THE USE OF CO-PRODUCTS FROM BIOFUELS AS A FEED SOURCE FOR CATTLE AND INFLUENCE ON THE MEAT INDUSTRY

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CHAPTER I

INTRODUCTION

Since January 2001, the United States population has witnessed a steady increase in gasoline prices. Consumers have seen steep spikes followed by gentle declines. According to data obtained from the U.S. Energy Information Administration/Annual Energy Review (2007), unleaded regular gasoline prices have increased 52.1% and unleaded premium gasoline prices have increased 54.7% from 2001 to 2007.

One hypothesis for the unsteady gasoline market may be the volatile price of oil. Reports by Kneller and Young (2001) and Sadorsky (1999) have indicated that economic growth and corporate stock prices respond negatively to increased prices of petroleum products. Another hypothesis, as stated by Ashton and Upton (2004), is that increasing world demand for oil and changing inventory levels are major factors in the increased price volatility.

Because of these upward-trending oil prices, the United States has intensified efforts to promote renewable fuel production. The U.S. Congress and many states have adopted numerous policies that increase the use of domestic ethanol and biodiesel in the U.S. transportation sector. Environmental regulations such as the Clean Air Act Amendments of 1990 have been passed to promote and encourage the use of renewable fuels to address air quality issues.

Alternative fuels such as biodiesel and ethanol appear to be increasing in popularity and abundance. According to the National Biodiesel Board (2009), the current U.S. annual production of biodiesel is 10.1 billion liters per year. This is far short of the 34 billion liters of ethanol produced in 2008 and the 37 billion liters expected to be produced in 2009 (Renewable Fuels Association, 2009). The use of biodiesel in vehicles requires modification to the engine, while gasoline/ethanol blends containing up to 10% ethanol by volume may be used in any vehicle without requiring modifications (U.S. Department of Energy, 2005).

Biodiesel seems to be more of a niche fuel and is less manufactured and sold than ethanol. Biodiesel is a renewable fuel produced from agricultural resources such as vegetable oils. According to the U.S. Environmental Protection Agency (2006), most of the biodiesel produced in the United States is made from soybean oil. However, canola oil, sunflower oil, recycled cooking oils, and animal fats are also used. Biodiesel can be used in pure form or blended in any proportion with conventional diesel. Through the process of converting vegetable oils into biodiesel, four main products are produced: methyl ester (biodiesel), glycerin, feed quality fat, and methanol. The glycerin and fats can be sold by the processing facility as additional income.

Currently, there are 170 operating ethanol biorefinery plants in the U.S., poised to produce over 37 billion liters in 2009 (Renewable Fuels Association, 2009). Ethanol is not the only product created by the fermentation process. According to the National Corn Growers Association (NCGA, 2009), every 25.4-kg bushel of corn produces 10.6 liters of ethanol and either 7.9 kg of distillers dried grains (dry mill process), or 7.3 kg of gluten feed and meal (wet mill process). These co-products are a good source of energy and

protein in livestock and poultry rations. The Renewable Fuels Association (2009) reported that in 2008 nearly 23 million metric tons of DG, 2.7 million metric tons of corn gluten feed, and 544,310 metric tons of corn gluten meal were produced by ethanol biorefineries.

The Environmental Protection Agency was directed by Congress to design a program that requires the blending of renewable fuels into the motor-vehicle fuel supply. That program is called the Renewable Fuel Standard (RFS). The RFS program requires the use of renewable fuels every year through 2012 (EPA, 2007). According to the EPA (2007), for year 2007, a minimum of 4% of fuel dispensed to U.S. motorists was from renewable sources. In May 2009 the EPA proposed revisions to the RFS program. The revised requirements establish specific volume standards for cellulosic biofuel, bio-mass biofuel, advanced biofuel, and total renewable fuel that must be used in motor-vehicle fuel each year (EPA, 2009). The first modification was the volume standard was increased beginning in 2008 from 20.4 billion liters to 34 billion liters produced (EPA, 2009). Under the new revision, 57.5 billion liters must be used in motor-vehicles in 2012; this is a little over half that was originally required by the RFS program in 2007. The volume is required to increase every year eventually reaching 136.2 billion liters by 2022 (EPA, 2009).

According to the EPA (2009), the impacts of the revised RFS program are expected to reduce dependence on foreign sources of crude oil, increase domestic sources of energy, while providing important reductions in greenhouse gas emissions. The increased use of renewable fuels such as ethanol, biodiesel and other renewable fuels are also expected to have the added benefit of providing an expanded market for agricultural

products such as corn and soybeans and open markets for the development of cellulosic feedstock industries and conversion technologies (EPA, 2009).

Biodiesel and ethanol production are going to impact the U.S. livestock industries. The objectives of experiments presented herein were to: 1) Compile information on types, volume, and location of FOG (fats, oils, and grease) from food processors in Oklahoma. This information can then be used in an economic analysis to determine the cost of handling and delivery of FOG from food processors to collection points or refiners. 2) Determine the impacts of feeding various levels of wet and dry distillers grains to yearling steers on carcass characteristics, palatability, shelf life and fatty acid profiles of *longissimus* muscle.

CHAPTER II

REVIEW OF LITERATURE

Alternative fuels and by-products

Biodiesel

Biodiesel is made through a chemical process called transesterification. Transesterification involves removal of glycerin from the triglycerides and replaces it with an alcohol to form fatty acid esters (Canakci, 2007; Dunford, 2007a). Essentially two main products are produced: methyl ester (biodiesel) and glycerol. The methanol can ultimately be recycled back into the system, while glycerol can be sold for additional income.

Biodiesel is a mixture of a large number of hydrocarbons and fatty acid esters (Sarin et al., 2009). Biodiesel is usually produced from food-grade vegetable oils that tend to be more expensive than diesel fuel (Canakci, 2007). Therefore, producing biodiesel in this manner is not economically feasible. Waste cooking oils, restaurant grease and animal fats are fairly inexpensive and represent one-third of the U.S. total fats and oil production (Canakci, 2007). They are typically collected, rendered and used almost exclusively in animal feed. Therefore, utilizing these low-cost feedstocks will make it feasible for biodiesel to be commercially viable.

Recycled grease products are commonly referred to as waste grease. Greases are generally classified into two categories: yellow grease and brown grease (Canakci, 2007). Yellow grease is produced from vegetable oil or animal fat that has been heated and used for cooking, and is required to have a free fatty acid (FFA) level no more than 15% (Canakci, 2007). Brown grease, sometimes called trap grease, will have FFA levels exceeding 15%, it may be sold at a discounted price, or blended with low FFA materials to meet yellow grease specifications (Canakci, 2007). According to the National Renerderers Association (2008), over 1.1 billion kg of waste fats are collected annually from restaurants and fast food establishments in the U.S.

A concern with utilizing waste restaurant oils is during food frying, these oils are used at very high temperatures, therefore causing various chemical reactions such as hydrolysis, polymerization and oxidation, which change the physical and chemical properties of the oil (Canakci, 2007). Tyagi and Vasishtha (1996) found that the FFA level of fresh soybean oil changed from 0.04% to 1.15% after 70 h of frying at 190°C. Free fatty acids are soluble in biodiesel and can further compromise oxidative stability during storage (Dunford, 2007a). It is necessary to remove or reduce the FFA levels of oils by either a chemical neutralization, which involves treatment with sodium hydroxide (NaOH) or potassium hydroxide (KOH), or physical deacidification, performed under a vacuum and requiring steam (Canakci and Van Gerpen, 1999; Dunford, 2007b). Canakci (2007) also found that viscosity increased. The greater the viscosity, the less willingly the oil flows.

In summary, the greatest challenge to biodiesel use is the cost compared to conventional diesel. However, there is a tremendous amount of restaurant waste oils

available for biodiesel production. Utilizing these oils to produce biodiesel will reduce the cost and stimulate the biodiesel market.

Ethanol

Recent elevated prices in oil and gasoline have driven the rapid expansion of the alternative fuels industry. Corn-based ethanol production has sky-rocketed. With approximately 170 biorefiners currently operating, ethanol production in 2009 is poised to reach well over 37 billion liters (Renewable Fuels Association, 2009). At present, it takes one bushel of corn, roughly 25.4 kg, to produce 10.5 liters of ethanol (National Corn Growers Association, 2009). Through newly developed technologies, corn producers are able to produce substantially more corn per acre of land, thus helping to meet the growing demand for food and fuel. In 1998, the average corn production per acre was 134.4 bushels; ten years later, in 2008, farmers produced an average of 153.9 bushels per acre (National Corn Growers Association, 2009). Research done by Schilcher (2008) reports that the U.S. corn market in 2015 expected to support 56.7 billion liters of ethanol; leaving 12.3 billion bushels of corn available for feed, food and export markets. With the incorporation of biotechnology-derived seeds to computer-equipped combines, corn-based ethanol is well in position to remain the lowest-cost ethanol production per gallon (Schlicher, 2008).

Corn dry-milling process

Greater than 80% of existing ethanol plants in the U.S. use a dry-grind process (Tao and Aden, 2009). According to Schlicher (2008), dry-grind ethanol plants in 2006 produced 18.5 billion liters of ethanol, approximately 72% of the total U.S. ethanol production. Dry-grind processes are less capital and energy intensive than wet-milling;

however, they only produce ethanol and distillers dried grains (DDG) (Tao and Aden, 2009).

The actual process (Appendix A) begins with corn being hammer milled and mixed with water and amylase enzymes to form a slurry (Davis, 2001). The mixture is then cooked and mixed with additional enzymes and yeast so the dextrose can metabolically turn into ethanol and carbon dioxide (Davis, 2001). The ethanol is concentrated and purified through a series of distillation and dehydration steps, while the by-product solids are dried through a series of drying steps (Tao and Aden, 2009). As a result, protein increases from 10 to 30%, fat from 4 to 12%, fiber from 12 to 36%, and phosphorus (P) from 0.3 to 0.9% of dry matter (DM) compared to corn (Klopfenstein et al., 2008). Before the solids are dried they can be sold as wet distillers grains (WDG) for livestock feed.

Corn wet-milling process

Wet-milling has developed into an industry that seeks optimum use and maximum value from each corn kernel (Davis, 2001). Wet-milling facilities are structured to produce a number of products, including starch, high fructose corn syrup, ethanol, corn gluten feed, and corn gluten meal (Tao and Aden, 2009). The ethanol yield is slightly lower at 9.5 liters per bushel (Tao and Aden, 2009).

Wet-milling (Appendix B) involves the corn kernel first being soaked in a mixture of water and sulfur dioxide through a process known as “steeping” to allow separation of the kernel components (Davis, 2001). Germ, fiber, gluten and starch are separated from one another through a series of screens, cyclones and presses (Tao and Aden, 2009).

Enzymes are added to the starch for hydrolysis to sugars, and the sugars can be fermented to ethanol (Tao and Aden, 2009).

Distillers grains

A co-product of the ethanol dry-milling process is distillers grains (DG), a high protein, high energy livestock feed (National Corn Growers Association, 2009). According to Tjardes and Wright (2002), corn distillers co-products offer the cattle industry a tremendous opportunity to reduce feed costs without sacrificing performance. A challenge with DG is that nutrient concentrations may be highly variable. Differences may be attributed to the corn, types of yeasts, fermentation efficiencies, drying processes and amount of solubles blended back into the co-product (Tjardes and Wright, 2002). Ham et al. (1994) reported the feeding values are different between WDG and DDG. Tjardes and Wright (2002) reported the total digestible nutrients (TDN) for WDG ranged from 70-110% while DDG was 77-88% expressed on a dry matter basis. Distillers grains tend to be low in calcium (Ca) levels but high in P and sulfur (S) amounts (Tjardes and Wright, 2002). Supplemental Ca may need to be provided to correct the P:S ratio, which should be approximately 2:1. According to Tjardes and Wright (2002), high levels of S, above 0.4%, in feed can lead to polioencephalomalacia (PEM). In a study conducted by Buckner et al. (2008), steers fed 50% DDGS showed signs of PEM and were removed from the experiment; the total S level was 0.6%. On the other hand, these co-products have significant concentrations of vitamins, such as A, D, E and B complex (Roeber et al., 2005). Vitamin E has shown to aid in prolonging shelf life of retail cuts.

Energy is the most important item in an animal's diet, and grains are a good source of energy due to their starch content (Church, 1991). Shand et al. (1998) reported

cattle fed a high energy diet tended to produce more palatable and flavorful beef than low energy diets. The higher inclusion levels of DG (> 15% of the diet dry matter) are primarily fed as an energy source (Erickson and Klopfenstein, 2002). The drying process of DG appears to reduce the energy value; therefore, feeding WDG at higher levels (30-40%) has shown to increase average daily gain (Vander Pol et al., 2006). Tjardes and Wright (2002) found the net energy for maintenance (NEm) of WDG was 1.98-2.42 Mcal/kg and DDG was 1.96-2.20 Mcal/kg. Additionally, the net energy for gain (NEg) for WDG was 1.54-1.76 Mcal/kg while DDG 1.47-1.54 Mcal/kg (Tjardes and Wright, 2002).

Storage is a major concern when using DG. Wet distillers grains contain up to 70% moisture, this product may freeze during winter months (Tjardes and Wright, 2002). Additionally, in warmer months, the WDG may mold and become useless for feed rations. Tjardes and Wright (2002) claim that DDG is easier to store since it contains only 10-12% moisture, the small particle size requires DDG to be stored in commodity bins or bulk feed tanks.

In summary, DG offer cattle producers an opportunity to potentially decrease production cost while maintaining performance levels. The high energy and moderate protein levels allow DG to become effectively incorporated into many feed rations. Nevertheless, careful consideration of nutritional properties, storage and economics need to be accessed.

Distillers Grains in Finishing Rations and the Impact on Meat Quality

Distillers by-products are an excellent feed source for cattle. Distiller by-products are available in 2 forms: WDG and DDG. The cost of DDG can become expensive for the ethanol plant (i.e. drying the DG) and in turn increases the price of the commodity. Therefore, WDG can be very advantageous for the cattle producer, as well as the ethanol plant.

Research has shown both positive and negative impacts of feeding DG in feedlot rations. A study completed by Daubert et al. (2005) fed heifers 0, 8, 16, 24, 32, or 40% sorghum wet distillers grains plus solubles (SWDGS) in diets based on steam flaked corn (SFC). Daubert et al. (2005) found heifers fed for 58 days improved feed efficiency by 9% when consuming 16% SWDGS. In contrast, Cole at al. (2006) fed SWDGS at levels of 0, 5, 10, or 15% and found a linear decrease in both gain and efficiency with increasing SWDGS concentration. The same study by Cole et al. (2006) compared 10% SWDGS and 10% corn WDGS and found no difference in performance. Buckner et al. (2008) found cattle fed 30% WDGS had higher average daily gain (ADG) than the control diet which consisted of no distillers grains. Additionally, Benson et al. (2005) found that ADG tended to be greater for cattle consuming 25% DDG compared to the control diet. A study conducted by Al-Suwaiegh et al. (2002) documented that steers fed either corn WDG or SWDG, fed at 30% inclusion level, had increased efficiency of gain compared with those steers fed dry-rolled corn.

Economic analysis as described by Buckner et al. (2008) found that regardless of corn prices, cattle fed any level of dried distillers grains plus solubles (DDGS) from 10% to 40% resulted in greater marginal returns per steer compared with feeding

predominately dry rolled corn (DRC). Buckner et al. (2008) concluded that the optimum level of DDGS inclusion for performance is 23% to 24% of diet.

Carcass Characteristics

A study by Corrigan et al. (2007) documented that optimum hot carcass weight (HCW) can be achieved at 40% WDGS in DRC based diets, 27.5% WDGS in high moisture corn (HMC) based diets, and 15% WDGS in SFC based diets. Al- Suwaiegh et al. (2002) found HCW was heavier for steers fed corn WDG or SWDG than steers fed the control diet which consisted of DRC. Buckner et al. (2008) found a quadratic response in HCW as levels of dry distillers grains plus solubles (DDGS) increased. However, Depenbusch et al. (2008) observed a 3.5% reduction in HCW when 25% WDGS was fed in SFC diets. Conversely, in studies by Larson et al. (1993), Lodge et al. (1997), and Koger (2004), HCW was not affected by the inclusion of DG in feed rations.

Ham et al. (1994) reported feeding 40% WDG or 40% DDGS did not impact fat thickness, yield grade, or quality grade. Benson et al. (2005) fed steers either cracked corn, 15% DDGS, 25% DDGS or 35% DDGS, and found that backfat significantly increased linearly as level of DDGS in the diet increased, while yield grade and HCW tended to increase. Additionally, Koger (2004) reported that feeding 20 or 40% DDGS resulted in carcasses with greater fat thickness and higher yield grades.

Degree of marbling is one of two factors used in determining quality grade in cattle. Vander Pol et al. (2004), along with Koger (2004), reported that marbling score was not affected by DG levels when fed at 20 and 40% of the diet. Corrigan et al. (2007) indicated cattle fed 40% WDGS had the lowest numeric marbling score.

These studies indicate that DG's are a suitable feed ingredient for finishing steers based on carcass characteristics. However, a few studies documented an increase in fat thickness as the level of DG increased; therefore, careful attention should be paid to days on feed and terminal endpoints. Including DG in rations at higher levels will allow for greater use of DG from increased ethanol production.

Lean Muscle Color

Muscle color, as perceived in retail display conditions, is one of the most important selection criteria for many consumers. Liu et al. (1995) along with Mancini and Hunt (2005) stated color is a primary factor influencing meat purchasing decisions because consumers use discoloration as an indicator of beef quality, especially freshness and wholesomeness. Smith et al. (2000) revealed nearly 15% of retail beef is discounted in price due to surface discoloration which relate to annual losses of $1 billion.

According to O'Sullivan et al. (2002 and 2003) and Gray et al. (1994), feeding regime can affect meat color, quality, flavor and lipid oxidation. A visual appearance score of 3 (moderately undesirable) is when a steak is assumed to be discounted in retail display. Roeber et al. (2005) found steaks from Holstein steers fed 25% WDG had a lower percentage of steaks receiving appearance score of 3 at 138 h of simulated retail display than other dietary treatments; SBM, 12.5% DDG, 25% DDG, 50% DDG, or 50% WDG. The steaks from steers fed 25% WDG also had significantly greater a^* values, indicating a more red steak than other dietary treatments except for 12.5% DDG (Roeber et al., 2005). A second experiment by Roeber et al. (2005) found a greater percentage of steaks from Holstein steers fed 40% DDG and 40% WDG having an appearance score of 3 than steaks from the control diets supplemented with either SBM or urea, 10% DDG,

10% WDG, and 20% WDG. Koger (2004) found no differences on subjective color evaluation scores of ground beef patties from steers fed 20% DDG or 40% DDG. Mancini and Hunt (2005) attributed feeding effects on color to the relationship between lipid and pigment oxidation, particularly the instability of polyunsaturated fatty acids (PUFA).

Fatty Acid Composition

Fatty acids contribute notably to various aspects of meat quality and are vital to the nutritional value of meat (Wood et al., 2008). In addition to flavor, fatty acid composition is also of importance to human health. Foods provide a diversity of saturated (no double bonds), monounsaturated (one double bond) and unsaturated (two or more double bonds) fatty acids in the diet. Beef has been criticized for a greater concentration of saturated fatty acids (SFA) compared with PUFA; thus, an unhealthy choice for today's society (Wood et al., 1999). Conversely, increasing the PUFA level of beef is a challenge due to the hydrogenation by rumen microbes, which are sensitive to unsaturated fatty acids (Jenkins, 1993). Linoleic acid is found at high levels in concentrate feeds such as grains and oilseeds (Wood et al., 2008). Porsgaard and Hoy (2000) found corn oil was highly abundant in linoleic acid. The NRC (1996) reports that DDGS contains approximately 3 times the oil content of corn. Harfoot, (1981), found that DDGS has a significant amount of linoleic acid (18:2), approximately 30% to 60% of total fatty acid. A study by Lancaster et al. (2007) found that inclusion of DDGS at 15% in the diet increased linoleic acid 40% when compared to the control diet. Additionally, Gill et al. (2005) reported an increase in linoleic acid concentration in steaks from steers fed diets containing DG than those steaks from steers fed SFC diet. Furthermore, Gill et

al. (2005) found that steaks from steers fed WDG had lower proportions of linoleic acid compared with steers fed DDG.

Hegsted et al. (1965) documented saturated fats, specifically lauric (12:0), myristic acid (14:0), and palmitic (16:0) are the primary SFA responsible for increasing plasma low-density lipoprotein (LDL) and total cholesterol levels. Stearic acid (18:0) is unique in that it appears to be neutral in regards to cholesterol levels (Grundy, 1994; Kris-Etherton and Yu, 1997). A study by Gill et al. (2008) found greater proportions of margaric (17:0) and stearic (18:0) acid in steaks from cattle fed DG when compared to cattle fed SFC. However, increased concentrations of 17:0 and 18:0 are not a major concern since they do not aid in increasing human plasma cholesterol levels (Baghurst, 2004).

In summary, diet plays a crucial role on the fatty acid composition of beef. As previously mentioned, altering fatty acids levels especially PUFA, cause a negative effect on shelf life of steaks. Also higher proportions of PUFA increase the likelihood of off-flavors developing.

Lipid Oxidation

Oxidative rancidity is a result of several chemical reactions involving atmospheric oxygen and lipids. Oxidation of lipids is one of the culprits of quality deterioration in meat (Gray et al., 1996). According to St. Angelo (1996), lipid oxidation occurs in a series of reactions in which a free radical is formed when a hydrogen atom is removed from a fatty acid. St. Angelo (1996) also found that lipid oxidation can be initiated by light, temperature, enzymes, metals, metalloproteins, and microorganisms. Lipid substrates play a role not only in meat color but also in the formation of off-flavors

The most common lipid substrates for oxidative rancidity in foods are fatty acids (Schmidt, 2000). Polyunsaturated fatty acids (PUFA) are more likely to be involved in lipid oxidation (Johns et al., 1989).

Malonaldehyde has been identified as the product of lipid oxidation (Beuge and Aust, 1978). A procedure developed by Beuge and Aust (1978) uses thiobarbituric acid to react with malonaldehyde and finally absorbance can be read on a spectrophotometer at 531 nm to determine levels of lipid oxidation. Campo et al. (2006) identified a value of 2.3 mg malonaldehyde/kg as the point where rancid flavors overpower beef flavor. Gill et al. (2005) found thiobarbituric acid reactive substance (TBAR) concentrations in steaks from steers fed DDG had greater amounts of oxidation than those from steers fed WDG. Koger (2004) observed a significant amount of lipid oxidation in ground beef patties from steers fed 40% WDG or DDG than the control diet. Gill et al. (2005) also found proportions of PUFA in steaks from DDG were higher than those of steaks from WDG treatments. Thus, by altering the fatty acid profiles and increasing PUFA proportions, the quality of meat seems to decline.

Sensory Characteristics

On-farm nutrition management has the potential to influence meat quality characteristics. The most important environmental factor influencing meat flavor is feed source (Shahidi and Rubin, 1986). Feed consumed by cattle (forage or concentrate) can modify meat quality and consumer acceptance through the quantity of feed energy available to the animal and the nutrient composition of the feed (Muir et al., 1998).

Taste and eating satisfaction is important to consumers. In the U.S., many consumers have acquired a taste for grain-fed beef; thus, beef having a grassy flavor can

be considered a quality defect (Young and Baumeister, 1999). The flavor of red meat develops during cooking through degradation and reactions of water-soluble compounds (Melton, 1990; Shahidi and Rubin, 1986). A major component of flavor is caused by aromatics as volatile substances are released, such as those in the fatty tissues, as food is eaten (Meilgaard et al., 1991).

Jenschke et al. (2007) indicated cooked beef cuts with greater degrees of lipid oxidation typically express a livery-like off-flavor. As stated previously, PUFA are more susceptible to oxidation, and therefore, increasing PUFA proportions may increase the incidence of a liver off-flavor. Roeber et al. (2005) found feeding DDGS and WDGS had no significant impact on palatability of the meat based on a consumer taste panel. However, Jenschke et al., (2007) conducted a trained taste panel and found a tendency for liver-like off-flavor to emerge, reporting the off-flavor occurred most frequently in steers fed 0% and 10% WDGS, while animals fed 30% and 50% WDGS had the lowest incidence.

Tenderness

Tenderness is another sensory factor that is considered an important trait of meat quality. According to Boleman et al. (1995) and Miller et al. (2001), consumers would be willing to pay higher prices for beef as long as it is guaranteed tender. Unfortunately, tenderness is a highly variable characteristic. This wide variability may be a reason for consumer dissatisfaction and reduction in beef (Destefanis et al., 2008). Therefore, tenderness inconsistency is a priority issue for the meat industry (Koohmaraie, 1996).

Tenderness can be evaluated by objective methods such as instrumental or with trained sensory panels, or by subjective methods such as with a consumer taste panel

(AMSA, 1995). Sensory panels can be expensive, time consuming and difficult to organize. Therefore, instrumental methods for assessing the force in shearing, penetrating, biting, mincing, compressing, and stretching the meat have been developed (Lawrie and Ledward, 2006). The most widely used instrumental method is the single blade shear test of the Warner-Bratzler shear force (WBSF) type (Culioli, 1995).

As discussed in previous sections, diet can play a critical role in altering meat quality. In a study by Roeber et al. (2005), WBSF values were not different among dietary treatments: corn-corn silage diet with soybean meal (SBM) or diets formulated with 12.5% DDG, 25% DDG, 25% WDG, 50% DDG, or 50% WDG. Koger (2004) also found no differences in WBSF values when evaluating inclusions levels of 20% WDG, 40% WDG, 20% DDG, or 40% WDG in finishing rations. Gill et al. (2008) found no differences in WBSF values among dietary treatments, which included: SFC, corn dry or wet DG, and sorghum dry or wet DG and alfalfa hay. Brandt et al. (1992) also found no differences in WBSF values of steaks from steers fed SFC or steam-flaked sorghum.

In conclusion, as the upward demand for alternative fuels, such as ethanol, increases, the abundance of DG will continue to grow. As research shows, DG can be incorporated into feeding programs without significant detrimental affects to final product quality. Higher inclusion levels of DG increase PUFA levels in steaks, which help to promote a healthier product. On the other hand, PUFA are more susceptible to oxidation, which shorten shelf life. Therefore, further research evaluating the impact of increasing levels of DG on carcass characteristics, meat quality, retail case life and fatty acid composition is needed.

CHAPTER III

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, abundance of distillers grains (DG) is on the rise. The objectives 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. Steers (n = 176) were assigned to one of five treatment groups: steam flaked corn (SFC), 10% DDG, 10% WDG, 20% WDG or 30% WDG. Steaks, 2.54 cm, 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% and 30% WDG had a higher proportion of polyunsaturated and n-6 fatty acids than 10% WDG. Data suggest that feeding WDG at higher levels, 20% or 30%, does not affect sensory attributes, however, retail display of strip loin steaks from those treatment groups had a shorter shelf life. 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

Increased ethanol production has led to an abundance of a by-product, distillers grain, which has given cattle feeders the option to consider DG as a feed source. Traditionally, distillers grains 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.

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.

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 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 color affects 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 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

Cattle

One hundred seventy-six yearling steers (avg. initial body weight = 317-362 kg) 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: 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 12th and 13th 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 (IMPS 180) were collected, vacuum packaged, and placed in ice chests for transit back to OSU Robert M. Kerr Food and Agricultural Products Center. Strip loins were aged 14 d postmortem at 2°C.

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 (-20°C) for subsequent fatty acid profiling and pre-display thiobarbituric acid reactive substance (TBAR) analysis. A 2.54 cm 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 -20°C 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 2°C ± 1°C, 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 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 -20°C for analysis. Lipid peroxidation was determined by the modified method of Buege and Aust (1978) (See appendix C). 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, frozen 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 D). Identification of the fatty acids were made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of an external standard ran simultaneously. Methyl ester peaks from samples were calculated as percentages of called fatty acids.

Objective Tenderness Determination

One 2.54 cm steak was cut from each strip loin for Warner Bratzler shear force (WBSF) determination. Steaks were allowed to temper at 4°C for 24 h. Steaks were cooked on an impingement oven (model 1132-000-A; Lincoln Impinger, Fort Wayne, IN)

at 180°C to an internal temperature of 70°C. 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 were allowed to cool for 24 h before conducting shear force analysis. Six cores, 1.27 cm, were 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 (kg) 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 2.54 x 2.54 cm 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 served to 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 controlled room with red lights. 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. A pivot table was then used to determine at which hour 75% of steaks were deemed moderately unacceptable (48 h). 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 3.1. No differences in treatment ($P > 0.05$) were found in adjusted fat thickness, and USDA yield grade. Carcasses from steers fed DDG had a higher ($P < 0.05$) marbling score ($Sm^{16} \pm 11.8$) than carcasses from steers fed WDG ($Sl^{86} \pm 6.7$). Koger (2004) found that cattle fed DDG 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%.

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 3.2 and Table 3.3. 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 3.3). Steaks from cattle fed 10% and 20% WDG had higher ($P < 0.05$) b^* values, which indicates more yellowness, than 30% WDG (Figure 3.1). On the other hand, L^* and a^* values were not significantly different (Table 3.2). Previous research indicated that steaks from control cattle fed SFC had lower

L^*, greater a^*, and 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 3.4). 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 3.5). 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 had lower ($P < 0.05$) Warner-Bratzler shear force values than 20% WDG (3.81 ± 0.3 kg vs. 4.31 ± 0.3 kg, respectively) (Figure 3.2). 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

No differences were found in total saturated fatty acids (SFA), or total monounsaturated fatty acids (MUFA), however, differences in individual fatty acids were detected (Table 3.6 and 3.7). Cattle fed SFC had higher ($P < 0.05$) levels of margaric (17:0) than cattle fed DG (Table 3.6). 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 (Figure 3.3). Conversely, Koger (2004) reported higher levels of PUFA's in the LM from cattle fed 40% distillers grain as compared to cattle fed 20% distillers grains. Higher ($P < 0.05$) levels of n-6 fatty acids can be found in 20% and 30% WDG steaks compared to 10% WDG (Figure 3.4). Linoleic acid (18:2) tended to be higher ($P = 0.09$) in 20% and 30% WDG (Table 3.8), 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 replacing a percentage of steam flaked corn with distillers grain in feed rations without causing detrimental effects 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 20% WDG. Beef from cattle fed 20% or 30% WDG will tend to have higher proportions of polyunsaturated fatty acids and therefore may be more susceptible to oxidation resulting in a shortened shelf life. However, color data does not show a significant difference among treatments in overall acceptability of steaks during retail display. 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 3.1. Least squares means ± SEM and main contrasts for carcass data (n = 176).

Treatments[1]	Adj. fat thickness, cm	Ribeye area, sq. cm	Marbling Score[2]	USDA Yield Grade
SFC	1.35 ± 0.76	93.03 ± 2.19	382.94 ± 11.63	3.1 ± 0.17
10% DDG	1.27 ± 0.76	90.64 ± 2.19	416.76 ± 11.63	3.1 ± 0.17
10% WDG	1.30 ± 0.74	94.32 ± 2.13	400.00 ± 11.30	2.9 ± 0.16
20% WDG	1.37 ± 0.74	87.68 ± 2.13	381.11 ± 11.30	3.3 ± 0.16
30% WDG	1.35 ± 0.76	88.45 ± 2.19	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}, 350 = slight^{50}, 400 = small^{00}, 500 = modest^{00}, 600 = moderate^{00}

Table 3.2. Least squares means ± SEM and main contrasts for instrumental color analysis of strip loin steaks under retail display (n = 174).

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 3.3. Least squares means ± SEM and main contrasts for visual color evaluation of strip loin steaks for muscle color, surface discoloration and overall appearance at 48 h (n = 174).

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	
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 3.4. 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 (n = 174).

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 3.5. Least square means ± SEM, and main contrasts for Warner-Bratzler Shear (WBSF) force and sensory characteristics of strip loin steaks (n = 174).

Treatments[1]	WBSF (kg)	Tenderness Overall[2]	Livery Flavor[3]
SFC	4.01 ± 0.13	5.55 ± 0.08	1.12 ± 0.02
10% DDG	4.08 ± 0.14	5.53 ± 0.08	1.09 ± 0.02
10% WDG	4.13 ± 0.13	5.69 ± 0.08	1.09 ± 0.02
20% WDG	4.33 ± 0.13	5.74 ± 0.08	1.12 ± 0.02
30% WDG	3.83 ± 0.13	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 3.6. Least square means ± SEM and main contrasts for total saturated fatty acid concentrations and individual fatty acids found in *longissimus* muscle (n = 81).

	Treatment[1]					Main Contrasts (*P* values)			
Fatty Acids	SFC	10DDG	10WDG	20WDG	30WDG	Dry vs. Wet	10D vs. 10W	% WDG	SFC vs. DG
Total SFA[2]	48.46 ± 8.65	48.81 ± 8.07	48.02 ± 9.30	50.60 ± 12.50	48.19 ± 9.55	0.96	0.82	0.64	0.87
10:0, capric	0.05 ± 0.005	0.04 ± 0.005	0.04 ± 0.005	0.03 ± 0.005	0.03 ± 0.005	0.73	0.35	0.51	0.05
13:0, tridecanoic	0.03 ± 0.002	0.03 ± 0.002	0.02 ± 0.002	0.03 ± 0.003	0.02 ± 0.003	0.21	0.29	0.61	0.53
12:0, lauric	0.14 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.55	0.44	0.61	0.03
14:0, myristic	3.14^{a} ± 0.14	3.16^{a} ± 0.14	2.94^{ab} ± 0.14	2.68^{b} ± 0.14	2.58^{b} ± 0.14	0.009	0.27	0.06	0.05
15:0, pentadecanoic	0.68^{a} ± 0.03	0.63^{a} ± 0.03	0.61^{ab} ± 0.03	0.55^{b} ± 0.03	0.56^{b} ± 0.03	0.03	0.43	0.07	0.002
16:0, palmitic	25.44 ± 0.37	25.38 ± 0.37	25.24 ± 0.37	24.48 ± 0.37	24.37 ± 0.37	0.12	0.80	0.08	0.17
17:0, margaric	1.82^{a} ± 0.06	1.69^{ab} ± 0.06	1.69^{ab} ± 0.06	1.58^{b} ± 0.06	1.57^{b} ± 0.06	0.26	0.95	0.14	0.009
18:0, stearic	13.19^{b} ± 0.33	12.87^{b} ± 0.33	12.99^{b} ± 0.33	13.71^{ab} ± 0.33	14.37^{a} ± 0.33	0.03	0.78	0.01	0.42
22:0, behenic	0.38 ± 0.05	0.29 ± 0.05	0.30 ± 0.05	0.24 ± 0.05	0.31 ± 0.05	0.90	0.89	0.54	0.09
23:0, triosanoic	0.07 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.24	0.10	0.49	0.52
24:0, lignoceric	0.22 ± 0.22	0.52 ± 0.26	0.34 ± 0.29	1.07 ± 0.34	0.79 ± 0.26	0.46	0.61	0.49	0.11

[a,b] 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

Table 3.7. Least square means ± SEM and main contrasts for total monounsaturated fatty acid concentrations and individual fatty acids found in *longissimus* muscle (n = 81).

	Treatment[1]					Main Contrasts (*P* values)			
Fatty Acids	SFC	10DDG	10WDG	20WDG	30WDG	Dry vs. Wet	10D vs. 10W	% DG	SFC vs. DG
Total MUFA[2]	45.06 ± 2.99	46.15 ± 2.17	46.59 ± 2.25	45.53 ± 2.99	45.40 ± 3.04	0.69	0.65	0.18	0.26
14:1, myristoleic	0.63^{abc} ± 0.05	0.72^{a} ± 0.05	0.65^{ab} ± 0.05	0.55^{bc} ± 0.05	0.49^{c} ± 0.05	0.01	0.32	0.05	0.66
16:1, palmitoleic	3.06^{ab} ± 0.13	3.15^{a} ± 0.13	3.10^{ab} ± 0.13	2.75^{bc} ± 0.13	2.65^{c} ± 0.13	0.04	0.78	0.02	0.32
17:1, heptadecenoic	1.32^{a} ± 0.05	1.21^{a} ± 0.05	1.24^{a} ± 0.05	1.07^{b} ± 0.05	1.07^{b} ± 0.05	0.16	0.70	0.009	0.004
18:1 9c, oleic	32.98 ± 0.71	33.85 ± 0.71	34.79 ± 0.71	34.01 ± 0.71	34.47 ± 0.71	0.48	0.35	0.53	0.09
18:1 11c, *cis*-vaccenic	1.87 ± 0.13	1.94 ± 0.13	1.92 ± 0.13	1.88 ± 0.13	1.97 ± 0.13	0.91	0.90	0.95	0.71
20:1, gadoleic	0.07 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.59	0.26	0.23	0.36

[a,b] 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] MUFA = calculated sum of fatty acids presented in this study that contain one double bond

Table 3.8. Least squares means ± SEM and main contrasts for total polyunsaturated fatty acid concentrations and individual fatty acids found in *longissimus* muscle (n = 81).

	Treatment[1]					Main Contrasts (*P* values)			
Fatty Acids	SFC	10DDG	10WDG	20WDG	30WDG	Dry vs. Wet	10 D vs. 10 W	% DG	SFC vs. DG
Total PUFA[2]	9.73 ± 2.93	9.15 ± 1.86	8.94 ± 1.98	11.53 ± 3.74	9.99 ± 3.19	0.23	0.83	0.04	0.83
Total CLA	0.56 ± 0.25	0.48 ± 0.15	0.51 ± 0.15	0.54 ± 0.18	0.57 ± 0.26	0.30	0.69	0.44	0.51
Total omega-3	1.67 ± 0.76	1.46 ± 0.45	1.57 ± 0.43	1.77 ± 0.68	1.68 ± 0.67	0.24	0.63	0.41	0.75
Total omega-6	7.97 ± 2.35	7.57 ± 1.61	7.33 ± 1.57	9.65 ± 3.54	8.23 ± 2.57	0.24	0.78	0.03	0.75
18:2, linoleic	6.36 ± 0.43	6.04 ± 0.43	5.99 ± 0.43	7.10 ± 0.43	6.69 ± 0.43	0.27	0.93	0.09	0.85
18:2tt, linoelaidic	0.23 ± 0.07	0.45 ± 0.07	0.26 ± 0.07	0.28 ± 0.07	0.29 ± 0.07	0.26	0.05	0.23	0.25
18:3, α-linoleic	0.42 ± 0.03	0.38 ± 0.03	0.38 ± 0.03	0.37 ± 0.03	0.42 ± 0.03	0.85	0.99	0.81	0.38
20:2, eicosadienoic	0.11 ± 0.39	0.11 ± 0.39	0.11 ± 0.39	0.99 ± 0.39	0.13 ± 0.39	0.85	0.55	0.38	0.61
20:4, arachidonic	0.62 ± 0.09	0.47 ± 0.09	0.38 ± 0.09	0.66 ± 0.09	0.47 ± 0.09	0.75	0.47	0.09	0.22
20:5, eiscosapentaenoic	1.22 ± 0.14	1.02 ± 0.14	1.14 ± 0.14	1.35 ± 0.14	1.22 ± 0.14	0.19	0.55	0.42	0.83
22:6, docosahexaenoic	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.35	0.33	0.70	0.53
CLA 9,11	0.08 ± 0.03	0.11 ± 0.03	0.04 ± 0.03	0.10 ± 0.03	0.08 ± 0.03	0.41	0.15	0.18	0.59
CLA 10,12	0.48 ± 0.06	0.36 ± 0.06	0.46 ± 0.06	0.43 ± 0.06	0.49 ± 0.06	0.12	0.24	0.87	0.63

[a,b] 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] PUFA = calculated sum of fatty acids presented in this study that contain two or more double bonds

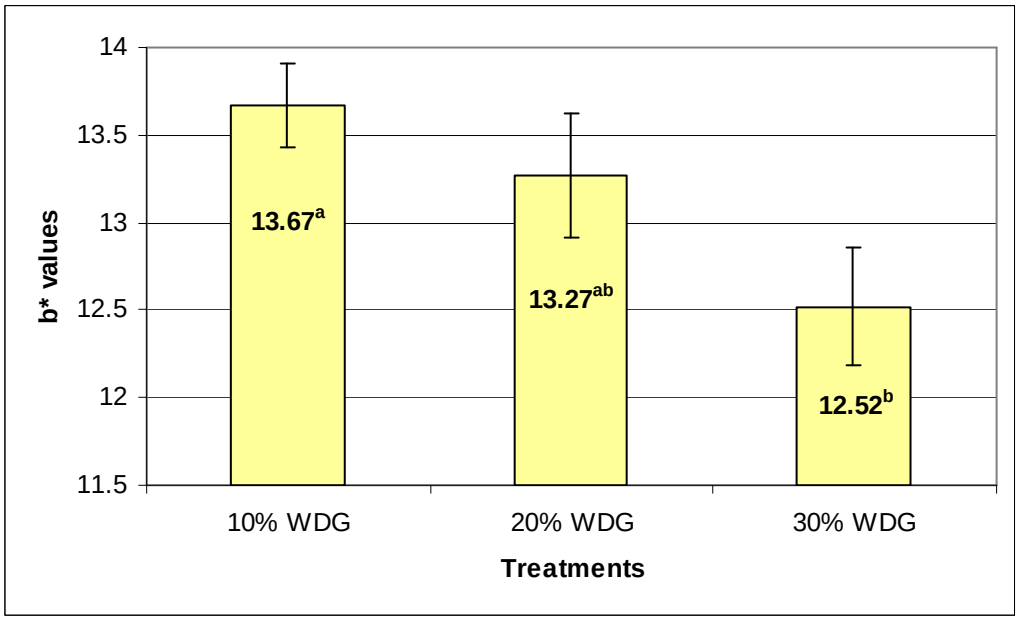

Figure 3.1. Varying percentage of WDG effects on b* value. Least squares means with the same letter are not different ($P > 0.05$).

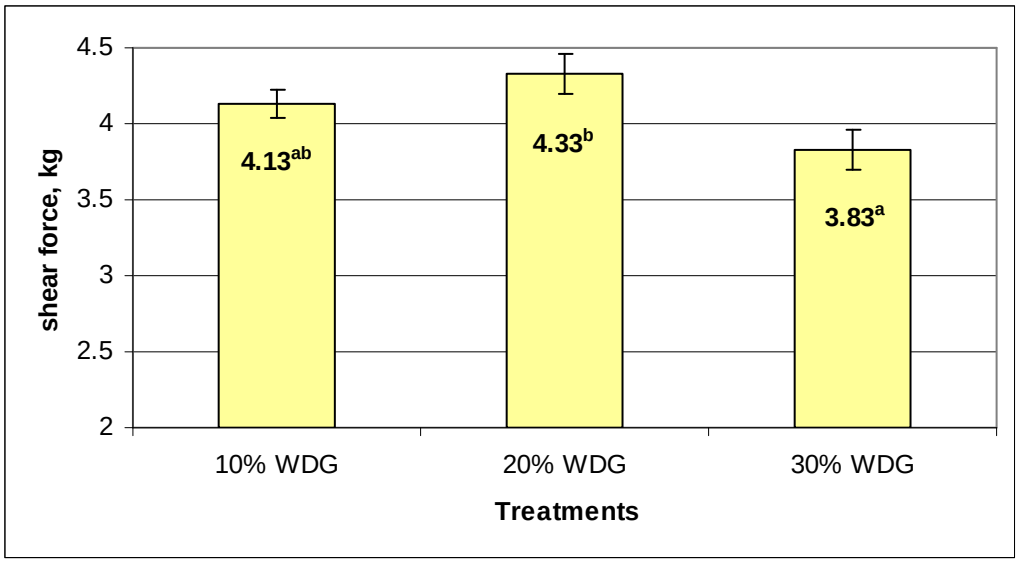

Figure 3.2. Warner-Bratzler shear force analysis of strip loin steaks from varying percentages of WDG. Least squares means with the same letter are not different ($P > 0.05$).

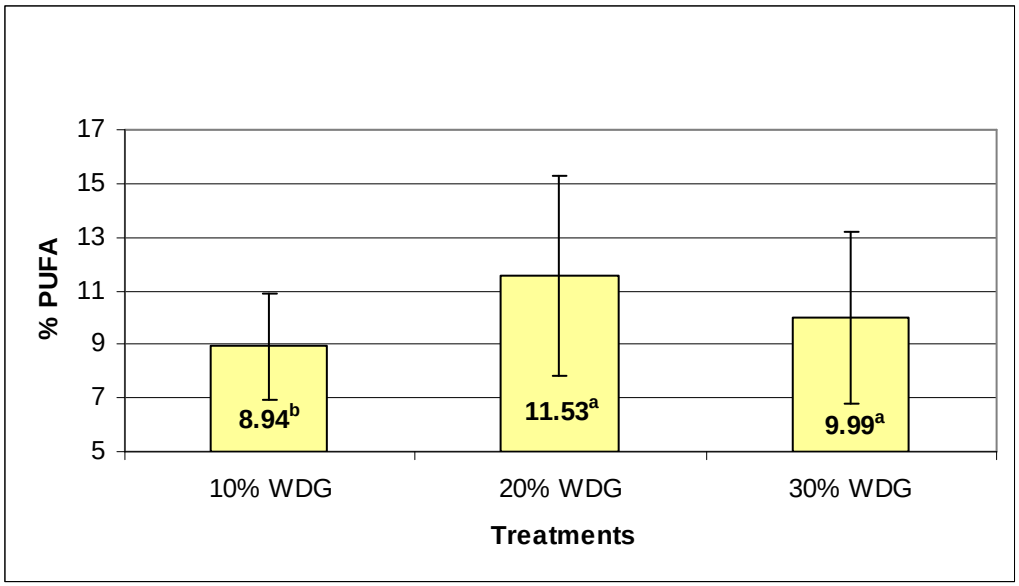

Figure 3.3. Total polyunsaturated fatty acid composition of strip loin steaks from varying percentages of WDG. Least squares means with the same letter are not different ($P > 0.05$).

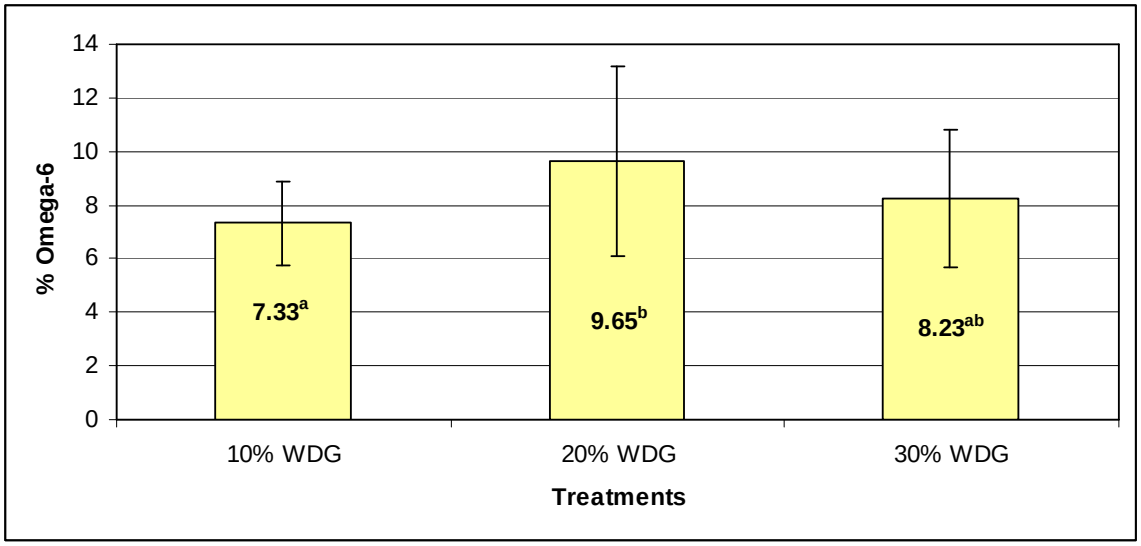

Figure 3.4. Omega-6 fatty acid composition of strip loin steaks from varying percentages of WDG. Least squares means with the same letter are not different ($P > 0.05$).

CHAPTER IV

PROS AND CONS TO A FOG (FATS, OILS, AND GREASE) BIODIESEL PLANT

Executive Summary

The idea of using vegetable oil for fuel has been around as long as the diesel engine. Rudolph Diesel, a German engineer, introduced the diesel engine, experimented with fuels ranging from powered coal to peanut oil (Radich, 2004; Canakci, 2007). For many years these engines were adapted to burn petroleum distillate (Radich, 2004). However, in 1970 as oil price rose, research interest into alternative fuels expanded (Canakci, 2007).

The cost of the feedstock used in biodiesel production is the main economic factor in regards to profitability and success of biodiesel. The most common sources of oil for biodiesel production in the U.S. are soybean oil and yellow grease (primarily recycled restaurant cooking oil). According to the USDA Agricultural Marketing Service (2009) the average price of soybean oil is approximately $0.75/kg while the price for yellow grease is $0.51/kg. At the current time yellow grease is cheaper than soybean oil. But in biodiesel production there is also the issue of increased use of catalyst and the increased cost of removing glycerol from yellow grease, so in the end it may not be the “cheaper” feedstock. Commercially manufactured biodiesel from yellow grease meets industry specifications. Yellow grease is a serious candidate for biodiesel production in the future.

According to the National Renderers Association (2009), a combination of greases and animal fats represent one third of the U.S. total fats and oil production, but soybean oil alone represents more than half of U.S. production. Because of its size and availability, the soybean processing industry currently dominates biodiesel production. However, the production of biodiesel from grease can be expected to benefit from a cheaper raw material cost, and inevitability reduce the overall cost of biodiesel and add to the diversity of these low cost raw materials. As grease value goes up it will eventually reach some equilibrium so that biodiesel costs from oil or grease will be close.

Introduction

Biodiesel is a clean burning alternative fuel produced from domestic, renewable resources that can be used alone or in a blend with conventional diesel (Biodiesel Resource, 2008). It can be used in compression-ignition engines with no major modifications. Biodiesel is simple to use, biodegradable, nontoxic, and essentially free of sulfur and aromatics (National Biodiesel Board, NBB, 2009).

In 2001, the American Society for Testing and Materials (ASTM) created a new standard for biodiesel, D6751, making it possible for car manufactures to have consistent biodiesel to test in their diesel engines. The Environmental Protection Agency (EPA, 2009) has also helped with standardizing biodiesel for on-road use. Biodiesel is the first and only alternative fuel to have a complete evaluation of emissions results and potential health effects submitted to the EPA under the Clean Air Act 211. Both Tier I and Tier II tests have been completed to establish biodiesel standards as a clean alternative to diesel. In December 2007, the Texas Commission on Environmental Quality (TCEQ) approved biodiesel blends compromised of 5% or less by volume

biodiesel and 95% or more by volume diesel fuel, commonly referred to as B5 (NBB,2009).

Biodiesel is the only alternative fuel to have fully completed the health effects testing requirements of the Clean Air Act (NBB, 2009). The use of biodiesel results in a substantial reduction of unburned hydrocarbons, carbon monoxide, and particulate matter compared to emissions from diesel fuel, in addition the exhaust emissions of sulfur oxides and sulfates from biodiesel are essentially eliminated compared to diesel (EPA, 2002).

Biodiesel is an easy "drop-in" replacement fuel, however, there are a few disadvantages to biodiesel, especially in regards to the vehicle. Biodiesel has a solvent effect that may release deposits accumulated in the fuel lines from years of petroleum diesel use, and these deposits may initially clog fuel filters (Biodiesel Resource, 2008). The same solvent effect can occur in holding tanks and pipelines. Also using biodiesel in older year model cars, pre-1994, may be ill advised because of the solvent effects may corrode natural rubbers used in hoses and seals (Biodiesel Resource, 2008). Biodiesel has some performance disadvantages. The performance of biodiesel in cold conditions is worse than that of petroleum diesel, and biodiesel made from yellow grease is worse than soybean biodiesel in this regard (Radich, 2004). At low temperatures diesel fuel forms wax crystals, which can clog fuel lines and filters. The "cloud point" is the temperature at which a sample of the fuel starts to appear cloudy, indicating hydrocarbons crystals have begun to form (Dunford, 2007a). At even lower temperatures diesel fuel becomes a gel and cannot be pumped. The "pour point" is the temperature at which the fuel ceases to

flow (Dunford, 2007a). The cloud point and pour point for biodiesel are higher than that of petroleum diesel (Radich, 2004).

Components of Fats, Oils and Grease (FOG)

The word “grease” may refer to yellow grease, choice white grease, edible or inedible tallow, lard, trap grease, poultry fat, or even hydrogenated vegetable oils (Canakci, 2007). In general all greases and oils are classified as lipids. Webster’s Dictionary describes fats as “organic compounds made up of carbon, hydrogen, and oxygen that are naturally occurring in animal fats and in plants and are soluble in organic solvents but not water”. Chemically, fats are classified as *triglycerides* (Canakci, 2007).

Oils are generally considered to be liquids at room temperatures, while greases and fats are solid at room temperature. Many animal fats and hydrogenated vegetable oils tend to be solid at room temperature (Canakci, 2007). Both hydrogenated and non-hydrogenated vegetable oils are used in commercial food frying operations.

Recycled grease products are referred to as waste grease. Greases are generally classified into two categories, yellow grease and brown grease. Yellow grease is manufactured from spent cooking oil and other fats and oils collected from commercial or industrial cooking operations. Spent oil may be vegetable oil or animal fat that has been heated and used for cooking a wide variety of meat, fish or vegetable products (Canakci, 2007). After a period of time the cooking oil is replaced with fresh product. At that time rendering companies may collect the spent cooking oil.

According to Canakci (2007), renderers will filter out the solids and heat the spent cooking oil to drive out moisture until it meets industry specifications for yellow grease. It may be sold as is, or blended with other grease products depending on the

specifications of the customer. Yellow grease is required to have a free fatty acid (FFA) level of less than 15% (Canakci, 2007). Yellow grease is often sold to livestock feed and pet manufacturing companies. According to Hunter and Applewhite (1993), the infrastructure for yellow grease collection is well established and it is estimated that 70% to 95% of the available yellow grease is now being collected in many metropolitan areas. Most heavy users of cooking oils probably use the service of a collection and/or rendering company.

Brown grease, sometimes referred to as trap grease, is collected from grease traps that are installed in industrial, commercial or municipal sewage facilities to separate grease and oil from water (Canakci, 2007 and Dunford, 2007a). Grease traps are sealed containers installed in sewer liners in a manner that allows the lighter grease and oil to float to the top of the trap, the water will then flow under the grease to the main sewer or water treatment area. Grease traps are installed so that the container can be emptied periodically. Many rendering plants will not process trap grease because of the possibility of contamination from soap or cleaning agents (Canakci, 2007).

Chemical Composition of Various Waste Oils and Animal Fats

Very few data are available in the literature for the actual composition of feedstocks at rendering plants. Canakci (2007) displayed a detailed chemical analysis of samples of rendering plant feedstocks and final products collected from Simonsen Rendering Co. in Quimby, IA. The moisture level of restaurant grease samples varied widely, with unprocessed restaurant grease being as high as 18.06%. The FFA levels also varied from 0.7% to 41.8%. Canakci (2007) found from this data that to convert waste restaurant grease into biodiesel must be very vigorous and capable of tolerating a wide

range of feedstock properties. Canakci (2007) also found that FFA levels of finished greases varied from about 8.8% to 25.5%. Zumbado et al. (1999) also found that fatty acid composition of restaurant greases varied depending on the original frying oil used. A paper by Avila et al. (2000) indicated that yellow grease contains relatively high proportion of unsaturated fatty acids, approximately 45% oleic acid (18:1).

Time of year may also play a role in variation among animal fats. Canakci (2007) found that from May to October, the FFA level exceeds 15%, so it must be blended with fat from other sources to meet the yellow grease specifications. Animal carcasses tend to degrade more rapidly during hot weather. However, the fatty acid profile and iodine number did not vary much during the same time period (Canakci, 2007). The iodine number measures level of unsaturation of the fat.

Biodiesel Production Process

Biodiesel can be made from a variety of animal or vegetable fats and oils. These oils or fats can be converted to fatty acids, which in turn are converted to esters. According to Radich (2004), the oils and fats can be converted directly into methyl or ethyl esters using an acid or base to catalyze the transesterification reaction. The preferred reaction used by biodiesel production facilities is base catalysis (Radich, 2004 and Dunford, 2007b). During the process, the fat is reacted with alcohol, typically methanol, in the presence of a catalyst, sodium hydroxide (NaOH); the alcohol reacts with the fatty acids to produce the mono-alkyl ester (biodiesel) and glycerol (Radich, 2004) (Appendix E). According to the National Biodiesel Board (2009), the base catalyzed reaction is the most economical for several reasons: low temperature and pressure required during processing, a very high conversion (98%) with minimal reaction

time, and a direct conversion to biodiesel with no intermediate steps. The methanol is recycled back through the system and the glycerol, if refined, can be sold separately to generate additional income.

The input level is approximately 12% alcohol, 1% catalyst, and 87% fats or oils, in turn the process would generate 86% biodiesel, 9% glycerol, 4% alcohol, and 1% feed quality fats (NBB, 2009). According to Canakci (2007) 0.45 kg of most fats and oils can be converted into 0.45 kg of biodiesel. The National Renderers Association (2008) reported the U. S. produced 1.1 billion kg of yellow grease from 1995-2000, that would be enough to make approximately 1301.8 million liters of biodiesel.

Pros and Cons of Fats and Oils

Given the wide range of fats and oils that can be used in biodiesel production, the relative price and availability of such individual products will have an impact on which raw material is most profitable at any given point. Bumper crops, crop failures or natural disasters in various parts of the world may increase or decrease the price and availability of certain fats and oils.

Legislation and regulation may also impact the price and availability of all fats and oil products for the production of biodiesel. The Renewable Fuels Standard would require the use of 18.9 billion liters of ethanol and biodiesel by the year 2012 (NBB, Federal Register, 2009). This will have significant impact on demand for fats and oils, thus increasing the price of such materials. Another important feature of the federal legislation includes a tax incentive for small-scale biodiesel producers as well as a blender's credit of $0.275/liter for blending biodiesel with petroleum diesel. These tax incentives may also be enhanced by state tax incentives for biofuel production.

The physical and chemical characteristics of various fats and oils may affect certain properties of biodiesel. In food frying, vegetable oils can undergo various chemical reactions such as hydrolysis, polymerization, and oxidation; thus, altering the physical and chemical properties of the oil (Canakci, 2007). Tyagi and Vasishtha (1996) found the percentage of FFA increased due to hydrolysis of triglycerides in the presence of food moisture and oxidation. All biodiesel is required to meet specifications designated as ASTM D-6751, therefore, any processing design for grease products should guarantee that the final product will meet those specifications.

Current Joint Endeavors in Oklahoma

In August 2007, Houston-based oil company ConocoPhillips teamed up with meat producer Tyson Foods Inc. to make renewable diesel fuel from beef, poultry and pork by-products. Renewable diesel and biodiesel use the same feedstocks such as animal fats and vegetable oils, but have different processing methods and create chemically different products. The two companies plan to make as much as 662 million liters of renewable diesel per year. Tyson Foods Inc. has access to approximately 1 billion kg of animal fat annually, the equivalent to 20,000 barrels a day of feedstock that can be turned into renewable fuel. Also according to the Tyson corporate website (2009), their next step is a joint venture with Syntroleum, a Tulsa-based synthetic fuel technology company, to produce synthetic fuels made from renewable feedstocks. Syntroleum contributes their gas-to-liquid technology expertise while producing and developing synthetic fuel for the U.S. Air Force and Department of Defense.

Texas County has long been recognized as the leading agricultural county in the state of Oklahoma. Now it is also Oklahoma's top biofuel county. High Plains Bioenergy,

a joint venture between Seaboard Foods and Oklahoma City-based Musket Corporation, held its grand opening ceremony in early spring 2008. The plant at full capacity will produce 113.5 million litters of high quality biodiesel fuel annually. The biodiesel plant will use animal fats, including pork fat from Seaboard Foods' Guymon processing plant, and vegetable oils as the feedstock for biodiesel. Seaboard Foods President Rod Brenneman (2008) stated that the biodiesel plant was a result of Seaboard employees experimenting with ways to add value to pork fat.

Feasibility of an FOG-based Biodiesel Plant in Oklahoma

The goal of the food waste utilization team at Oklahoma State University was to find alternative used for food waste that are economical and beneficial to the environment. Fats, oils and grease are an important, high-volume, energy-rich waste product of the food industry and was selected as a model for the waste utilization study. According to Bowser et al. (2005) food industry generation of waste FOG exceeds 90,718 metric tons annually. It was not known how much FOG are available in Oklahoma, or if it is feasible to collect and utilize in a biodiesel refinery. Therefore, the objective of the study was to compile information on the types, volume, location and current value of FOG from food processors in Oklahoma.

A survey was generated with questions pertaining to type of waste generated, volume and current method of disposal. Company name and location were kept completely confidential. Oklahoma food companies and rendering companies were contacted by phone, email or letter and asked to volunteer information for the survey. If there were no response in a week, another phone call, letter or email was sent to the companies.

Unfortunately, only 2 companies replied to the questions on the survey. It was determined that other companies did not want to divulge such proprietary information. Therefore, statistical analysis could not be performed on 2 survey questioners.

Conclusion

The growth and popularity of biodiesel seems to be increasing. As more companies join together to find new ways to add value to a "waste" product the amount of biodiesel being produced will expand. As Americans eat out more than cook at home, the supply of yellow grease should remain steady. However, even with the lower priced feedstock, the profitability of a FOG (fats, oils and grease) biodiesel plant will rely heavily upon tax credits and incentives.

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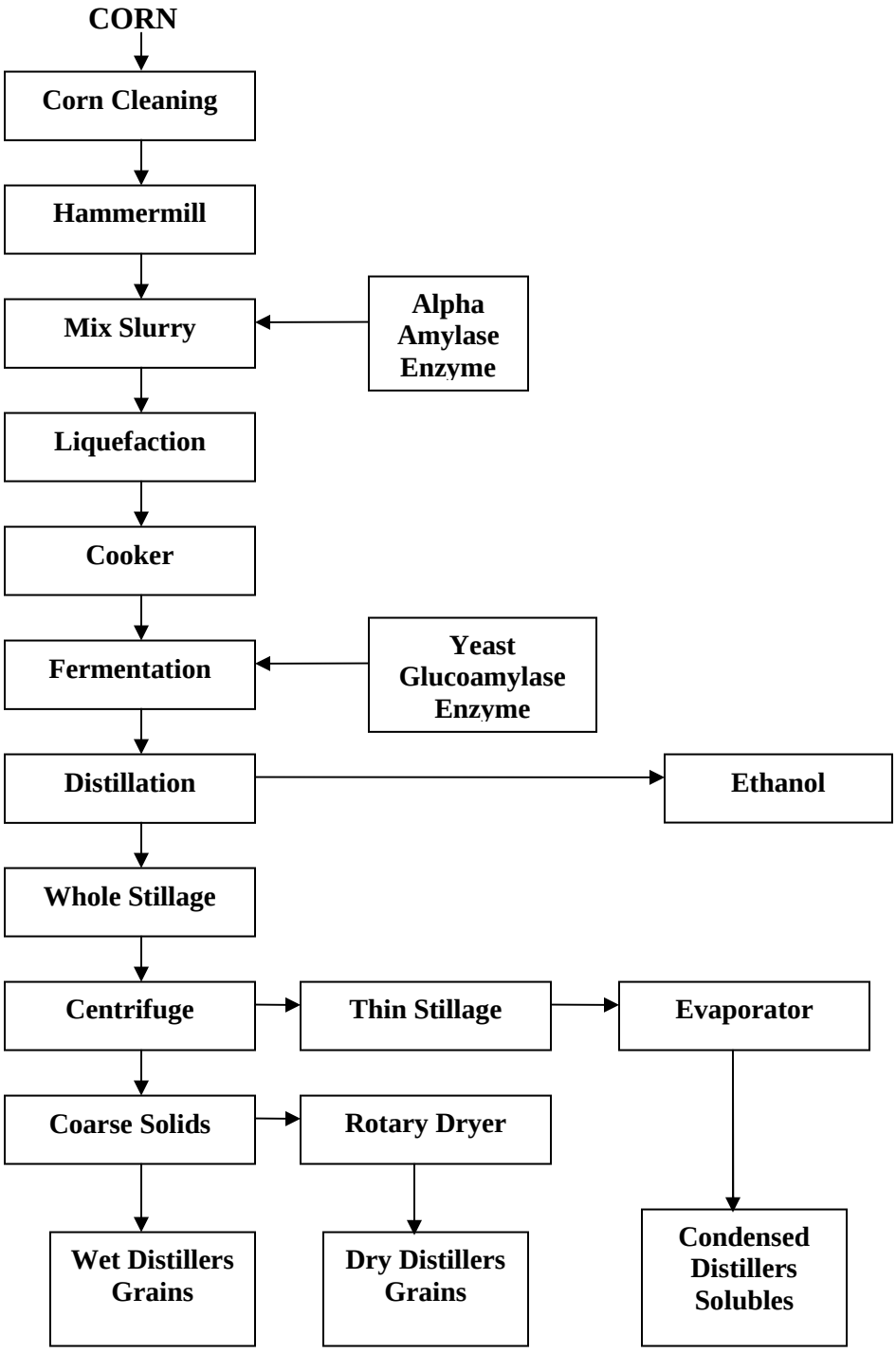
APPENDICES

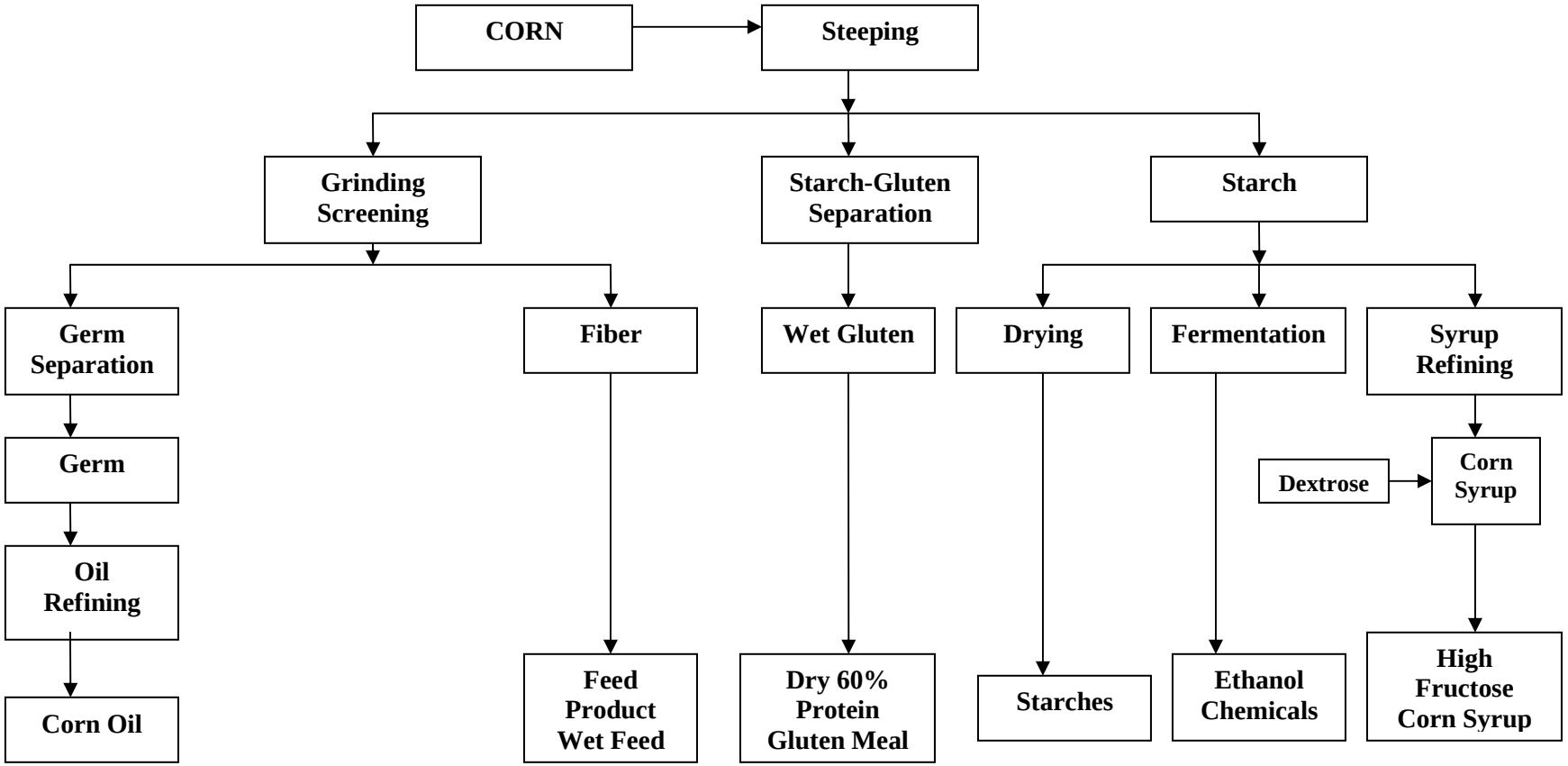
Appendix A

Ethanol and related co-products dry-milling production process diagram



Appendix B

Ethanol and related co-products wet-milling production diagram



Appendix C

Operating Procedure for TBAR Analysis; Modified Buege and Aust, (1978)

*Note: Mix reagents the day before analysis.

TCA/TBA Solution

- Place 2.88 g thiobarbituric acid (TBA) solution in 1000 mL beaker
- Add approximately 700 mL deionized water
- Place beaker onto stirring hot plate
    - Heat on low setting and stir with a magnetic stirrer until TBA dissolves
    - Remove from heat and let cool
- Add 150 g trichloroacetic acid (TCA) into beaker and stir
- Pour into 1000 mL volumetric flask and let set at room temperature
- Bring to 1000 mL volume with deionized water
- Store in cooler overnight

Tetraethoxypropane (TEP) Solution

- Stock solution- dilute 0.1 mL of TEP to 100 mL of deionized water
- Working solution- dilute stock solution 1:2.96 TEP:Water

10% BHA Solution

- Dissolve 10 g of BHA in 90% ETOH
- Store in cooler

TEP Standards

- The following table shows the solution amounts

Tube #	Vol. TEP (ml)	H_20 (ml)	TBA/TCA (ml)
Blank	0	2	4
1	0.010	1.99	4
2	0.020	1.98	4
3	0.040	1.96	4
4	0.060	1.94	4
5	0.080	1.92	4
6	0.100	1.90	4

- Place tubes in hot water bath for 15 min
- Remove and place into cool water bath for 10 min
- Read standards on spectrophotometer at 531 nm

TBAR Procedure:

- Cut sample into small pieces and weigh out 10 g
- Place into Waring blender and add 30 ml of cold deionized water
- Blend for approximately 30 sec
- Pour slurry into a disposable tube
- Centrifuge sample for 10 min at 3000 rpm
- Place 2 ml of supernatant into 16x125mm glass disposable tube
- Add 4 ml of TBA/TCA reagent and 100 µl BHA into each tube
    - Vortex tubes to disperse solutions
- Place glass tubes in hot water bath for 15 min
- Remove and place rack in cold water bath for 10 min
- Centrifuge glass tubes for 10 min at 3000 rpm
- Read samples on spectrophotometer at 531 nm

Appendix D

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

Derivitizations

- 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

Hexane Extraction

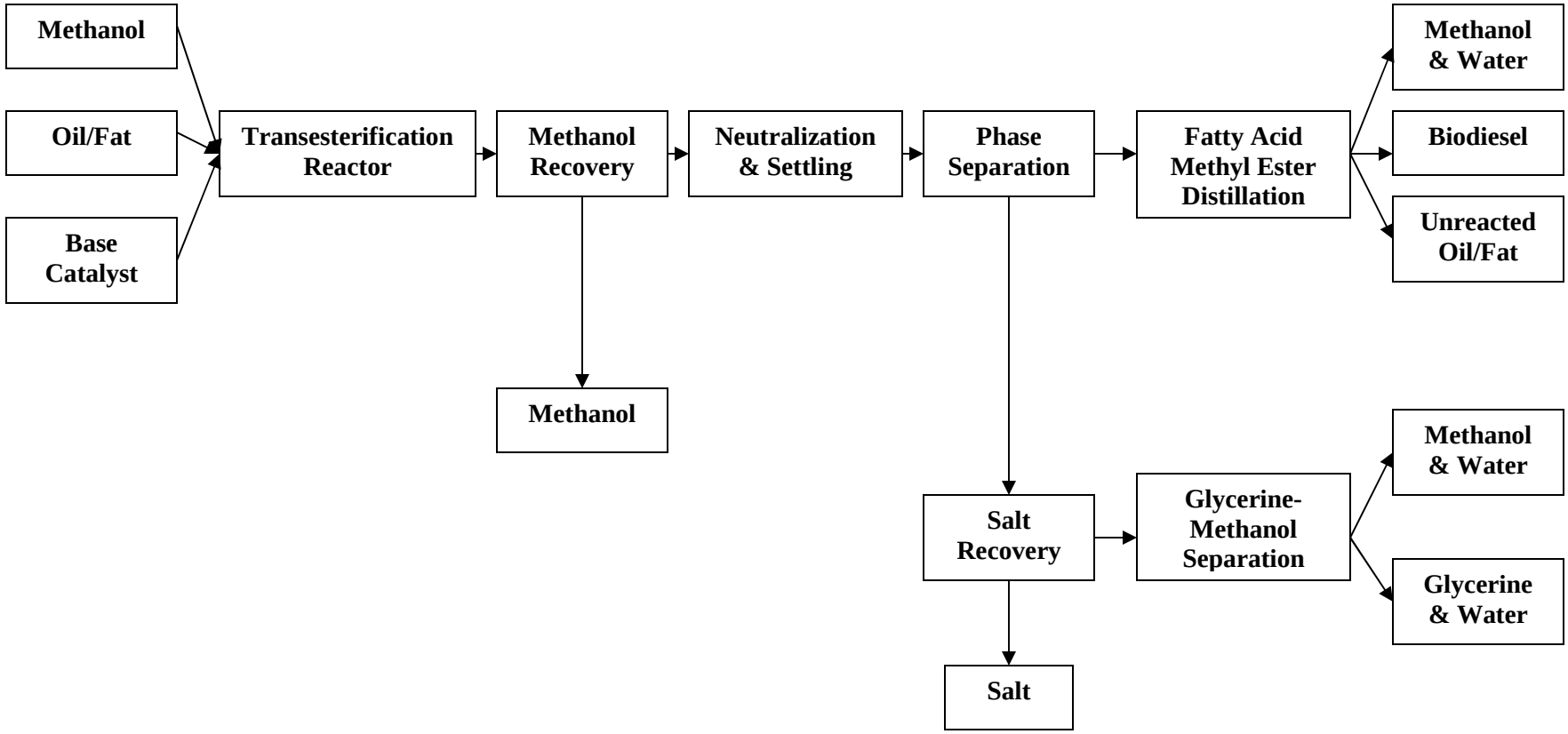
- Add 2 mL hexane, mix by inverted vials 2-3 times, collect upper phase into new silicate vials (repeat twice more)
- Dry samples down under N_2 (can freezer here if 200 µl are left in vial)

Biosil Mini-columns

- 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

Appendix E

Biodiesel and by-products processing diagram



Oklahoma State University Institutional Review Board

Request for Determination of Non-Human Subject or Non-Research

Federal regulations and OSU policy require IRB review of all research involving human subjects. Some categories of research are difficult to discern as to whether they qualify as human subject research. Therefore, the IRB has established policies and procedures to assist in this determination.

1. Principal Investigator Information

First Name: Lea Ann	Middle Initial:	Last Name: Kinman
Department/Division: Animal Science		College: Agriculture
Campus Address: 106 Animal Science building		Zip+4: 74078
Campus Phone: 744-7336	Fax:	Email: lea.kinman@okstate.edu
Complete if PI does not have campus address:		
Address:		City:
State:	Zip:	Phone:

2. Faculty Advisor (complete if PI is a student, resident, or fellow) ☐ **NA**

Faculty Advisor's name: Deb Van Overbeke		Title: Assisstant Professor
Department/Division: Animal Science		College: Agriculture
Campus Address: 1040 Animal Science building		Zip+4: 74078
Campus Phone: 744-9262	Fax:	Email: deb.vanoverbeke@okstate.edu

3. Study Information:

A. Title Economic feasibility of utilizing food processing FOG (fats, oils and grease) generated in Oklahoma as biodiesel feedstock.

B. Give a brief summary of the project. (See instructions for guidance)

C. Describe the subject population/type of data/specimens to be studied. (See instructions for guidance)

Oklahoma State University Institutional Review Board

Request for Determination of Non-Human Subject or Non-Research

4. **Determination of "Research".**
45 CFR 46.102(d): *Research* means a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. Activities which meet this definition constitute research for purposes of this policy whether or not they are conducted or supported under a program which is considered research for other purposes.

One of the following must be "no" to qualify as "non-research":

A. Will the data/specimen(s) be obtained in a systematic manner?
☐ No ☒ Yes

B. Will the intent of the data/specimen collection be for the purpose of contributing to generalizable knowledge (the results (or conclusions) of the activity are intended to be extended beyond a single individual or an internal program, e.g., publications or presentations)?
☐ No ☒ Yes

5. **Determination of "Human Subject".**
45 CFR 46.102(f): *Human subject* means a living individual about whom an investigator (whether professional or student) conducting research obtains: (1) data through intervention or interaction with the individual or (2) identifiable private information. Intervention includes both physical procedures by which data are gathered (for example venipuncture) and manipulations of the subject or the subject's environment that are performed for research purposes. Interaction includes communication or interpersonal contact between investigator and subject. Private information includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (for example, a medical record). Private information must be individually identifiable (i.e., the identity of the subject is or may be ascertained by the investigator or associated with the information) in order for obtaining the information to constitute research involving human subjects.

A. Does the research involve obtaining information about living individuals?
☒ No ☐ Yes
If no, then research does not involve human subjects, no other information is required.
If yes, proceed to the following questions.

All of the following must be "no" to qualify as "non-human subject":

B. Does the study involve intervention or interaction with a "human subject"?
☐ No ☐ Yes

C. Does the study involve access to identifiable private information?
☐ No ☐ Yes

D. Are data/specimens received by the Investigator with identifiable private information?
☐ No ☐ Yes

E. Are the data/specimen(s) coded such that a link exists that could allow the data/specimen(s) to be re-identified?
☐ No ☐ Yes
If "*Yes*," is there a written agreement that prohibits the PI and his/her staff access to the link?
☐ No ☐ Yes

Oklahoma State University Institutional Review Board

Request for Determination of Non-Human Subject or Non-Research

6. **Signatures**

Signature of PI [signature] **Date** 1/24/08

Signature of Faculty Advisor [signature] **Date** 1/24/08
(If PI is a student)

☒ Based on the information provided, the OSU-Stillwater IRB has determined that this project **does not** qualify as human subject research as defined in 45 CFR 46.102(d) and (f) and **is not subject to oversight by the OSU IRB.**

☐ Based on the information provided, the OSU-Stillwater IRB has determined that this research **does** qualify as human subject research and **submission of an application for review by the IRB is required.**

[signature]
Dr. Shelia Kennison, IRB Chair

1/25/08
Date

VITA

Lea Ann Kinman

Candidate for the Degree of

Master of Science or Arts

Dissertation: IMPACTS OF ALTERNATIVE FUELS AND CO-PRODUCTS ON THE AGRICULTURAL INDUSTRY

Major Field: Food Science

Biographical:

Personal Data: Born in Stillwater, Oklahoma, on July 13, 1977, the daughter of Jerold and Nancy Kinman.

Education: Graduated from Prue High School, Prue, Oklahoma in May 1995; received Associate of Science degree in Animal Science from Eastern Oklahoma State University, Wilburton, Oklahoma in May 1997; received Bachelor of Science degree in Animal Science from Oklahoma Panhandle State University, Goodwell, Oklahoma in May 1999. Completed the requirements for the Master of Science degree with a major in Agricultural Sciences at Texas A&M University-Commerce in May 2001. Completed the requirements for Doctorate of Philosophy degree in Food Science at Oklahoma State University in December of 2009.

Experience: Employed by Northeast Texas Farmers Coop in Sulphur Springs, Texas as a Customer Service Representative. Employed by Pilgrim's Pride Inc. in Mt. Pleasant, Texas as a Broiler Service Technician. Employed by Oklahoma State University, Department of Animal Science as a graduate research and teaching assistant.

Professional Memberships: American Meat Science Association, Institute of Food Technologists, American Society of Animal Science, North American College Teachers of Agriculture. North American Scholar Consortium Honor Scoiety.

Name: Lea Ann Kinman Date of Degree: December, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE INFLUENCE OF FEEDING VARIOUS LEVELS OF WET AND DRY DISTILELRS GRAINS TO YEARLING STEERS ON CARCASS CHARACTERISTICS, MEAT QUALITY, FATTY ACID PROFILE AND RETAIL CASE LIFE OF *LONGISSIMUS* MUSCLE

Pages in Study: 72 Candidate for the Degree of Doctor of Philosophy

Major Field: Food Science

Scope and Method of Study: Due to increased production of ethanol, frequency of distillers grains (DG) is on the rise. The objective was to determine the effects of wet (WDG) or dry (DDG) distillers grains on final product quality. Steers (n = 176) were assigned to one of five treatment groups: steam flaked corn (SFC), 10% DDG, 10% WDG, 20% WDG or 30% WDG. The objectives 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 l*ongissimus* muscle. Steaks, 2.54 cm, were cut from strip loins and identified for simulated retail display, Warner-Bratzler shear force (WBSF) analysis, sensory panel determination, and fatty acid composition.

Findings and Conclusions: 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% and 30% WDG had a higher proportion of polyunsaturated and n-6 fatty acids than 10% WDG. Data suggest that feeding WDG at higher levels, 20% or 30%, does not affect sensory attributes, however, shelf life of strip loin steaks from those treatment groups had a shelf life. Further research needs to be conducted to evaluate methods that aid in increasing shelf life of steaks from cattle fed higher rates of WDG.

ADVISER'S APPROVAL: Deb VanOverbeke