THESIS

MANAGEMENT STRATEGIES FOR IMPROVED PRODUCTION PRACTICES TO MAXIMIZE EFFICIENCY ASSOCIATED WITH LIVESTOCK PRODUCTION

Submitted by

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ABSTRACT

MANAGEMENT STRATEGIES FOR IMPROVED PRODUCTION PRACTICES TO MAXIMIZE EFFICIENCY ASSOCIATED WITH LIVESTOCK PRODUCTION

With the ever-increasing world population of over 7 billion and subsequent increase in urbanization, it is crucial for the livestock sector of agriculture to move in the direction of sustainability. Appropriate changes in production practices ensure adequate production with fewer resources to meet the needs of the consumer. Multiple improvements within various management categories are essential to increase animal efficiency and economic gain, improved utilization of natural resources and reduce resulting environmental impacts. The National Air Quality Site Assessment Tool (NAQSAT), originally launched in 2010, provides its users the ability to qualitatively assess how effectively producers are mitigating harmful air emissions in site-specific beef, dairy, swine, broiler chicken, laying hen and turkey production facilities. The air emissions deemed to be of the greatest concern were odor, particulate matter (PM), ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4) and Volatile Organic Compounds (VOCs). Eight management categories are considered: animal housing, diet, manure handling, storage and application practices, mortality and road management. The tool enables users to run hypothetical scenarios to identify potential unintended consequences of management modification prior to making costly changes. The NAQSAT has since then been reviewed and updated by experts according to the most up-to-date knowledge and research to create version 2.0. The tool has expanded to include the horse species and air emission nitrous oxide (N_2O). Following the implementation of the tool, users are directed to potentially applicable NRCS practices pertinent to their management goals within a given management category/emission of concern. Ideally this

will guide users to reformed practices for continued sustainability in today's production environment.

In order to accommodate this movement towards sustainability, diet modifications to the typical feedlot diet have been explored;126 corn fed cross-bred steer calves (initial BW 529.5kg ± 10.7) were supplemented a rumen bypass fat during the last 60 days of the finishing period to evaluate its effects on feedlot performance, carcass characteristics and intramuscular fatty acid composition. Steers were blocked by initial, BW 9 head/pen (n = 7 pens / treatment), at the South Eastern Colorado Research Center (SECRC) in Lamar, CO. Pens were randomly assigned one of two treatment groups: 1) a control diet consisting of a regular corn based finishing ration (CON) and 2) rumen bypass fat treatment consisting of the control diet + Megalac-R/head/day (BF). Diets were formulated to be isocaloric and isonitrogenous. Animals were fed twice daily at 110% of the previous daily *ad libitum* intake. Feed bunks were cleaned and orts were collected weekly. Dry matter content was analyzed and diet samples were collected weekly for proximate analysis. Individual live weights were recorded and blood samples were collected on d -54, -10, 27 and 60 and 61. Feedlot performance and carcass characteristics were assessed (table 2). Initial BW was included in statistical analysis as a covariate. Steers fed the CON diet had a greater level of performance for most of the parameters measured; the CON treatment had greater DMI (10.14kg vs. 8.77kg; $P<0.02$) and tended to have greater ADG (1.699kg vs. 1.469kg; $P<0.09$) (table 2). Final BW was not significantly different between treatment groups ($P<0.16$). On d 62, steers were transported to a commercial slaughterhouse where carcass characteristics were assessed. Hot carcass weight was not significantly different between treatments ($P< 0.19$). Marbling score ($P<0.04$) and quality grade ($P<0.02$) were greater for steers fed the CON diet than those fed BF. The *L. dorsi* area tended to be greater ($P<0.10$) in steers fed CON (87.60cm^2) than those fed BF

($84.88 cm^2$). Furthermore, laboratory analysis showed that UFA palmitoleic acid (C16:1) and oleic acid (C18:1 c9) had Trt x Time interactions in the blood serum (table 4). At d 60, C16:1 was significantly increased in the CON group whereas C18:1 was significantly increased in the BF treatment (Table 4). These data suggest that rumen bypass fat may be added to finishing diets without significant reduction in final body weight, although there may be modest reductions in marbling and quality scores. More research is needed to elucidate the potential mechanism for these reductions.

Key words: Air quality, management practices, rumen bypass fat, biohydrogenation, sustainability

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My friends, Alicia Hornback and Kristin Craddock, you have witnessed both my brightest and darkest moments yet have never left my side. I could not ask for better friends.

DEDICATION

I dedicate my thesis work to my parents, Karen Theisen and Mark Warner. Through your encouragement, both near and far, I find my strength from within.

Mama, you are an inspiration; your strength and compassion never cease to amaze me. Although I am certainly my father's daughter and can be difficult to handle, you've never denied me a listening ear when I have needed it the most and have always provided a shoulder to cry on. I am forever grateful for the qualities you have instilled in me and I will count myself blessed if I become even half of the woman you are. I love you.

Daddy, your words of motivation echo in my memory daily. You helped me learn from your mistakes so I wouldn't make them myself. You have always encouraged me to embrace the person that I am and to follow my dreams. It has been a rough road without you by my side but you are forever in my heart. I will always be your little angel, I love you.

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REVIEW OF LITERATURE

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Confined Animal Feeding Operations

The US Environmental Protection Agency (2012) defines Animal Feeding Operations (AFOs) as agricultural operations where animals are kept and raised in confinement for at least 45 days within a 12 month period. About 15% of all AFO's are considered Concentrated/ Confinement Animal Feed Operations (CAFOs), which fit all of the criteria of an AFO plus regulations outlined for large, medium or small CAFO, respectively. Distinction between large, medium and small is based on a strict range of animal units (U.S. EPA, 2012).

Such CAFOs were initiated in the 1930's with hog operations (Ikerd, 2007). Regulations were developed as early as the 1970's under the National Pollutant Discharge Elimination System (NPDES). The Clean Air Act (CAA), established in 1970 and amended in 1977 and 1990, allows the EPA to create National Ambient Air Quality Standards in order to regulate air emissions (U.S. EPA, 2014a). The Air Quality Compliance Agreement for Animal Feeding Operations was established in 2005 to reduce air pollution and to ensure the compliance with the CAA (U.S. EPA, 2013a). Due to the large numbers of animals congregated within a small area of land in CAFOs, there are increased potential environmental impacts: air, water and land quality (U.S. EPA, 2014b).

Odor

Odor is subjective; it is a matter of perception that differs among individuals and varies based on concentration, intensity and longevity. Livestock odor may results from incomplete anaerobic decomposition of manure generated by livestock and poultry (Pfost et al., 1999). Three main sources of odor production within livestock operations are animal housing, manure storage and land application. There are many variables affecting odor emissions; animal density, management practices, landscaping, neighboring facilities or houses, perception and tolerance by neighbors. Contributing factor to nuisance odor are 1) odor intensity, concentration or strength, 2) frequency of odor detection over a time frame, 3) duration in which the odor is detected and 4) offensiveness of the odor (Jones, 1992). Rainfall events and warm temperatures significantly increase odor concentrations (Watts and Tucker, 1993; Watts et al., 1994; Miller and Varel 2001).

Health Concerns

Odor is a nuisance pollutant and is not regulated under the Federal Clean Air Act. However, odor intensity and dust concentration are positively correlated (Sweeten et al., 1988). Emissions contributing to odor within livestock facilities include volatile organic compounds, hydrogen sulfide and ammonia and may result primarily from incomplete anaerobic fermentation of manure (Mackie et al., 1998; Powers et al., 1999; Zahn et al., 2001).

Measuring Odor

Odor intensity has been quantified by defining the odor unit (OU), the mass of odorants in 1 m^3 of air at the odor detection threshold (ODT) with an efficiency of 50% recognition (European Committee for Standardization, 2002). Approaches for emission measurement varies upon the source of emission; point source (i.e. mechanically ventilated building vent) or area source (i.e. compost pile).

Quantifying odor emissions from animal operations poses a challenge as there are many sources, variables and components. Measurement techniques include sensory methods, analytical methods and electronic noses. Olfactometry, a human sensory method of measuring odor, is the most commonly efficient way to quantify odor (Xue and Chen, 1999). It is the process by which trained personnel are presented with diluted or undiluted odors according to standardized procedures of "ascending concentration series." The dilution to threshold (DT) is the non-odorous airflow rate divided by the odorous airflow rate at which the trained personnel appropriately detect the odorous airstream (McGinley, 2001).

Individual gas concentrations may be measured by sampling ambient air using equipment such as electronic noses or scentometers (Miner and Stroh, 1976; Sweeten et al., 1977, 1983, 1991). Electronic sensors mimic human olfactory recognition and are used to measure individual gases that may be present in very low concentrations (Lacey, 1998). Odorous compound concentration may be measured. Techniques frequently used are gas chromatography and mass spectrometry (White et al., 1971; Hammond et al., 1974).

Mitigation Techniques to Reduce Odor

Many similarities may be seen between odor and particulate matter management techniques. Via manipulations in animal housing, manure storage units, diet and land application, odor and particulate matter can be minimized. Landscaping placed along the property line may variably reduce downwind concentration of dust and particles depending on the material used (Powers, 2004d).

Animal Housing

Within animal housing, filtration and biofiltration methods efficiently trap particles. Installation of mechanical filters in livestock and poultry housing facilities provides an outlet for air where a filter collects odorous compounds that may carry dust particles. Odor dilution threshold is decreased 40-70% by mechanical filtration; trapping 45% of particles 5-10 µm in size and 80% of particles larger than 10 µm (Powers, 2004d). Research has indicated that providing dry litter in poultry facilities will inhibit anaerobic bacterial activity thus reducing production of odor (Jiang, 2000).

Manure Storage

Odor emissions may be abated by solids separation, anaerobic digestion, impermeable and permeable manure covers and aeration. Solid waste is separated by sedimentation, screening, filtration or centrifugation. Anaerobic digestion systems also may effectively degrade odorous compounds in swine manure, as seen by Welsh et al. (1977). Laboratory results by Powers et al. (1997) found a linear relationship between decreased odor intensity of dairy manure and increased hydraulic retention time (HRT). Wilkie (2000) found corresponding results; a three day HRT resulted in a 94% reduction in odor produced by flushed dairy manure.

Odor emissions are greater following a rainfall event which may create conditions optimal for odor production (Lunney and Lott, 1995), therefore, maintaining an aerobic environment may result in reduced odor emission. Composting, aerating surface manure and mixing contents is a common and inexpensive practice. While these procedures decrease odor, they may in turn increased the amount of nitrogen volatilized as ammonia (Powers, 2004d).

Impermeable and permeable covers are a useful mechanism for minimizing odorous gas release from areas known to have high odorous outputs, such as manure piles and retention ponds. Impermeable covers are materials such as polyethylene while materials used for permeable covers include straw, cornstalks, etc. Research indicates that covering manure storage areas with impermeable and permeable covers may reduce odor emissions by up to 90% or 40-50%, respectively (Powers, 2004d; Hudson et al., 2006).

Land Application

Techniques used in land application to mitigate odor emissions are rapid incorporation and injection of manure, proper irrigation and timing of manure application. Injection of manure after application can effectively reduce odor 50-75% as opposed to broadcast application. Irrigation with spray systems close to the canopy and those with low-rise nozzles, low-pressure or trickling characteristics have shown to be the best methods to inhibit distribution of odorous compounds (Powers, 2004d). Moisture management has conflicting results for dust and odor; while increasing moisture content reduces dust emission, it worsens odor concentrations. Alternatively, oil sprinkling may reduce odor emissions (Pfost et al., 1999).

Diet Manipulation

Manipulations to an animal’s diet may efficiently reduce concentration of odorous compounds as well as excretions that contribute to odor production. Primary odorous compounds identified to be associated with manure decomposition are volatile fatty acids, ammonia, indoles, phenols and sulphur-containing compounds (Mackie et al. 1998). According to Zhu et al., (1999), carbohydrate and protein decomposition in manure result in bacterial growth and thus contributing to odor production. Powers (2004d) recommends decreasing the CP composition in swine diets to reduce odor precursors in manure. In feedlot cattle, Miller and Varel (2001) have attributed VFA production to starch fermentation. Research has shown that starch output and subsequent odor production may be reduced by feeding high-moisture ensiled maize plants as opposed to dry rolled maize (Archibeque et al., 2006).

Particulate Matter (PM)

Particulate matter, also known as PM or dust, is variable in size, physical properties and composition. Primary concerns associated with PM are related to air visibility and negative health effects. Livestock housing facilities, manure storage sites and land application sites are prime generators of particulate matter. The primary cause of dust in animal housing was found to be feed, while particulates also result from soil, bedding, dry manure, animal dander, hair, feathers, bacteria, endotoxins, etc. (Koon et al., 1963, Anderson et al., 1966, Curtis et al., 1975b, Heber and Stroik, 1988, Heber et al., 1988). Air emissions are highly variable and factors affecting the contribution of each source depend on the type of animal, design of the confinement facility, the methods of manure handling, weather conditions, geographical location, etc.

Particulate matter is a “criteria” pollutant regulated under the federal Clean Air Act and is classified by two categories based on size. Fine particles with aerodynamic equivalent diameters (AEDs) $\leq 2.5\mu m$ are referred to as $PM_{2.5}$ while PM with AED $>10\mu m$ are PM_{10}. Due to the health and environmental concerns in it which is poses, $PM_{2.5}$ is regulated by U.S. EPA under the National Ambient Air Quality Standards (NAAQS) (U.S. EPA, 2012a). Particulate matter 2.5 can be emitted directly into the atmosphere or formed as secondary particles containing nitrates, sulfates, organic chemicals, metals, soil, dust and allergens (U. S. EPA, 2013 b, c). Any PM larger than 10 µm is not regulated by the U.S. EPA.

The amount of PM emitted differs among facility and may be impacted by many factors. Swine and poultry facilities attribute a large portion of their PM emission to dry, ground fed grains and dried forages (Casey et al, 2006). Pelleting reduces dust emissions, silage generate very little PM and veal calves on liquid diets generate no PM emissions (U.S. EPA, 2001). Solid floors are less effective at trapping dust particles than open-mesh floors, which allow waste products to be undisrupted by animal activity (Carpenter and Fryer, 1990, Dawson 1990). Open feedlots and storage facilities may have a higher rate of PM depending upon manure coverage (Bicudo, 2003). There is minimal data on particulate size in confined animal buildings but PM size is thought to depend on the animal sector, confinement method and building ventilation. Naturally ventilated buildings, used for dairy and beef cattle, have lower PM emissions than those ventilated mechanically, which are typically used for poultry, swine and veal (U. S. EPA, 2001).

Health Concerns

There are National Ambient Air Quality Standards (NAAQS) set by the United States Environmental Protection Agency (U.S. EPA) in regulation of PM due to the public health and environmental concerns associated with its emissions (U. S. EPA, 2012b, c). Respiratory distress of occupational workers is associated with dust components, such as feed particles, dander, endotoxins found in manure and ammonia produced by the breakdown of urea (Donham et al., 1986; Von Essen and Romberger, 2003). When inhaled, coarse PM_{10} deposit in the upper airways of the respiratory tract while fine $PM_{2.5}$ deposit in the lower airways in the lungs. Potential human health risks include cardiovascular and pulmonary distress Environmental effects of PM include decreased visibility, acidification of water reservoirs, altered nutrient composition of soil and damage to vegetation (U.S. EPA, 2013b).

Within the confined swine industry, as much as 74% of workers have reported nasal related health conditions (Donham et al., 1977). Organic dust toxic syndrome (ODTS) has also been reported as a result of exposure to organic dust (Vogelzang et al., 1999; Seifert et al., 2003).

Similarly to other emissions, PM emission differs among facilities. Takai et al. (1998) reports dust concentrations and emission rates for various species in multiple European countries. Overall inhalable and respirable dust concentrations were found to be greatest in poultry housing (3.60 and 0.45 mg/m^3, respectively), lesser in swine (2.19 and 0.23 mg/m^3, respectively) and least in cattle housing (0.38 and 0.07 mg/m^3, respectively). This same trend was seen in dust emission rates; poultry housing had the greatest overall mean inhalable and respirable dust emission rates (3165 and 504 mg/h (500 kg), respectively), followed by swine (567 and 59 mg/h (500 kg), respectively), and then cattle (145 and 24 mg/h (500 kg), respectively). Varying seasonal effects were seen amongst species (Takai et al., 1998).

Measurement Techniques for Particulate Matter

Federal Reference Methods (FRMs) exist for regulation of PM_{10} and $PM_{2.5}$, collected at 50% efficiency (62 Fed. Reg. 38651-38701). Particulate matter is measured according to size, hence the naming of criteria pollutants, $PM_{2.5}$ and PM_{10}. Most commonly used sampling techniques involve collection over a length of time using size-specific inlets and sampling media. Continuous mass concentration measurements are possible using methods such as the Tapered Element Oscillating Microbalance (TEOM 1400A; Rupprecht and Patashnick, Albany NY). Other measurement techniques involving photometers or nephelometers use light scattering (Sloane, 1984; Sioutas et al., 2000).

Mitigation Techniques to Reduce Particulate Matter

Many similarities may be seen between particulate matter and odor management techniques. Via manipulations in animal housing, manure storage units, diet and land application, odor and PM can be minimized. Landscaping is also a method to reduce downwind concentration of dust and particles and in turn reduced odor transmission (Powers, 2004d).

Animal Housing

Within animal housing, filtration and biofiltration methods effectively trap particles from confinement buildings. Ionization, the process by which ionized gases bind particulate matter and wet scrubbing also decreased PM emission (U. S. EPA, 2001).

Open Lots

Greater dust concentrations are common in the evening due to the increase animal activity and steady atmospheric conditions (Parnell et al., 1999) Methods to reduce PM concentrations are increasing stocking density (Auvermann et al., 2002) and/or increasing feedlot surface moisture content. Particulate matter concentrations are minimized by 55-80% by increased feedlot surface moisture, i.e. rainfall events or management practices such as sprinkling (Bonificacio et al., 2011). Increasing moisture content of feedlot surface to decrease PM exacerbates odor. Thus, Sweeten and Lott (1994) have established the optimal moisture content of 25-45% on a wet basis to minimize PM and odor simultaneously. Amosson et al. (2006, 2007, and 2008) estimated the relative costs of initial investment, annual fixed, operational and total costs between solid set sprinklers, traveling gun sprinklers and water trucks for different size feedlots. Each had varied greatly by cost labor intensity and practicality.

Manure Storage

Dust emissions may be effectively reduced and pen conditions improved by proper and frequent removal of uncompacted manure (Razote et al., 2006, Auvermann, 2009). However, as with all air emissions, tradeoffs exist. If surface manure is removed too often or with improper equipment, underlying layers of compacted manure covering mineral soil may be damaged causing future pen maintenance to be problematic.

Impermeable and permeable covers effectively reduce dust concentrations in the atmosphere. Impermeable covers include materials such as polyethylene. The effectiveness of permeable covers, made from materials such as straw, cornstalks, etc., varies based on material used and depth of material used. It required more frequent reapplication than impermeable covers and varies greatly in cost depending upon material used (Powers, 2004b).

Maintaining an aerobic environment may also reduce odor emission and odor carried particulates. Aerating surface manure and mixing contents is a common and inexpensive practice. Composting, another method of manure management, is common but very labor intensive. While these procedures decreased odor, it in turn increased the amount of nitrogen volatilized as ammonia (Powers, 2004d).

Land Application

Because odorous compounds carry dust particles, techniques used in land application to mitigate odor emissions subsequently reduce the spread of PM as well. Injection of manure after application can effectively reduce odor 50-75% as opposed to broadcast application. Irrigation with spray systems close to the canopy and those with low-rise nozzles, low-pressure or trickling characteristics have shown to be the best methods to inhibit distribution of odorous compounds. Moisture management has conflicting results for dust and odor; while increasing moisture content reduces dust emission, it worsens odor concentrations. Oil sprinkling may effectively reduce PM emissions (Powers, 2004d).

Diet Manipulation

Manipulations to an animal’s diet may efficiently reduce PM emissions. The addition of fat (i.e. rumen bypass fat) or vegetable oil to swine or cattle diets may reduce PM by making feedstuff and manure stickier. Replacing soybean meal with full-fat soybeans in swine confinement facilities have shown a reduction in dust concentration up to 30-40% (Powers, 2004b). Modification to feed delivery, using enclosed trucks and covered feeders or pelleting feed may lessen PM emissions (U. S. EPA, 2001).

Ammonia (NH_3)

Ammonia, or NH_3, is known for its colorless, pungent and reactive characteristics (NAQSAT). Ammonia exists as a liquid or in a gaseous state. Ammonia is highly soluble and when chemically combined with water forms ammonium hydroxide. Anhydrous ammonia is ammonia without water. Anhydrous ammonia has a boiling point of -28°F. Liquid anhydrous ammonia is less dense than water while anhydrous ammonia gas is less dense than air. Although anhydrous ammonia is considered to be inflammable, when ammonia vapor is highly concentrated it becomes flammable, which is more likely when it’s accompanied by oil or combustible materials (OSHA).

Health effects of anhydrous ammonia include eye, lung and skin irritation. Depending on the means of exposure, it may cause mild irritation or severe damage to the eyes. Respiratory tissue may be damaged by liquid anhydrous ammonia causing symptoms such as a cough, trouble breathing, congestion or death. The severity of skin effects is dependent upon exposure length and concentration. Mild irritation, burning or tissue damage may occur. Such freeze-dry burns mimics frost bite and spreads until the chemical is diluted (OSHA).

The permissible exposure limit (PEL) defined by the U.S. Occupational Safety and Health Administration (OSHA) is 50 ppm averaged over an 8 hours period. The human detection threshold for ammonia is 20 ppm (OSHA, 2008).

In 1994, Battye et al. reported 50-80% of ammonia volatilization in the United States is result of agricultural activity. More recently, the U. S. EPA (2004) estimated 48.7% of national ammonia emission is result of animal husbandry operations, with cattle and poultry contributing the most.

In livestock production facilities, significant ammonia emissions are result of land application, housing, grazing land and manure storage (Meisinger and Jokela, 2000) and vary based on animal type, management, land application techniques, manure storage type and treatment, wind speed, etc. (Arogo et al., 2001). Ammonia production results from the hydrolysis of urea via the enzyme urease, the breakdown of uric acid and degradation of undigested protein (Koerkamp et al., 1998). Nitrogen is excreted as urea in the urine and urease is excreted in feces of livestock while poultry excrete both urease and uric acid in their feces. Ammonia is promptly volatilized and emitted from manure. Ammonia volatilization is dependent upon ammonia concentration, temperature, humidity, manure storage duration, cover and treatment process (Olesen and Sommer, 1993). At a basic manure pH (>7.0), rate of ammonia volatilization increases, at acidic pH (<7.0), ammonia volatilization decreases and is primarily exists in its ionized form, ammonium (NH_4^+), which has a greater solubility due to the positive charge (U. S. EPA, 2001).

Health Concerns

Ammonia production may negatively affect human, animal and environmental health. It can react with other gases in the atmosphere and form ammonium aerosols [NH_4^+] and fine particulates such as ammonium nitrate and ammonium sulfate (Watson et al., 1998; Harris et al., 2001; Aneja et al. 2000). Environmental concerns of atmospheric NH_3 include those associated with excess N deposition leading cause soil acidification, increased nitrogen concentration in surface water, and lead to further eutrophication and changes to the native ecosystem (Dillon and Molot, 1989; Todd et al., 2004). In particular, the Rocky Mountain National Park (RMNP) has experienced an increase in N deposition over the past couple decades both from in-state and out-of-state sources (Malm et al., 2013).

Measurement Techniques for Ammonia

Many NH_3 detection techniques have been established and compared (Harrison and Kitto, 1990, Weibe et al. 1990). Samples are commonly collected using filters and analyzed via colorimetry or ion chromatography. Other methodologies, such as infrared Fourier transform spectrometry, have been used in swine facilities (Childers et al. 2001). Mass balance techniques have been used to quantify N and calculate residual ammonia emissions in Nebraska (Bierman et al., 1999; Erickson et al., 2000). Alternative approaches used by researchers in Texas include the flux-gradient method (Baek et al., 2006) or inverse dispersion model used by researchers(Flesch et al., 2007).

The backward Lagrangian stochasitic (BLS) model uses gas concentration, wind velocity and direction, atmospheric stability, and a specific source are to estimate fluxes of gases. This method was used in a study conducted in a feedyard in the Texas Panhandle (todd, et al., 2007). Measurements were taken via a tower quipped with acid gas washing. Ammonium in the sample was quantified using a calibrated flow injection analyzer (QuickChem FIA+ 8000, Lachat Instruments, Milwaukee, WI.) Within a different campaign also reported by Todd et al. (2007), NH_3 was continuously measured using a chemiluminescence analyzer (17C, Thermo Environmental Instruments, Franklin, MA).

Mitigation Techniques to Reduce Ammonia

Numerous factors influence NH_3 emissions, which may be species dependent. Those pertaining to housing type include confinement buildings, floor type and design, bedding material, ventilation and temperature. Other influences are diet, retention pond conditions, manure storage methods and animal age (Dewes, 1996; Ni, 1999; Todd et al., 2006, 2007).

Animal Housing

When assessing cattle housing types, NH_3 concentrations are often significantly higher in enclosed confinement buildings, exceeding 25 ppm (Omland, 2002) than in open-lots which rarely exceed 3 ppm (Todd et al., 2005). In a study comparing 34 dairy facilities, NH_3 concentrations were twice as great in free stall barns with liquid manure than those in tie stall barns with solid manure (Swensson and Gustafsson, 2002). These results are likely due to restricted activity of cows confined to tie stalls.

Another study comparing flooring type in cattle housing compared grooved flooring to concrete slatted floors. Swierstra et al (2001) found ammonia emissions to be 46% less in grooved flooring with 1.1m spacing than concrete slotted floors. Bedding type is another variable to consider; deep-bedded areas with 60% peat and 40% straw was found to more efficiently reduce ammonia emissions by 60% in comparison to areas using only long straw as bedding (Jeppsson, 1999).

Physical barriers, such as windbreaks and landscaping placed along the outer property parameter, are used to restrict air movement. Landscaping filters particle emissions, delays movement and lowers ammonia concentration. Cost of landscaping, its effectiveness and visual appeal varies upon type of vegetation (Powers, 2004a).

Similarly to odor and H_2S, biofilters effectively reduce NH_3 emissions (Nicolai and Janna, 1997, 1998) via trapping and microbial degradation using different types of media. Using different wood chip sized >20mm and 10-16 mm reduced ammonia emissions 89% and 95%, respectively (Sheridan et al., 2000). Bioscrubbers, which also rely on microbial degradation, may reduce NH_3 emissions by 89% (Lais, 1997). Ozonation, an intended reaction of ammonia and ozone to produce stable nitrogen gas, in swine finishing facilities have been found to reduce NH_3 emissions (Priem, 1977) by 58% (Bottcher et al., 2000).

Manure Storage

Method of manure storage impacts NH_3 emissions. Biocovers, such as those using layers of barley, wheat, oats or brome straw vary in cost and effectiveness. Studies have shown biocovers to reduce ammonia emissions from lagoons 17-54% (Zahn et al., 2001) and more specifically reduce emission rate 60-95% by using 15 cm of wheat straw over an anaerobic liquid dairy manure lagoon (Xue and Chen, 1999). Floating permeable and impermeable covers over swine manure tanks reduce NH_3 emissions 45-98% (Karlsson, 1996; De Bode, 1991).

Manure removal systems, flooring and bedding all influence emissions. By removing fecal matter, drying manure or litter more frequently, odor and ammonia emissions are decreased (Voermans et al., 1995, Groot Koerkamp et al. 1998b). Flushing solid floors in free-stall dairies reduce NH_3 emissions 14-70% in comparison to scraped or dirty floors (Kroodsma et al., 1993). The effectiveness of the flushing is dependent upon frequency, water quality and amount (Voorburg and Kroodsma, 1992; Hoeksma et al., 1993).

To mitigate ammonia emissions, management and dietary practices are effective. Pre-excretion and post-excretion strategies may be implemented; pre-excretion methods are used to reduce ammonia produced, and post-excretion for the management of manure (Powers, 2002). The use of manure handling systems to separate feces and urine effectively reduces ammonia production up to 80% while dietary manipulations also prove to be effective (Powers, 2004a).

Under acidic conditions, NH_3 volatilization is suppressed and is predominantly in the form of ammonium (NH_4^+). Therefore, under acidic conditions, manure acidification may temporarily suppress ammonia volatilization (U.S. EPA, 2001). Results from Herber et al. (1999) indicate a 70% reduction in ammonia emissions by flushing swine confinement facilities with acid liquid once to twice daily.

Research indicates other techniques to reduce NH_3 include anaerobic digestion of manure, the oxidation of liquid manure or use of other manure additives, i.e. potassium permanganate or peroxide, (U.S. EPA, 2001), urease inhibitors (Varel, 1999; Varel et al., 1999), sulfuric acid (Stevens et al., 1989), paper products (Subair et al., 1999) and alum (Moore et al., 1995; Cole and Parker, 1999; Meisinger et al., 2001) decrease NH_3 emissions. Ozonation may effectively reduce ammonia concentration in the air during the summer and winter (15and 50% respectively) (Priem, 1977). Environmental factors may influence emission rates. Correlations between greater NH_3 emissions and temperature (Anderson, 1995; Anderson et al., 1996; Voermans et al., 1996, Harper et al. 2000) have been reported.

Land Application

Of the total NH_3 emitted due to agriculture, Pain et al. (1998) reports 30% as the result of land application. According to Brunk et al. (1988), Morken and Sakshaug (1998), factors effecting NH_3 emissions during manure spreading are environmental factors (temperature, wind velocity, humidity and rainfall) during spreading, time intervals between spreading, manure type and technique of application.

Land application spreading techniques and corresponding NH_3 emissions have been extensively studied. Injection or immediate incorporation of manure into the soil most effectively minimizes NH_3 emissions compared to other application methods (Hoff et al., 1981; Sommer and Thomsen, 1993). Chadwick et al. (2000) reported a decrease in NH_3 emissions for shallow injection and low trajectory spreaders (85% and 40-75%, respectively) when compared to broadcast spreading. The use of band spreaders with rapid incorporation during liquid manure application effectively reduced gaseous emissions by 55-60% when compared to broadcast application with splash plate spreaders (Ministry of Agriculture FaF, 1992). Direct injection studies have found ammonia emission reduction of 87-98% (Burton, 1997). Slurry acidification prior to land application may reduce ammonia emission (Burton, 1997; Berg and Horing, 1997)

Research findings suggest that oil sprinkling, using vegetable oil (Zhang et al., 1996), soybean oil (Dorota et al., 2001), mineral oil (Derikx and Aarnik, 1993) or canola oil (Buscher et al., 1997) may significantly reduce NH_3 emissions.

Dietary Manipulations

As reported by Todd et al. (2007), 20-50% of nitrogen (N) in cattle diets is lost as urinary N. Avoiding feeding beyond the animal's nutritional N requirements and reducing the amount of N being excreted will in turn decrease the amount of ammonia produced and available for volatilization. Ample research has correlated excess CP content in a feed ration to excess nitrogenous losses. Research has shown that reducing CP content in feed from 13% to 11.5% during the finishing period of livestock results in a 39% decrease in NH_3 emission from an artificial feedyard surface (Todd et al., 2006) and a 25% decrease in apparent N volatilization (Cole et al., 2006). Similar findings reducing dietary CP from 13% to 11% resulted in reduced urine and fecal N losses by 60-200% (Cole et al., 2005). Alternative research reduced CP from 13.5% to 11.62% and found a 21-40% reduction in NH_3 fluxes Galles et al. (2011). Thus, feeding cattle to their nutritional requirements, not beyond, will yield decreased NH_3 emission and volatilization.

Reports have shown a 28-79% decrease in NH_3 emissions of swine through diet modifications (Sutton et al., 1999). Similarly to cattle, studies have shown N excretion to decrease in swine with reduced CP diets that contain synthetic amino acids (Hartung and Phillips, 1994; Canh et al., 1997, 1998; Hayes et al., 2004). Other research has linked a decrease in CP to reduced NH_3 concentrations in broiler housing (Elwinger and Svensson, 1996; Ferguson, 1998; Gates, 2000).

A decrease in NH_3 emission has been reported in beef and dairy cattle in diets with reduced CP; a 28% decrease in NH_3 emission in beef (Klopfenstein and Erickson 2001) and 15-30% decrease in dairy (James et al. 1999). Burkholder (2004) suggests feeding steam-flaked maize instead of dry rolled maize to dairy cows to increase N digestibility, deceased N output and consequently decreased NH_3 loss.

Altering an animals' diet may decrease manure volume and resulting emissions. Nitrogen excretion in animal waste results from excess undigested protein within the diet. Jongbloed and Lenis (1992) report 70% of dietary protein to be excreted for growing swine, 80-90% for beef cattle and 55% for broilers. Increased nitrogen intake and excretion is result of greater dietary CP concentrations in cattle (James et al., 1999) and is commonly decreased with lesser CP concentrations (Paul et al., 1998; James et al., 2000).

Dietary additions may also reduce ammonia. Powers (2004a) suggests the addition of fermentable carbohydrates an ammonia binding additives. Studies have shown the inclusion of pressed sugar beet silage (Canh et al., 1997), adipic acid supplementation (Van Kempen, 2000) or yucca extract (Sutton et al., 1992) to swine diets reduce NH_3 emissions.

Careful consideration should be taken when formulating a ration to decrease air emissions; it is essential to find a median to maintain production efficiency. Decreasing CP content may unintentionally reduce productivity of an animal. An extended finishing period may result, which may in turn increase overall emissions produced by that animal.

Hydrogen Sulfide (H_2S)

Hydrogen Sulfide, abbreviated H_2S, is a colorless, flammable and hazardous gas commonly known for its distinctive rotten egg odor detectable at low concentrations. It exists in both gaseous and liquid forms. Hydrogen sulfide is denser than air therefore is in higher concentration in low-lying areas. . It is soluble in water and so can be easily oxidized to SO_2 and then to sulfate and transported by water far distances. If H_2S is ignited, highly toxic gases such as sulfur dioxide are produced (OSHA, 2005). Hydrogen sulfide is the product of bacterial action; sulfate is reduced and sulfur-containing organic compounds are broken down under anaerobic environments (Hill, 1973).

Health Concerns

Hydrogen sulfide has been recognized for decades as an occupational hazard from fermenting manure (Morse et al., 1981) At low concentrations, H_2S is a mild eye, nose, throat and respiratory irritant (OSHA, 2005). However, high concentrations and repeated exposure pose serious health risks; it is responsible for the highest number of manure-related deaths for animals and humans (Lorimor, 1994). The permissible exposure limit (PEL) of H_2S for occupational workers defined by OSHA is 10 ppm for and 8 hour time period (ACGIH, 1992). Hydrogen sulfide can be distinguished by 80% of people at a concentration of 30 ppm (Schiffman et al., 2001). Hydrogen sulfide levels greater than 100ppm is labeled as immediately dangerous to life and health (IDLH) (OSHA, 2005). Field (1980) reports symptoms such as headache and dizziness at a concentration of 200 ppm for 60 minutes, severe headache, nausea and insomnia at 500 ppm for 30 minutes and the lethal concentration of 1000 ppm.

Measurement Techniques for Hydrogen Sulfide

Hydrogen Sulfide is a common concern in swine facilities. Concentration of H_2S can be measured via many techniques; wet chemistry techniques, gas analyzers and monitors. Gas chromatography is commonly used for collected samples (Banwart and Bremner, 1975; Powers et al. 2000). Field measurements can be easily obtained using portable equipment.

The Jarome H_2S Analyzer (model 631-X, Arizona Instrument, Phoenix, AZ) is used to measure ambient H_2S concentrations around livestock facilities (Wood et al. 2001) with 6-8% efficiency. The Zellweger MDA Single Point Air Monitor (SPM) has an accuracy of 20% and has been used to collect ambient H_2S concentration at swine facilities (Bicudo et al., 2002). The TEI Model 45C H_2S Analyzer has also frequently been used at swine facilities over wastewater lagoons (Zahn et al., 2002).

Mitigation Techniques to Reduce Hydrogen Sulfide

A positive correlation between H_2S concentration and odor dilution threshold (DT) has been reported in livestock production facilities (Fakhoury et al., 2000; Guo et al., 2000). Many similarities exist in the mitigation techniques due to the interconnectedness amongst emissions. There are often tradeoffs associated with the management practices.

Animal Housing

Many strategies to reduce H_2S emissions focus on the reduction of sulfur containing compounds. Filtration and biofiltration effectively trap emissions and allows for aerobic degradation. Such techniques have been reported to successfully reduce H_2S emissions by 90% and NH_3 by 74% in a swine facility and similar results in a dairy facility. Lesser abatement was found in a poultry facility due to increased PM emissions (Powers, 2004c). In order to create optimal conditions for aerobic digestion, factors such as oxygen concentration, temperature, residence time and moisture content must be considered (Powers, 2004c).

Oil sprinkling reduced H_2S emissions by 40-60% and reduced odor 40-70% in a study out of Minnesota (Powers, 2004c). Other Techniques to control H_2S emissions are similar to those of NH_3 mitigation. They include biofiltration (Nicolai and Janni, 1997, 1998; R.E. Nicolai and R.M. Lefers 2006), bioscrubbing (Nishimura and Yoda, 1997), and the use of activated carbon (Bagreev et al. 2001), ozone (Fitament et al., 2000; Masuda et al., 2001), and non-thermal plasma (Goodrich and Wang, 2002).

Manure Storage

Implementing manure management techniques such as frequent manure removal, manure compositing, manure storage covers, additives and dietary manipulations, may effectively reduce H_2S emissions. According to research, more frequent manure removal, every other week instead of every 6 weeks, can decrease H_2S by 79% (Heber et al., 2001). Composting maintains the aerobic conditions for H_2S reduction (Powers, 2004c).

Research has demonstrated the efficiency of that implementing manure storage covers to reduce H_2S emissions. Permeable covers reduce emissions by reducing the effects of radiation and wind on manure storage. For example, geotextile membranes (0.3mm) with a straw topcoat (8 or 12 in.) reduces H_2S emissions by >70% (Bicudo at al., 1999). Miner and Pan (1995) found that permeable blankets or zeolite covering manure storage may reduce H_2S emissions by up to 90%. Other studies over a seven week time period found 5-10cm wheat straw covering anaerobic liquid dairy manure to efficiently reduced H_2S emission by 95% (Xue et al., 1999). The effectiveness of permeable covers is dependent upon cover depth and frequency of replacement.

Impermeable covers block gaseous losses; inflated plastic covers reduced H_2S emissions of manure storage tanks by 95% (Mannebeck, 1985; Zhang and Gaakeer, 1996).

Chemical additives are commonly used to raise manure pH, which effectively reduce H_2S emission but may subsequently increase NH_3 emissions. Arogo (1997) attributes the decreased H_2S emissions to 1) the conversion of sulfite into intermediate forms, inert metallic sulfide or bisulfide ions and 2) the eradication of bacteria that produce sulfide or alteration of the bacterial environment. Studies suggest a reduction in H_2S emissions with the addition of hydrogen peroxide to manure (Ritter et al., 1995). Chen and Xue (1998) reported an 80% reduction of H_2S concentration and emission rate with the addition of hydrogen peroxide and potassium permanganate (0.5%) to an anaerobic dairy liquid manure lagoon. Alternatively, raising manure pH above 9.5 using lime may eradicate H_2S emissions (Miner, 1980; Day, 1966).

Land Application

Gaseous emissions, including H_2S, NH_3 and VOCs, may be reduced via rapid incorporation of manure into the soil at time of land application. The use of band spreaders with rapid incorporation during liquid manure application effectively reduced gaseous emissions by 55-60% when compared to broadcast application with splash plate spreaders (Ministry of Agriculture FaF, 1992). Hydrogen sulfide reductions are extrapolated from field tests in Iowa indicating a 50-75% reduction in odor with manure injection practices instead of broadcast application (Powers, 2004c).

Dietary Manipulation

Dietary manipulations prevent excretion and thus emission of H_2S. Numerous studies have correlated a reduction in H_2S with reduced dietary CP, sulfur containing mineral supplementation and minimal sulfur content in water (Powers, 2004c). A 40% decrease in H_2S

emission of swine corresponds to a 4.5% dietary CP reduction with the addition of synthetic amino acids, while a 26.5% decrease in H_2S emission correspond a 2.7% dietary CP reduction with the addition of 10% soybean hulls (Kendall et al., 1999). Another study showed a reduction in H_2S and odor emissions from manure by reducing the sulfur content t in feed and water (Whitney et al., 1999).

Volatile Organic Compounds (VOC)

Volatile organic compounds (VOC's) are defined as any organic compound of carbon, with a few exceptions such as carbon dioxide, carbonic acid, and ammonia carbonate. Under aerobic conditions, VOC's are oxidized to carbon dioxide and water. Under anaerobic conditions, complex organic compounds are microbially degraded to VOC's which are then converted to methane and CO_2 via methanogenic bacteria. In the presence of methanogenic bacteria, most VOC's are metabolized to simpler compounds and therefore results in minimal VOC emissions. Inhibition of methane formation by low temperatures or high loading rates of volatile solids in liquid storage facilities causes excessive VOC build-up in manure and thus volatilization (U.S. EPA, 2001).

The World Health Organization categorizes VOC's based on how easily they are emitted. Very volatile organic compounds (VVOC) like propane have a low boiling point of 0 to 50-100^{0}C, volatile organic compounds (VOC) like formaldehyde have a boiling point of 50-100 to 240-260^{0}C, and semi volatile organic compounds (SVOC) like pesticides have a boiling point of 240-260 to 380-400^{0}C (WHO, 1989). There are four classes of VOC's; volatile fatty acids, indoles and phenols, amines and sulfur-containing compounds (U.S. EPA, 2001).

The U.S. EPA Clean Air Act identifies some VOC's as being hazardous air pollutants and regulates indoor and outdoor VOC compounds. VOC's are from household products like cleaning products, painting supplies, photography equipment and dry-cleaning products or in animal production facilities from livestock and poultry waste. Outdoor regulation is primarily for the prevention of atmospheric ozone, a component of photochemical smog. Ground level ozone can form by VOC's reacting with oxygen containing compounds in the atmosphere accompanying sunlight and contributes to climate change (U.S. EPA, 2001).

One of the earliest lists of volatile compounds associated with manure of cattle, poultry and swine was created by Kreis (1978) listing 32 VOC compounds from cattle waste.. These numbers continue to grow as now there have been 331 VOC compounds and fixed gases identified from swine facilities in North Carolina (Schiffman et al, 2001).

Volatile organic compounds are primarily produced in manure storage facilities where feces and urine breakdown under an aerobic environment. Silages are a major producer of VOC's, producing over 700 substances that may contribute to ozone formation, including ethanol and alcohols (Mitloehner et al., 2009). Ethanol is a product of yeast metabolism and its inhibition will limit the energy lost in the form of ethanol (McDonald et al., 1991). Yeast production may be inhibited under aerobic conditions by increasing the acetic acid content of silage using the heterolactic lactic acid bacteria (LAB) ensiling practice (Driehuis and Van Wikselaar Oude, 1996).

Health Concerns

Many VOC's may be hazardous to human health and vary based upon the nature of the compound, degree and length of exposure. Short-term, low concentration exposure cause symptoms such as headaches, dizziness, visual and memory impairments, fatigue, nausea, loss of coordination, eye, skin and respiratory irritation. Long-term exposure to VOC's can be detrimental to the liver, kidneys and central nervous system. Many organic compounds, i.e.

benzene and formaldehyde, have also been found to be carcinogenic (U.S. EPA, 2012b). High concentrations of VOC's can affect the olfactory system and have severe toxic effects (Shiffman and Nagle, 1992).

The Occupational safety and Health Administration lists around 500 compounds in their permissible exposure limits (PELs). In order to protect workers in areas of VOC exposure, gas detectors with sensors may be used to detect hazardous concentrations of gases. VOC's are detected by broad-range sensors, which give an overall reading of detected classes of contaminants but cannot distinguish between the different compounds (Henderson 2004).

Measurement Techniques for Volatile Organic Compounds

Most commonly, gas chromatography and flame ionization detection is used (GC-FID). Emissions can be identified using this technique by observing retention time (Westberg and Zimmerman, 1993). Another commonly used measurement techniques for VOC's is a hot-bead pellistor type combustible gas sensor. They detect gas by oxidizing them on an active bead within the sensor. This oxidation causes the bead to heat and therefore can be used to quantify the amount of gas in the atmosphere to be used as the basis for instrument reading. These instruments typically display % lower explosive limit (LEL) increments with hazardous levels of 5-10% (Henderson, 2004).

Analysis from a swine facility in North Carolina identified over 300 of assorted VOC compounds with acids, phenols and aldehydes in largest qualities. Measurement techniques were gas chromatography and mass spectrometry. VOC's from AFOs and waste management systems measured from 0.60 mg/m^3 to 108 mg/m^3 depending on how recently the facility was cleaned (Schiffman et al. 2002).

Volatile Organic Compound concentrations can be measured by proton-transfer-reaction mass spectrometry (PTR-MS). Advantages of this chemical ionization technique are little compound fragmentation, high frequency measurements, quick response times and its ability to detect several VOCs (Lindinger et al., 1998). Other options to measure VOC emissions are ppbRAE method with a photoionization sensor or the nonspecific photoionization detector (PID) (Maughan et al., 2005).

Accurate measurement downwind from livestock facilities may be difficult. Many VOC's which are emitted from livestock facilities are produced elsewhere as well; i.e. aldehydes are also produces via the incomplete combustion of fossil fuels (Marnett, 198).

Mitigation Techniques to Reduce Volatile Organic Compounds

In order to control VOC emissions, factors influencing their production, such as temperature, sunlight and decomposition of organic materials, must be considered.

VOC emissions from fresh cattle manure can be reduced via the application of the dry, granular acid salt, Sodium bisulfate (SBS) to animal bedding material. Ethanol and methanol emissions are reported to be reduced by 61% and 58% while having a 60% reduction in ammonia emission by the use of SBS. Anaerobic digestion of manure variably reduces NH_3, H_2S, VOC and CH_4 (U.S. EPA, 2001). The Oxidation of liquid manure or other manure additives, i.e. potassium permanganate or peroxide, may reduce gaseous emissions (U.S. EPA, 2001).

Methane (CH_4)

Methane, CH_4, is a greenhouse trace gas, which contribute greatly to climate change and global warming due to its ability to absorb infrared radiation and trap heat within the atmosphere (Lashof and Ahuja, 1990). It is a simple asphyxiant, non-reactive, odorless, colorless, less dense than air and is highly flammable (Airgas, 2013). Although methane gas does occur naturally in the atmosphere, up to 60% of global methane emissions is a result of livestock animals (Ellis et al., 2007; NRC, 2002) and has an atmospheric lifetime of 12 years (Forster et al., 2007; IPCC, 2001).

Fermentation, the process by which microorganisms breakdown digesta prior to gastric stomach, is the primary source of ruminant methane gas production. Specifically, methanogens function in the rumen and hindgut yielding methane due to normal enteric fermentation of feedstuffs by utilizing hydrogen and carbon dioxide (Hungate et al., 1970). Up to 89% of methane generated by ruminants emitted into the atmosphere via eructation (Murray et al., 1976), which can represent a 2-12% energy loss derived from feed (Johnson and Ward, 1996). Each year domesticated ruminants are responsible for the production of up to 86 million metric tons (Tg) of methane; 18.9 Tg of which is attributed to dairy cattle, 55.9 Tg to beef cattle, and 9.5 Tg to other smaller ruminants (Johnson and Ward, 1996). While individually each livestock animal contributes a relatively insignificant amount of methane, The U.S. EPA estimates that collectively livestock animals are responsible for 28% of the global methane emission related to human activity (U.S. EPA, 2007).

The cow-calf subdivision of the U.S. livestock industry is responsible for the greatest methane emission, followed by dairy and then feedlots/stockers (58%, 23% and 19%, respectively). This is due to sub-optimal management and lower dietary quality in comparison to dairy or feedlot areas (U.S. EPA, 2007).

Based on research conducted by Murray et al. (1976), of the CH_4 emitted by the ruminant, the rumen and hindgut are responsible for 87% and 13%, respectively. Of the ruminally produced CH_4, 95% of it can be attributed to eructation (Murray et al., 1976).

Health Concerns

Methane inhalation exacerbates acute or chronic respiratory conditions and is a simple asphyxiant (MSDS, 2013). There is no PEL outlined by OSHA.

Measurement Techniques for Methane

Mass balance techniques are used to measure CH_4 and are typically used to assess a group of animals. It entails measuring the difference in CH_4 concentrations entering and exiting an enclosure. Air flow rate is measured using anemometers or tracer gases (Persily, 1988; Howard, 1991). The most common means of CH_4 analysis is via gas chromatography and flame ionization detection is used (GC-FID) (Steele at al., 19876). Both ambient air and collected samples may be analyzed by this technique.

Methane emissions sampling methods are classified as direct or indirect. Direct methods use the live enclosed animals and require specialized housing and equipment. Direct techniques include the calorimeter system and energetics methods (Vermorel 1989; Johnson *et al*. 2003). Alternatively, indirect methods such as open-circuit respiration chambers created by Pettenkoffer in 1892 (McLean and Tobin, 1987), use tracers. Other methods include headboxes and facemasks, each with their limitations. The open-circuit indirect calorimeter enables the researcher to measure inspiration of O_2, and production of CO_2 and CH_4 (Young et al., 1975). Johnson et al. (1994) developed the sulfur hexafluoride (S F6) tracer technique, which is idea for measuring methane in grazing, free-range cattle.

Mitigation Techniques to Reduce Methane

Methane production represents a loss of carbon and therefore a reduction in energy efficiency. By improving livestock production and efficiency via nutritional and genetic developments methane production can be minimized (U.S. EPA, 2007).

Manure Storage

Similarly to other emissions discussed, more frequent manure removal and appropriate manure storage covers will reduce CH_4 emissions.

Dietary Manipulations

Methane production can be minimized by grinding and pelleting forages (Blaxter, 1989). The addition of lipids to the ruminant diet decreased CH_4 emissions. Lipid supplementation decreases CH_4 losses by the increased biohydrogenation of unsaturated fatty acids, shift in VFA concentration towards propionate, and via protozoal inhibition (Johnson and Johnson, 1995). Methanogenesis was decreased with the addition of long chained PUFA (Czerkawski et al., 1966). Supplemental tallow or soybean oil was found to decrease CH_4 production by minimizing fermentable substrate (Swift et al., 1948; Haaland, 1978;Van der Honing et al., 1981).

The addition of ionophores to beef diets, i.e. monensin, has been shown to decrease DMI by up to 6%, decrease the acetic: propionic acid ration and temporarily decreased CH_4 production (Goodrich et al., 1984).

Nitrous Oxide (N_2O)

Nitrous oxide, or N_2O, is produced via nitrification and denitrification, which are the biological reductions of ammonia and nitrate or nitrite, respectively. Under different conditions the source of N_2O production may vary. According to Pahl et al. (2001), under anaerobic conditions the nitrate reducing process of denitrification predominates and under aerobic conditions the ammonia reducing process of nitrification predominates. The U.S. EPA attributed 69% of the total United States N_2O emission in the year 2011 to agricultural soil management and 5% to N breakdown from livestock urine and feces (U.S. EPA, 2014c).

Health Concerns

The greenhouse gas, N_2O, is a leading environmental concern. Nitrous oxide, along with other greenhouse gas contributors CO_2 and CH_4, absorb infrared radiation. Houghton et al. (1992) reports that N_2O has the infrared radiation absorbing efficiency 200 times greater than that of $CO_{2.}$ The photochemical decomposition of N_2O to NO contributed to ozone reduction.

Measuring Nitrous Oxide

Nitrous oxide may be measured using ambient air or collected stored samples. The most commonly used measurement technique used for N_2O is gas chromatography and electron capture detection (GC-ECD) (Robertson et al., 2000). Others include tunable diode laser spectroscopy (TDLS) and Fourier transform infrared spectroscopy (FTIR).

Mitigation Techniques to Reduce Nitrous Oxide

Although N2O is variable among species, the primarily sources are unpaved drylots of dairy and beef cattle and as result of land application. Nitrous oxide emissions are affected by drainage and plant update. Optimal anaerobic conditions for denitrification are obtained by poorly drained soils. Excess land application of manure, i.e. during the non-growing season, provides excess nitrogen for plant uptake (U.S. EPA, 2001).

Animal Housing

Chadwick et al. (1999) assessed N_2O emissions from animal housing in the UK, which ranged from 0.4 to 26 g N_2O AU^{-1} d^{-1}. Emissions differed upon species; N_2O emissions were the least from swine housing and greatest from poultry housing. Within dairy housing, slurry-based systems have less N_2O emissions than housing using straw bedding (Chadwick et al., 1999). Weekly discharge of ground manure pits reduced N_2O emissions, as reported by Osada et al. (1998).

Manure Storage

Nitrous oxide emissions vary based upon method of manure storage and time of the year. Stockpiled cattle manure contributes nearly double the N_2O emission than poultry (Chadwick 1999). Covered cattle slurry produced a maximum of 25 mg N m^{-2} d^{-1} during the summer (Sommer et al., 2000). Nitrous oxide flux from stored solid dairy manure was measured by Brown et al. (2000); samples were collected within 30 cm of the manure pile surface and the mean daily flux was reported to be between 0 and 330 mg N m-2 d-1, which was variable due to water content and redox potentials.

Nitrous oxide has been measured from composted swine and cattle manure. Kuroda et al. (1996) found N_2O to only contribute 10% of the total nitrogen content in manure. Sommer (2001) attributes less than 0.3% of the total nitrogen to N_2O to composted cattle bedding. For composted swine litter, Sommer and Moller (2000) found increased N_2O emissions when high density bedding material was used. The effects of bedding material on N loss during feedlot cattle manure composition was determined by Hao et al. (2004); N_2O emissions were greater for wood chip bedding than straw bedding, 0.084 kg N Mg^{-1} and 0.077 kg N Mg^{-1}, respectively.

Land Application

Nitrous oxide loss from land application is minimal if applied appropriately and at agronomic rates (U.S. EPA, 2001). Of the N applied during land application of dairy and swine manure, a mere 0.3% and 0.4% were lost as N_2O, respectively (Chadwick et al., 1999). Other data presented by Sharpe and Harper (2002) found an increased flux of N_2O following irrigation of swine lagoon liquid; N_2O flux was increased from 0.0016 mg N2O-N m-2 d-1 to between 2.5 and 3.8 mg N2O-N m-2 d-1.

Diet manipulation

Greenhouse gas emissions have been studied extensively in swine facilities. Results have shown feeding to the animals' nutritional requirements, and not above, will result in reduced N and C excretion and emissions from waste (Sutton et al., 1999; Lenis and Jongbloes, 1999). Thus nutritional modifications have been explored. Reducing the protein content in a diet minimizes N_2O emissions from stored manure according to Kulling et al. (2001) and may effectively reduce greenhouse gases without hindering animal performance (Ball and Mohn, 2003). Clark and associates (2005) caution that when reducing protein, the diet may become deficient in amino acids. Therefore the supplementation of synthetic amino acids is necessary to keep a balanced diet (Clark et al., 2005).

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CHAPTER II

REVIEW OF LITERATURE

SUPPLEMENTATION OF RUMEN-PROTECTED FAT IN CORN-FED BEEF STEERS

Beef quality, and therefore economic value, is correlated to site of adipose tissue deposition; directly related to increased fat deposition intramuscularly and inversely related to subcutaneous adipose tissue (Pickworth, 2011). Adipose tissue development and deposition is variable based on many factors including breed, physiological status, stage of development and finishing systems (Pavan and Duckett, 2008). Thus adipose tissue development, synthesis, genomic factors and dietary influences have been thoroughly examined.

Adipose Tissue

Adipose is connective tissue derived from the mesoderm of the embryo, which gives rise to a fat precursor, the adipoblast. Growth of adipose tissue may occur by hyperplasia, hypertrophy or the recruitment of additional cells to adipose tissue (Singh et al., 2007). Adipoblasts proliferate and eventually differentiate into preadipocytes. Lipid droplets formed in the preadipocyte go on to form a large globule and thus become a mature adipocyte (Otto and Lane, 2005).

Lipogenesis

Lipids may be synthesized de novo or from the dietary sources. Adipose tissue is the primary site of lipogenesis in the non-lactating ruminant (Ingle et al., 1972). According to Smith and Crouse (1984), substrate preference for lipogenesis differs based on depot site. Acetate provides the majority of the acetyl units for in vitro lipogenesis in subcutaneous adipose tissue, while glucose is the primary precursor in intramuscular adipose tissue, 70-80% and 50-75% acetyl units respectively (Smith and Crouse, 1984). Different regulatory processes involved in lipogenesis of fat depots may allow for manipulation of lipid deposition site. Rate of fatty acid synthesis is dependent upon the substrate. Rate of fatty acid synthesis increased with age in subcutaneous, intramuscular and intermuscular adipose tissue when expressed on a cellular basis using acetate or lactate as the substrate. With the exception of acetyl-CoA carboxylase, lipogenic enzyme activity tended to increase with age for all three of the aforementioned adipose depots (Whitehurst et al., 1981).

The metabolic pathways of fatty acid synthesis has been studies extensively (Saggerson, 1977; Berg et al., 2002). While lipogenesis in non-ruminants typically takes place in the liver, the primary site of de novo FA synthesis occurs in the adipose tissue of non-lactating ruminants and mammary tissue of lactating ruminants (Ingle et al., 1972). NADPH is a reducing equivalent for lipogenesis. It can be generated for use in FA synthesis via three pathways: The pentose phosphate, malate dehydrogenase and isocitrate pathways. According to Vernon (1981), in the non-lactating ruminant, glucose oxidation via the pentose phosphate pathways is responsible for 50-80% of NADPH, a reducing equivalent, required for fatty acid synthesis in adipose tissue.

Adipose Deposition

The three main depots for fatty acid deposition are; intermuscular (or seam fat), subcutaneous and intramuscular (or intrafascicular) fat. (Ingle et al., 1972). Site of adipose deposition is variable based on factors such as, physiological status, genomics, age, body weight, and nutrition. Fat partitioning of adipose tissue changes during growth; fat deposits intramuscularly at a greater rate later in life. Depending on breed, trait selection and other factors, intramuscular fat will typically increase exponentially until plateauing near time of slaughter (Vernon, 1981; Scollan et al., 2001).

Intramuscular adipose tissue depots have been shown to have smaller adipocytes (Hood and Allen, 1973; 1978) and a reduction in lipogenic rates (Hood and Thornton, 1980) than subcutaneous adipose. According to research by Smith and Crouse (1984), intramuscular adipose tissue is less sensitive to dietary manipulations than subcutaneous adipose tissue. This suggests a non-coordinated regulation of lipogenesis between intramuscular and subcutaneous depots with high grain fed cattle. Results of their data showed no effect of age or diet on enzyme activity and glucose incorporation into FA in intramuscular adipose tissue.

Genomics

Lipid metabolic genes such as those involved in lipogenesis, lipid uptake, fatty acid esterification, lipolysis and fatty acid oxidation may contribute to fatty acid deposition. Via genomic analysis, fatty acid synthase (FASN), Stearoyl-CoA desaturase (SCD) and Lipoprotein lipase (LPL) have been found to be more abundant in adipose tissue and identified as key genes in ruminant lipid metabolism (Bakhtiarizadeh et al., 2013). Both LPL and FASN are important enzymes in adipocyte lipogenesis. Their mRNA expressions were found to be greater in subcutaneous fat than in the intramuscular fat tissue (Pickworth et al., 2011). This indicates that subcutaneous adipose tissue has more opportunity for fat deposition than intramuscular adipose tissue.

Leptin is a protein hormone synthesized by the adipocytes in white adipose tissue (Della-Fera et al., 2001; Liefers et al., 2002). Therefore, the amount of circulating leptin in the blood corresponds to the amount of fat in the body. The leptin gene and circulating leptin concentration hve been positively correlated to marbling in cattle is (Nkrumah et al., 2005;

Geary et al., 2003). Leptin also plays an important in the regulation of food intake and body weight; it is an inhibitor of appetite and/or stimulatory of satiety.Stearoyl-CoA desaturase 1 (SCD1) is an enzyme involved in catalyzing the desaturation of saturated fatty acids to monounsaturated fatty acids (MUFA) (Li et al., 2013). Previous research has indicated that Japanese Black cattle, or Wagyu, deposit a greater amount of intramuscular adipose (Yamazaki, 1981) and a higher concentration of MUFA in subcutaneous adipose tissue than Angus cattle (Sturdivant et al., 1992). Thus it is supposed that the stearoyl-CoA desaturase gene expression may be responsible for this difference in fatty acid composition. In addition, it has previously been found that increasing n-3 PUFA composition in the diet caused a significant reduction in SCD expression in subcutaneous adipose tissue of Holstein bulls (Hiller et al., 2011). Moreover, alpha-linolenic acid (C18:3n-3) inhibited SCD gene expression in the adipocytes of mice (Sessler et al., 1996).

In addition to the aforementioned enzymes and genes, the esterification genes glycerol-3-phosphate acyltransferase 1 (GPAT1) and adipose triglyceride lipase (ATGL) have been found to have great mRNA abundance and therefore may be major variables in predicting intramuscular deposition in the LM tissue (Jeong et al., 2012).

Alternative evidence suggests that mutations in the growth/differentiation factor-8 (GDF8), a gene negatively correlated to skeletal muscle development, may cause an increase in muscle (McPerron et al., 1997). Thus, selecting for increased muscle growth results in fewer and lower growth rates of “islands” of fat cell development and smaller adipocytes in marbling deposits result in less intramuscular fat deposition (Wegner et al., 1998).

Lipid Digestion

In ruminants, lipid digestion begins in the reticulorumen. Short chain FAs are absorbed through the rumen wall, enter the blood and are shuttled to the liver and oxidized. As TG from the diet enters the rumen, lipases cleave FA's from the glycerol backbone. The free fatty acids are then susceptible to microbial biohydrogenation, the addition of two H+ ions, and are later absorbed and deposited within muscle in the saturated form (Laugh and Smith, 1976).

Lipids entering the small intestine are primarily long chain, saturated non esterified fatty acids (NEFA) and phospholipids (Noble, 1981). For proper digestion, bile and pancreatic secretions are required to solubilize and emulsify FAs. Following their absorption into the intestinal cell, they enter the lymph system packaged as very low density lipoproteins (VLDL) or chylomicrons. Via plasma lipoproteins, lipids are transported to tissues (Bauchart, 1993).

Unsaturated fatty acids are toxic to rumen microbes and hinder digestion, specifically fiber digestion. Rumen homeostasis has a pH of 5.5-6.5 to provide optimal conditions for microbial function. Grain consists of high starch content and is rapidly digested. This results in increased rumen acidity, causing high amounts of lactobacilli, which can ultimately lead to acidosis. Unsaturated fatty acids in the rumen have the potential to depress DMI, impair fiber ingestion, is toxic to the microbial population, and it may impair rumen fermentation (Palmquist and Jenkins, 1980; Pantoja et al., 1994). Therefore biohydrogenation is beneficial to maximize microbial population and activity in the rumen. Within the dairy industry, the maintenance of rumen health is critical for optimal long-term production. However, in beef production, muscle laydown and intramuscular fat deposition is the primary objective.

Rumen Bypass Fat

Dietary fat is a great source of energy; supplementation of rumen protected fat increases apparent FA digestibility (Palmquist, 1991) and was originally used to meet the high energy requirements of high producing dairy cattle. Rumen protected fats, originally created by Jenkins and Palmquist (1984), are insoluble lipids formed by the reaction of palm fatty acid distillate and calcium hydroxide. They are commonly termed calcium soaps (or salts) and have low solubility in the rumen and thus are less susceptible to biohydrogenation (Laugh and Smith, 1976). Unsaturated fatty acids are more readily digestible than SFA; therefore post ruminal dissociation of calcium soaps increases UFA availability and absorption and lead to improve production efficiency. Wu et al. (1991) found that dairy cows fed protected fats made from palm oil, as opposed to animal-vegetable oil, more effectively prevent against biohydrogenation and increase digestion of FA.

Megalac, a granular supplement, was the first bypass fat product on the market and is an efficient energy source known to enhance animal performance. Megalac is commonly used in the dairy industry to provide the necessary energy required for the high producing dairy cow; it provides 2.96 Mcal per pound, is equal to 270% total digestible nutrients (TDN) and is greater than 85% digestible in the small intestine (Church and Dwight Co. Inc., 2011a). Research has indicated that with proper supplementation of Megalac, it increases average milk yield (Andrews et al., 1991), net energy for lactation, (USDA, 1991) and reproduction efficiency (Staples, 1998). Recommendations are based on physiological status of the animal with a maximum of 0.907 kg/head/day (Church and Dwight Co. Inc., 2011b).

The dissociation of calcium soaps into their calcium ion and free fatty acids is variable; the FA chain length, degree of saturation, and rumen pH will affect how quickly FA dissociates from the calcium backbone. According to Block et al. (2005) in trials using bypass fat in dairy cattle, it has a pK value of approximately 4.5, which is the pH at which 50% of the calcium salt dissociates. Shorter chained and unsaturated FA have a slightly higher pK value; a greater proportion of these calcium soaps will dissociate at a given rumen pH. Bypass fat functions optimally at a rumen pH of 5.5-6.5 (Sukhija and Palmquist, 1990) at which 60-90% of the calcium soaps remain intact and are able to escape biohydrogenation in the rumen for subsequent digestion in the abomasum and UFA absorption in the small intestine (Block et al., 2005).

Due to consumer pressure to minimize negative health implications of red meat consumption, research to optimize the fatty acid composition of ruminant products has been conducted in order to minimize the saturated fatty acid content and increase unsaturated fatty acids in ruminant products while maintaining typical grain-based feedlot finishing diets. One way proposed to do this is to increase the intramuscular unsaturated fatty acid concentration in grain fed steers by supplementing rumen bypass fat during finishing period. Based on the literature, rumen bypass fats protect unsaturated fatty acids from the harsh environment of the rumen allowing them escape biohydrogenation, therefore entering the small intestine as unsaturated and being more efficiently utilized by the ruminant (Church and Dwight Co. Inc., 2011a). It is hypothesized that rumen bypass fat supplementation high in PUFA during finishing period will increase circulating PUFA in blood as well as in meat in steers on high corn-based finishing rations.

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CHAPTER III

NATIONAL AIR QUALITY SITE ASSESSMENT TOOL VERSION 2.0

INTRODUCTION

Version 1.0 of The National Air Quality Site Assessment Tool (NAQSAT) was initiated in 2007, led by Dr. Wendy Powers of Michigan State University and completed in 2010. The purpose of NAQSAT is to allow producers and their advisors of livestock and poultry production facilities, including the species beef, dairy, swine, broiler chickens, laying hens and turkeys, to qualitatively and anonymously assess the mitigation of air emissions at their particular facility. The air emissions of the greatest concern are odor, particulate matter (dust), ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4) and Volatile Organic Compounds (VOCs). Eight management categories are assessed: animals and housing, feed and water, collection and transfer, manure storage, land application, mortalities, on-farm roads and perception.

The NAQSAT provides users the ability to run a hypothetical scenario to see how particular modifications to their facility may positively or negatively change air emissions prior to performing costly management or infrastructural changes. With this feature, tradeoffs and unintended consequences regarding all of the emission gases may be identified. Each air emission result, within each management category, is displayed as an individual green/white box according to how well producers are implementing management practices to mitigate emissions once the base survey is completed. The tool qualitatively assesses a facility based on the existing infrastructure and is not meant to be used comparatively between multiple facilities.

As concern over health and environmental impacts of livestock production raises, so does the need for a broad, nationally applicable tool for anonymous self-evaluation. Advancements in knowledge and scientific findings are ever-growing. Further development of the NAQSAT version 2.0 will allow livestock, poultry and equine producers to identify problematic management areas according to the most current knowledge available from expert scientists around the country. Subsequently they will be directed towards management strategies to maximize stayability, profitability and animal production.

MATERIALS AND METHODS

This study was collaboration between the United States Department of Agriculture's Natural Resource Conservation Service, the Department of Animal Science at Colorado State University in 2012 to create version 2.0 of the National Air Quality Site Assessment Tool.

Experimental Design

Version 2.0 of NAQSAT was initiated in 2012, led by Dr. Shawn Archibeque of Colorado State University and completed in 2014. Expert Scientists from around the United States collaboratively reviewed the science and knowledge pertaining to their area of expertise. Biannually, NAQSAT team members gathered to review and advance the entire tool.

RESULTS

All aspects of the tool were reviewed and updated by experts according to the most up-to-date knowledge and research. In addition to the update, the species horse was added to the tool.

Upon viewing the results page, by clicking on a particular emissions green/white bar, a popup page appears with pdf links to the NRCS practice standards that may be applicable to this particular air emission. This is meant to guide the user to practice standards that may or may not be implemented at their facility which could assist in reaching management goals. A post-survey questionnaire has been implemented of a to gain feedback by the user for even further advancements of the tool.

DISCUSSION

Via the use of the NAQSAT version 2.0, livestock producers and their advisors are provided a qualitative evaluation of the management practices at their facility with full anonymity. The user may run a “what if” scenario to discover tradeoffs and unexpected consequences of changed management practice that otherwise may not have been considered. Specific concerns pertaining to particular management practices and/or emissions of concern may be assessed. Following utilization of the NAQSAT, the users are directed to potential NRCS practices that may assist in their management goals. The aim of NAQSAT is improve the management practices appropriate for site-specific facilities to reduce air quality impacts associate with livestock production. This is turn will maximize the efficiency of livestock production.

CHAPTER IV

SUPPLEMENTATION OF RUMEN PROTECTED FAT IN CORN FED BEEF STEERS

INTRODUCTION

In February 2014, the United States Department of Agriculture National Agriculture Statistics Service (USDA NASS, 2014) reported a total of 10.8 million cattle and calves in U.S. feedlots (with capacity of 1,000+ head) for slaughter. Typically, the majority are fed high concentrate diets, which is ideal for rapid intramuscular adipose deposition, a characteristic desired for better meat quality (Vernon, 1981). Ruminant fat is an important dietary energy source consisting of predominantly saturated fatty acids (SFA), palmitic acid (C16:0) and stearic acid (C18:0), and primary unsaturated fatty acid (UFA) oleic acid (C18:1) (Whetsell et al., 2003). Naturally occurring *trans* fatty acids found in animal products are conjugated linoleic acids (CLA) and vaccenic acid (18:1, *trans*-11), (Hodgeson et al., 1996). They are produced from PUFA via biohydrogenation (Kepler et al., 1996), and are known to be beneficial to human health.

Trans fatty acids are commonly known for their negative health implications. However, naturally produced trans fats found in animal products are structurally different than those found in processed foods (Belury, 2002) and in fact, may decrease health risks (Willett et al., 1993). Conjugated linoleic acids are isomers of linoleic acid (18:2) with conjugated double bonds, most notably *cis*-9, *trans*-11-octadecadienoic acid (*c*9, *t*11-18:2) (Ha et al., 1987, 1990). The American Dietetic Association suggests that CLA may alter cancer carcinogenesis (1999).

Research has indicated that CLA have anticarcinogenic effects (Ha. et al., 1990; Ip et al., 1994; Whigham et al., 2000; Belury et al., 2002; Parodi et al., 2002), reduce risk of diabetes (Moloney ey al., 2004), enhance immune response (Ip et al., 1994, Nugent et al., 2005, Riserus et al., 2004, Moloney et al., 2004), reduce LDL plasma cholesterol levels, and suppress cholesterol-induced atherosclerosis (Lee et al., 1994; Nicolosi and Laitinen, 1996). While Long-chain omega-3 fatty acids have been correlated to improved immune and cardiovascular function in humans (Jump et al., 1997; Clarke, 2001; Kris-Etherton, 2002) as well as improved reproductive efficiency and carcass characteristics of ruminant animals (Mattos et al.,2000; Ponnampalam et al., 2001).

According to numerous studies, CLA has been found in greater concentration in intramuscular adipose of grass-fed beef cattle than in grain-fed (French et al. 2000; Lawless et al., 1998; yang et al., 2002; Nuernberg et al., 2005). Alternatively, research indicates that grain-fed beef has a greater SFA concentration (Realini et al., 2004) and a lesser PUFA: SFA ratio than grass-fed beef (Duckett et al., 1993).Saturated fatty acids may have negative health implications, such as increased serum low-density-lipoprotein (LDL) cholesterol concentration and increase risk of coronary heart disease (Keys, 1970). Due to the correlation between grain-fed beef and negative health implications, the World Review of Nutrition and Dietetics strongly advocates for producers to improve the lipid profile: increasing dietary CLA and long-chained omega-3 fatty acids while minimizing SFA and trans fatty acid intake (Simopoulos, 1998). It is recommended to decrease the intake of SFA from 15% down to 10% of total energy intake and to increase the PUFA: SFA ratio to greater than 0.4 (Department of Health, 1994). However, while increased PUFA:SFA is advised, oxidative stress has been associated with increased PUFA intake due to increased susceptibility to lipid peroxidation (Lee et al., 1989).

With aims to increase the SFA concentration of beef intramuscular adipose, research is being conducted in order to minimize the SFA concentration and increase the UFA concentration in ruminant products while maintaining typical, cost efficient, grain-based feedlot finishing diets. One experimental method includes the supplementation of rumen-protected fats. They are insoluble lipids that bypass the rumen and are utilized as an energy source upon absorption in the small intestine. Fat supplementation increases apparent FA digestibility (Palmquist, 1991) and was originally used to meet the high energy requirements of high producing dairy cattle. Unsaturated fatty acids are more readily digestible than SFA; therefore increased UFA absorption may improve production efficiency.

The objective of this particular study is to improve meat fatty acid composition in grain fed beef steers by the inclusion of rumen bypass fat to grain-based finishing rations. UFA are protected from the harsh environment of the rumen allowing them to escape microbial fermentation and biohydrogenation, therefore entering the small intestine as unsaturated and being more efficiently utilized by the ruminant (Richard Webster Nutrition LTD). Through extrapolation of bypass fats efficiency in dairy cattle, it is hypothesized that rumen bypass fat supplementation high in UFA during finishing period will increase the circulating UFA in blood and consequently deposition into intramuscular meat in steers fed high corn-based finishing rations.

MATERIALS AND METHODS

This study was collaboration between the Colorado Corn Administrative Committee, the Colorado Growers Association and the Department of Animal Science at Colorado State

University in 2012 to investigate the supplementation of rumen protected PUFA to increase muscle PUFA in corn fed beef steers.

Experimental Design

A total of 126 cross-bred steer calves (initial BW 529.5kg ± 10.7) were housed at SECRC (Southeastern Colorado Research Center, Lamar, CO). The Institutional Animal Care and Use Committee (IACUC) approved the care and use of animals for Colorado State University for this trial. Steers arrived at SECRC 90 days prior to trial commencement and offered ad libitum access to alfalfa hay for back grounding. Steers were processed for ear tags, initial body weight and rectal temperatures were recorded. Steers were ranked by body weight and housed 9 steers/pen (n = 7 pens / treatment).

Dietary Treatments

Following the 90 day adaptation period, on June 12, 2012, each pen was randomly assigned to one of two treatment groups; rumen bypass fat treatment (control diet + Megalac-R/head/day) or control diet (regular corn based finishing ration) (Table 1). The bypass fat (BF) diet was formulated to be isocaloric and isonitrogenous. Both diets contained 13.5% CP. Based on Megalac-R feeding recommendations for dairy cattle and the maximum allowable fat concentration recommended by feedlot nutritionists (Wagner, 2014), rumen bypass fat supplement was mixed into the total mixed ration (TMR) at 4.305% DM. Treatments were randomly assigned to pens such that 63 animals (7 pens with 9 head/pen) received same diet. Treatment lasted a total of 60 days. Animals were fed twice daily (approximately 7am and 5pm) at an estimated 110% of the previous daily ad libitum intake. Diets were mixed in a truck mounted feed processer. Prior to feeding, rumen bypass fat was added to the feed processor. Feed bunks were cleaned and orts collected on a weekly basis before morning feeding. Dry matter content was analyzed. Diet sample was collected weekly following morning feeding and analyzed for proximate analysis.

Sample Collection

By pen, steers were herded individually through the squeeze chute located at SECRC. Body weights were recorded and blood samples collected on d -54, -10, 27, 60 and 61. Approximately 12ml of blood was taken from the jugular vein using 20 gauge, 1 ½ mL vacutainer needles at each collection for fatty acid analysis. Serum from each sample was collected and stored in -80^{0}C for fat determination. Live weighs were recorded for two consecutive days prior to slaughter. Steers were transported and harvested at a commercial slaughterhouse (JBS Swift Slaughterhouse, Greeley, CO) on d 62 and were slaughtered according to industry-accepted procedures.

Muscle and adipose samples were collected from the *longissimus dorsi* regions, flash frozen and stored at -80^{0}C for fatty acid composition determination before chilling and were immediately placed into whirlpaks and into liquid nitrogen for flash freezing..

Animal harvest and Carcass Evaluation

Upon slaughter, carcasses were weighed to obtain HCW. Muscle and adipose samples were dissected from the *Longissimus dorsi* region, flash frozen and stored at -80°C for subsequent pulverization and fatty acid determination. Following the carcass-chilling period, carcass quality characteristics were assessed according to standard techniques (USDA, 1997) by trained Colorado State University personnel; marbling, quality grade, fat thickness, LM area, KPH, preliminary yield grade, final yield grade and dressing percent.

Fatty Acid Composition

Three steers were randomly selected from each experimental unit (pen). Muscle and adipose tissue were pulverized using a Waring blender . Blood serum samples were pooled for each experimental unit. Total lipid from blood serum and intramuscular adipose was extracted using the procedures outlined by Folch et al. (1957) followed by methylation as described by Morrison and Smith (1964). Fatty acid methyl esters (FAME) were determined by gas chromatography (model Hewlett-Packard, 6890 series II) equipped with a SP-2560 fused silica capillary column (100-m x 0.25-mm i.d.; Supelco Inc. Bellefonte, PA), with a series 7683 injector and flame ionization detector. Helium was used as the carrier gas with a flow rate of 2.1 mL/min. Fatty acids were identified by comparing the retention times displayed on the chromatographs to that of reference standards.

RNA Isolation and Quantitative RealTtime PCR (qtRT-PCR)

Total RNA from frozen adipose tissues was isolated with TRIzol reagent (Invitrogen, NY, USA). Isolated RNA was further purified by using RNeasy Mini Kit (Quiagen, CA, USA) with RNase-free DNase (Quiagen, CA, USA) and quantified by use of a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, DE, USA). Synthesis of cDNA was performed using iScriptcDNA synthesis kit (Bio-Rad, CA, USA). Real-time PCR was performed for the targets leptin, lipoprotein lipase (LPL), fatty acid synthase (FASN) and stearoyl-CoA desaturase (delta-9-desaturase, SCD) target mRNA and housekeeping GAPDH mRNA (Table 1). Each analysis was performed in 9 µL reaction mix, containing iQ SYBR green supermix (Bio-Rad, CA, USA), primer, and nuclease free water, and 1 µL cDNA. Real-time PCR was conducted using the LightCycler480 PCR system (Roche Applied Sciences, IN, USA) with 384-well plates. The PCR cycle conditions were as follows: 95℃ for 3 min, 40 cycles of 95℃ for30 sec, 61℃ for

30 sec, and 72℃ 15 sec followed by a melt curve analysis to confirm amplification of single cDNA products. The Ct values were obtained from the Lightcycler software (Roche Applied Sciences, IN, USA) and adjusted for GAPDH Ct values for each sample to determine ΔCt and relative expression of the target mRNA. Data were presented $2^{-\Delta\Delta Ct}$ (Schmittgen and Livak, 2008).

Statistical Analysis

This study was conducted as a completely randomized block design with repeated measures, consisting of two treatment groups with 63 animals per treatment, divided into 7 pens. In order to minimize the effect of initial body weight, steers were assigned into 1-7 weight blocks based off of body weight at d 0. Rumen bypass fat treatment was randomly assigned into 7 pens, while the other 7 were fed the control diet. Analysis of Variance (ANOVA) was performed on feedlot performance, fatty acid composition and continuous carcass data were analyzed on a pen mean basis using PROC MIXED of Statistical Analysis Software (SAS Institute, Inc., Cary, NC). The model includes fixed effect of treatment, random effect of body weight, USDA quality and yield grades, incidence of morbidity and mortality and fatty acid profile. The treatment difference was detected using a modified students test. Responses are reported using LSmeans ± standard error. Significance was determined at $P<0.05$ and tendency at $P<0.10$.

RESULTS

Feedlot performance and carcass characteristics are presented in table 2. Steers fed the CON treatment had greater DMI (10.14kg vs. 8.77kg; $P<0.02$) and ADG (1.699kg vs. 1.469kg;

P<0.09) (Table 2). Hot carcass weight was not significantly different between treatments (*P*<0.19). Marbling score (P<0.04) and quality grade (*P*<0.02) were greater for steers fed the CON diet than those fed BF. The *L. dorsi* area tended to be greater (*P*<0.10) in steers fed CON (87.60cm^2) than those fed BF (84.88cm^2). Gain: feed was slightly increased for the BF treatment group, but was not significant (P<0.74). Numerically, there was an increase in unsaturated FA in BF diet in blood and muscle, although this is not statistically significant (table 3 and 4).

The fatty acids palmitoleic acid (C16:1) and oleic acid (C18:1 c9) had a Trt x Time interaction in the blood (table 4). Oleic acid was significantly increased in the BF treatment at d60. Fatty acid composition in the CON diet, BF diet and Megalac-Rsupplement alone is shown in Table 5. The fatty acids in largest quantity are palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2).

The leptin mRNA expression tended (*P* < 0.0878) to be less in steers fed a BF diet when compared to those fed control diet (Fig. 1). There were no significant differences between the treatments in LPL (*P* = 0.7212), FASN (*P* = 0.4469) and SCD (*P* = 0.1411) mRNA expression.

DISCUSSION

Greater than 10 million cattle and calves are currently on feed for slaughter market in U.S. feedlots (USDA NASS, 2014), which feed 60-90% grain. Research has found a greater SFA concentration in grain-fed beef (Realini et al., 2004), which is shown to induce increased serum low-density-lipoprotein (LDL) cholesterol concentration and have been correlated to an increased risk of coronary heart disease (Keys, 1970).

Due to the correlation between grain-fed beef and negative health implications, minimizing dietary SFA intake is recommended to curb these risks (Department of Health, 1994). In comparison to grain-fed beef, grass-fed steers naturally produce leaner beef and have an increased UFA content, which are beneficial to human health. Grass finished beef are greater in conjugated linoleic acid and omega-3 FA (Noci et al., 2007; Nuernberg et al., 2005). Conjugated linoleic acids lower bad LDL cholesterol, have anti-carcinogenic effects and reduce the risk of obesity. Omega-3 FA decrease blood pressure and the likelihood of coronary heart disease (Keys, 1970). Thus consuming grass-fed beef with a greater UFA concentration may reduce the risk of diseases.

There are, however, limitations associated with grass finishing. It is not environmentally or economically feasible to raise all cattle on pasture. Hay quality is limited; it is variable based on season and differs in nutritional value making it more difficult to ensure adequate animal nutrition. High forage based rations require greater land and time requirements per animal. This poses a problem; as urban areas continue to grow, less land and fewer resources are available or viable for agricultural activity. In order to keep up with consumer demand, the industry must implement reformed management practices in order to ensure stayability. Thus, it is more practical to confine many animals to a smaller area and feeding more readily digestible feed (i.e. grain). This allows the animal to reach slaughter weigh more quickly and contribute less to environmental concerns. This is possible through improved management practices.

While grain-fed beef have undesirable characteristics regarding SFA concentration, grass-fed beef has its downfalls as well. Grass-finished beef is less palatable than grain fed beef (Daley et al., 2010), and may have darker meat and more yellow colored fat (Realini, 2004) The preferred taste of grain fed beef is due to the double bonds of the trans orientation in ruminant unsaturated fatty acids due to microbial fermentation in the rumen causing a lower melting temperature than saturated fatty acids (Wood et al., 2007).

Grain finishing is more cost effective for the producer and consumer as the animal reach as the animal reaches slaughter weight faster with greater IM fat deposition (marbling) and thus greater meat quality (Vernon 1981). Due to the improved production efficiency and fatty acid profile achieved using rumen bypass fats within the dairy industry (Staples, 1998; USDA, 1991; Andrews et al., 1991); it is supposed that it could potentially do so in beef cattle whilst alleviating human health concerns as well.

The CON and BF diets were formulated to be isocaloric and isonitrogenous. The rumen protected fat product used, Megalac-R, was included in the BF diet at the expense of primarily tallow (Table 1). Fatty acid composition was measured for the CON diet, BF, and Megalac-R supplement itself. Comparatively, the BF diet was high in palmitic acid (C16:0) and oleic acid (C18:1) (table 2). Both of which are constituents of palm oil.

The effects of rumen protected fat on feedlot performance and carcass characteristics were in favor of the CON treatment group. Dry matter intake was significantly greater (*P*=0.02) for the CON steers. This corresponds to the tendency of CON to have a greater ADG (*P*=0.07) in comparison to BF treatment. Increase feed intake and BW gain coincides with the tendency for a greater *l. dorsi* area in the CON treatment group (*P*=0.10). Decreased DMI of BF steers was not unexpected as previous research indicates that rumen bypass fat is known to slightly depress feed intake due to decreased palatability. Our results show that, although DMI decreased in BF steers, final body weight was not significantly different between CON and BF steers. Marbling score and quality grade were greater for the CON than BF (*P*=0.04 and *P*=0.02, respectively). This may be attributed to increased intramuscular fatty acid deposition in steers fed the CON diet as a result of greater feed intake and subsequently greater weight gain. Carcass characteristics were statistically analyzed as categorical data assuming binomial distribution; quality grade for all steers were either select or choice and either had slight or small marbling scores. These data suggest that rumen bypass fat can be added to finishing diets without significant reduction in final body weight, although there may be modest reductions in marbling and quality scores.

No significant effects of rumen protected fat on FA composition in muscle tissue were found (table 4). However, significant treatment by time interactions were observed between treatment groups for unsaturated fatty acids palmitoleic acid (C16:1) and oleic acid (C18:1 c9) in fatty acid composition in blood serum (P=0.02 and P=0.04, respectively) (table 5). At d 60, the BF diet had a significantly decrease in C16:1(P=0.02). Alternatively, at d 60, the BF diet was significantly increased (P=0.02) in C18:1 c9 than was the CON diet. This tradeoff may be explained by the high C18:1 concentration in the BF treatment diet. Day intervals in table 4 were not expressed for fatty acids where there was no treatment by time interactions.

Due to the treatment by time interaction seen in the blood serum at d60, a longer feeding period may be necessary for increased effects of rumen protected fat on the fatty acid composition of blood serum, which may result in alteration of muscle fatty acid composition. As FA enters the circulatory systems, free fatty acids are transported in the blood bound to albumin while other lipids are transported packaged as lipoproteins and delivered to tissues. Continued increase in circulating FA and thus increased delivery to tissues, will potentially produce a greater content of these FA in the muscle tissue. Further investigation with a longer feeding period is needed to elucidate rumen bypass fat supplementation effects in beef muscle.

The low rumen pH caused by high concentrate diets typically fed to feedlot cattle may contribute to minimal changes of fatty acid profile. Rumen protected fats have been effectively used within the dairy industry for years to meet the increased energy requirements of the lactating dairy cow. Due to the differences in feed formulation of dairy versus feedlot cattle, bypass fat function differs at the opposing rumen pH. High forage concentrations of dairy cattle yield an increased pH, while the higher concentrate diets of feedlot cattle decreases rumen pH. Rumen protected fats are more stable and work optimally at a normal rumen pH of 5.5-6.5. As pH decreases in the rumen, calcium soaps more readily dissociate leaving the free fatty acids susceptible to biohydrogenation into their saturated form for subsequent absorption. Therefore by increasing rumen pH more FA may stay intact with the calcium ion, escaping biohydrogenation and dissociate in the abomasum and duodenum for subsequent digestion. Research of this product being used in beef cattle is limited.

Leptin, a protein hormone synthesized and secreted by adipocytes in white adipose tissue (Della-Fera et al., 2001; Liefers et al., 2002), plays a role in the regulation of food intake as an appetite inhibitor and an inducer of satiety. Moreover, the bovine leptin gene has been shown to be related to marbling (Nkrumah et al., 2005). In this study, mRNA expression of leptin in adipose tissue was reduced by rumen-protected fat supplementation. This is reasonable in that steers fed the CON diet had increased DMI and marbling scores therefore have greater amounts of adipose tissue thus increased leptin production. This supposition is reinforced by the positive correlations existing between marbling score and serum leptin in cattle as reported by Geary et al. (2003). Reduced leptin expression in adipose tissue would decrease circulating concentration of leptin, therefore, it seems reasonable that marbling score could also decrease.

Other genes related to lipogenesis, including LPL, FASN and SCD were observed. LPL and FASN are important enzymes in adipocyte lipogenesis and have been found to have greater mRNA expression in the subcutaneous fat than in the intramuscular fat tissue (Pickworth et al., 2011). This may explain the similar levels of LPL and FASN expression observed from the frozen tissue between treatment groups. While marbling score of carcass did statistically differ between groups, similar fat thickness was observed in the present study.

Stearoyl-CoA desaturase 1 is an enzyme involved in the catalyzing the desaturation of saturated fatty acids to monounsaturated fatty acids (MUFA) (Li et al., 2013). It has previously been found that increasing n-3 PUFA composition in the diet caused a significant reduction in SCD expression in subcutaneous adipose tissue of Holstein bulls (Hiller et al., 2011). Moreover, alpha-linolenic acid (C18:3n-3) inhibited SCD gene expression in the adipocytes of mice (Sessler et al., 1996). However, result of the current study showed no significant inhibition of SCD gene expression in response to BF diet. Furthermore, MUFA composition of *longissimus dorsi* muscle was not affected by rumen-protected PUFA supplementation. In a study similar to this one, Wynn et al. (2006) observed no effect of Megalac supplementation on SCD mRNA expression level in sheep. Other investigators argued that there was no correlation between Δ9desaturase index and SCD gene expression in subcutaneous fat (Corazzin et al., 2012), and the Δ9desaturase index does not correctly reflect the enzyme activity in cattle (Archibeque et al., 2005).

Although the beef industry has been a target of public and consumer criticism, all forms of agriculture is what feeds the ever-growing world. As population increases, urban areas are growing and rural areas are diminishing. Therefore land availability for crop and livestock is limited and the demand for food is constantly increasing. Concern regarding where animal products are coming from and their health implications for human consumption continues to gain public attention. Grain-finished beef cattle have a higher concentration of saturated fatty acids yet grain takes less time and land to produce and allow for cattle to reach slaughter weight quicker than grass-finished cattle. If we can successfully minimize the biohydrogenation of PUFA into SFA and in turn increase intramuscular PUFA while maintaining the grain based diet and supplementing rumen protected fat, it could be a huge advancement within the beef and corn industries. This could also improve human health, decrease occurrence of cardiovascular disease and other known associated ailments and alleviate the concerns of the public.

These data suggest that rumen bypass fat can be added to finishing diets without significant reduction in final body weight, although there may be modest reductions in marbling and quality grade. Few significant differences were seen in the muscle and serum fatty acid profile. Adjustment of finishing diet formulation in favor of a more basic pH may increase PUFA in intramuscular adipose, although this may result in decreased ADG and therefore a longer time requirement to reach slaughter weight. Potential future research to consider for a significantly increase UFA content include feeding rumen bypass fat for an extended time period as opposed to the 60 days fed in this trial. Decreasing distiller's grains in the diet or adding bicarbonate would theoretically raise rumen pH and allow more UFA to pass through the rumen without becoming saturated.

Table 1. Experimental diet on DM basis.

	Treatment	
Ingredient Basis: % of DM	Control	BF[1]
Corn Silage 50% Gr	9.78	9.78
Corn Grain Flaked	75.56	76.62
Distillers Gr. + Soluble	4.56	4.23
Corn Steep	3.00	3.00
Urea	1.22	1.22
Tallow	3.79	---
Limestone	1.47	0.23
Potassium Chloride	0.19	0.21
Salt	0.25	0.25
Trace Mineral	0.11	0.16
Megalac-R	---	4.30

[1] Bypass fat. Diets were formulated to be isocaloric and isonitrogenous.

Table 2. Effects of rumen protected fat on feedlot performance and carcass characteristics

	Treatment			
Parameter	Control	Protected fat[1]	SE	P <
Initial BW, kg	537.9	521.1	10.7	0.29
Final BW, kg	594.0	570.3	11.2	0.16
ADG, kg	1.699	1.492	0.07	0.07
DMI, kg	10.14	8.772	0.36	0.02
Gain: Feed	0.168	0.171	0.01	0.74
HCW, kg	360.47	345.8	7.44	0.19
Marbling score[2]	402.9	374.1	8.89	0.04
Quality grade[3]	384.2	362.7	5.50	0.02
Dressing, %	60.67	60.63	0.24	0.90
Adjusted fat thickness, cm	1.035	0.997	0.08	0.73
LM area, cm^2	87.60	84.88	1.10	0.10
Yield grade	3.019	2.982	0.08	0.74

[1] Diets were formulated to be isocaloric and isonitrogenous.

[2] Practically Devoid = 100; Traces = 200; Slight = 300; Small = 400; Modest = 500; Moderate =600; Slightly Abundant = 700; Moderately Abundant = 800; Abundant = 900.

[3] Standard =200; Select = 300; Choice = 400; Prime = 500

Table 3. Effects of rumen protected fat on fatty acid composition of beef *longissimus dorsi* muscle (% of total fatty acids reported).

	Treatment			
Fatty acid	Control	Protected fat[1]	SE	P <
C14:0	3.67	3.44	0.15	0.29
C14:1	0.27	0.26	0.03	0.82
C15:0	0.64	0.59	0.05	0.74
C16:0	25.30	25.45	0.53	0.42
C16:1 c9	1.11	0.85	0.36	0.41
C17:0	1.60	1.46	0.17	0.67
C18:0	20.07	19.49	1.02	0.45
C18:1 c11-15	6.37	6.06	0.42	0.65
C18:1 c9	34.68	36.14	0.65	0.76
C18:1t (total)	3.71	3.53	0.21	0.84
C18:2 Total	1.77	1.76	0.10	0.44
C18:2t[2]	0.59	0.68	0.07	0.29
C18:3 n-3	0.22	0.24	0.01	0.74
C20:1 c11	0.18	0.14	0.02	0.38
C22:5 n-3	0.02	0.02	0.01	0.87
Saturated	51.09	50.30	0.18	0.86
Unsaturated	48.91	49.70	0.19	0.84
MUFA	46.31	47.00	0.12	0.68
PUFA	2.60	2.70	0.08	0.21
Sat.:Unsat.[3]	1.04	1.01	0.02	0.41

[1] Diets were formulated to be isocaloric and isonitrogenous.

[2] Includes C18:2 c9, t11, C18:2 t10 c12, C18:2 c11 t13, and C18:2 tt.

[3] Total saturated fatty acid to total unsaturated fatty acid ratio.

Table 4. Effects of rumen protected fat on fatty acid composition of plasma (% of total fatty acids reported).

	Treatment			P <		
Fatty acid	Control	Protected fat[1]	SE	Trt	Time	Trt x Time
C14:0	0.99	0.93	0.10	0.68	0.34	0.86
C14:1	0.69	0.68	0.09	0.98	0.17	0.87
C15:0	1.17	1.21	0.06	0.61	0.12	0.92
C16:0	21.46	19.46	0.91	0.15	0.16	0.79
C16:1	2.59	2.35	0.12	0.21	0.21	0.02
-54d	2.63	2.39	0.15	0.45	---	---
-10d	2.39	3.06	0.21	0.19	---	---
27d	2.68	2.02	0.14	0.08	---	---
60d	2.65	1.94	0.08	0.05	---	---
C17:0	2.61	2.49	0.13	0.53	0.004	0.67
C17:1	0.88	0.68	0.07	0.08	0.03	0.83
C18:0	14.06	14.01	0.83	0.97	0.50	0.84
C18:1 c9	5.92	6.77	0.65	0.37	0.001	0.04
-54d	8.86	7.36	0.91	0.26	---	---
-10d	4.50	4.08	0.74	0.75	---	---
27d	5.62	7.61	0.62	0.14	---	---
60d	4.70	8.02	0.45	0.02	---	---
C18:1t (total)[2]	2.57	2.60	0.16	0.88	0.07	0.85
C18:2t (total)[3]	3.34	3.48	0.05	0.06	0.80	0.67
C18:2	37.80	39.91	1.40	0.31	0.07	0.95
C18:3 n-3	1.13	1.06	0.14	0.77	0.01	0.96
C20:1 c11	3.05	2.66	0.52	0.61	0.17	0.64
C22:5 n-3	1.88	1.61	0.34	0.60	0.01	0.71
Saturated	40.28	38.08	1.20	0.21	0.13	0.69
Unsaturated	59.72	61.92	1.21	0.21	0.12	0.68
MUFA	12.64	13.09	0.78	0.69	0.03	0.27
PUFA	47.27	48.73	1.61	0.53	0.12	0.97
Sat.:Unsat.[4]	0.68	0.64	0.023	0.24	0.02	0.48

[1] Diets were formulated to be isocaloric and isonitrogenous.

[2] Includes C18:1 t10 and t11.

[3] Includes C18:2 c9, t11; t10, c12; c9, c-11; t9, t11.

[4] Total saturated fatty acid to total unsaturated fatty acid ratio.

Table 5. FA composition in CON diet, BF diet and Megalac-R supplement.

Fatty Acid	Control Diet	BF[1] Diet	Megalac-R
C14:0	0.41	0.17	1.41
C16:0	19.17	34.73	46.59
C16:1	0.35	0.35	0.60
C18:0	2.97	5.37	3.61
C18:1	24.16	31.27	33.94
C18:2	42.48	19.57	9.44
C18:3	8.22	6.72	4.42
C20:0	0.73	0.81	---
C20:1	0.70	0.40	---
C24:0	0.79	0.61	---

[1] Bypass fat. Diets formulated to be isocaloric and isonitrogenous.

Table 6. Real-time PCR primer sequences.

Target gene	Primer sequence	Accession #
GAPDH	F: GATTGTCAGCAATGCCTCCT R: GGTCATAAGTCCCTCCACGA	NM_001034034.2
Leptin	F: TGTGGCTTTGGCCCTATCTG R: CGGACTGCGTGTGTGAGATG	NM_173928.2
Lipoprotein lipase	F: ATACACCAACCAGGCCTTCG R: GCTTTGCCAAGTTTCAGCCA	NM_001075120.1
Fatty acid synthase	F: CTGCCGAAGACAGGGATTGT R: TGTACAGCTTCTGCTGGTGG	NM_001012669.1
Stearoyl-CoA desaturase	F: CCTGTGGAGTCACCGAACC R: CAAAAACGTCATTCTGGAACGC	NM_173959.4

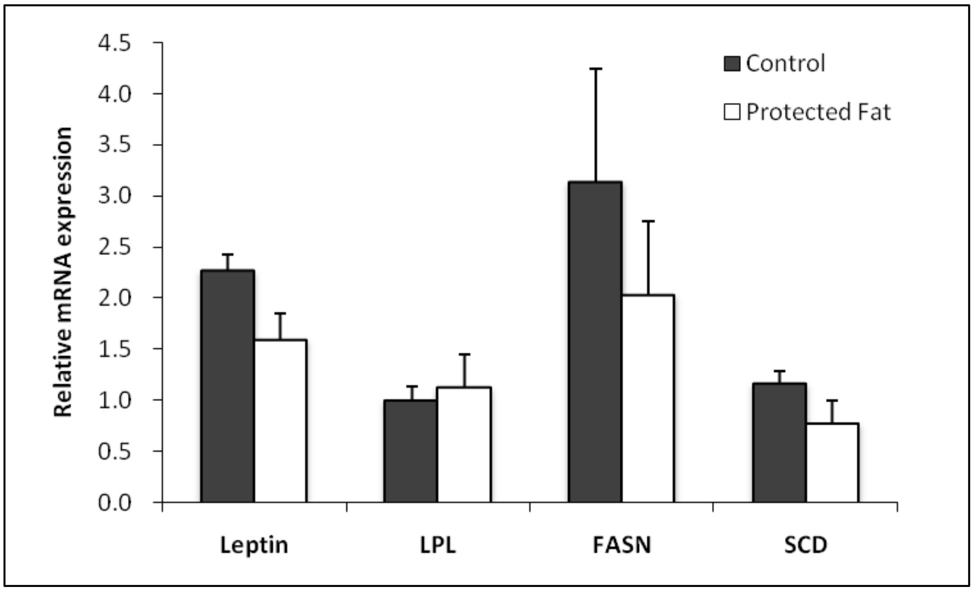

Figure 1. Differentially expressed genes involved in lipid metabolism of steers adipose tissue.[1]

[1] The data are expressed as the means ± S.E. Lipoprotein lipase, LPL; Fatty acid synthase, FASN; Stearoyl-CoA desaturase, SCD.

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