0.14	0.11	0.04	0.01	0.37	0.46
C18:1 cis-13	0.12	0.09	0.07	0.22	0.21	0.19	0.02	0.01	0.06	0.93
C18:1 trans-9	0.15	0.13	0.43	0.22	0.05	0.22	0.08	0.25	0.02	0.20
C18:1 trans-10 & 11	2.85 ^c	2.56 ^c	5.20 ^d	5.99 ^d	7.12 ^e	7.49 ^e	0.47	0.01	0.01	0.07
C18:1 trans-12	0.21	0.09	0.08	0.19	0.15	0.18	0.04	0.18	0.12	0.31
C18:1 trans-15	1.35	1.26	1.11	0.89	0.79	0.71	0.05	0.01	0.01	0.75
C18:2	5.29 ^c	6.60 ^d	8.21 ^e	5.82 ^{cd}	3.71^{f}	5.18 ^c	0.46	0.01	0.01	0.01
CLA c9,t11	0.11	0.11	0.13	0.31	0.46	0.44	0.04	0.01	0.14	0.14
CLA t10,c12	0.01	0.00	0.01	0.01	0.07	0.07	0.01	0.01	0.11	0.11
C18:3n3	0.16	0.10	0.12	0.06	0.08	0.09	0.02	0.03	0.61	0.18
C20:0	0.04	0.02	0.02	0.05	0.05	0.06	0.02	0.11	0.97	0.82
C20:1	0.33	0.29	0.29	0.52	0.32	0.38	0.04	0.01	0.01	0.11
C20:2	0.04	0.06	0.09	0.08	0.13	0.09	0.05	0.40	0.81	0.74
C20:4	0.86 ^c	1.92 ^d	1.56 ^{de}	1.55 ^{de}	1.10 ^{ce}	1.40 ^{cd}	0.24	0.62	0.38	0.02

Table 6. Effect of WDG^a concentration in high concentrate or high forage diets on Longissimus dorsi fatty acid composition (g/100 g).

C20:5	0.11	0.13	0.14	0.21	0.10	0.14	0.04	0.48	0.53	0.26
C22:0	0.36 ^{cd}	0.49 ^e	0.47 ^{ce}	0.49 ^e	0.34 ^d	0.45 ^{cde}	0.05	0.74	0.67	0.05
C22:1	0.39	0.00	0.00	0.34	0.00	0.00	0.21	0.92	0.15	0.99
C22:5	0.29	0.33	0.37	0.44	0.27	0.31	0.07	0.90	0.66	0.25
C22:6	0.01	0.00	0.00	0.02	0.00	0.04	0.02	0.17	0.40	0.38
C23:0	0.01	0.00	0.00	0.03	0.00	0.03	0.01	0.05	0.12	0.38
C24:0	0.01	0.00	0.01	0.02	0.00	0.02	0.01	0.12	0.22	0.61
Total SFA	45.78	46.93	45.63	47.60	48.41	47.10	0.87	0.04	0.31	0.98
Total MUFA	47.31 ^c	43.83 ^d	43.74 ^d	43.87 ^d	45.66 ^{cd}	45.10 ^{cd}	1.09	0.93	0.54	0.04
Total PUFA	6.90 ^{cf}	9.25 ^{de}	10.62 ^e	8.50 ^e	5.92°	7.77 ^{ef}	0.63	0.01	0.03	0.01
PUFA:SFA	0.15 ^{cf}	0.20^{d}	0.23 ^e	0.18 ^{cd}	0.12^{f}	0.16 ^{cd}	0.01	0.01	0.02	0.01
MCFA (< 15:1)	4.60	4.81	4.74	3.95	4.09	3.71	0.25	0.01	0.66	0.72
LCFA (> 16:0)	95.40	95.19	95.26	96.05	95.91	96.29	0.25	0.01	0.66	0.72
Atherogenic index	0.70	0.69	0.67	0.58	0.69	0.62	0.03	0.04	0.22	0.13

^aWDG = wet distillers grains. ^bPercentage of WDG in the diet. ^{cdef}Means within the same row with different superscripts differ (P < 0.01; interaction).



^{ab}Bars with different superscripts differ (P < 0.01)

^cHC = high-concentrate; HF = high-forage; 0, 20, 40 = percentage of wet distillers grains in the diet



^aHC = high-concentrate; HF = high-forage; 0, 20, 40 = percentage of wet distillers grains in the diet



^{ab}Bars with different superscripts differ (P < 0.01)

^cHC = high-concentrate; HF = high-forage; 0, 20, 40 = percentage of wet distillers grains in the diet



^{abcd}Bars with different superscripts differ (P < 0.01)

^eHC = high-concentrate; HF = high-forage; 0, 20, 40 = percentage of wet distillers grains in the diet



^{abcd}Bars with different superscripts differ (P < 0.01)

 e HC = high-concentrate; HF = high-forage; 0, 20, 40 = percentage of wet distillers grains in the diet.