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**EFFECTS OF ESSENTIAL OILS AND AMINO ACID PROFILE ON THE
GROWTH AND HEALTH OF RECEIVING CATTLE**

A Thesis
Presented to
The Faculty of the Hutson School of Agriculture of Animal Science
Murray State University
Murray, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
of Master of Science in Agriculture

by Kelsey Rai Machens
April 2018

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Abstract

Effects of Essential Oils and Amino Acid Profile on the Growth and Health of Receiving Cattle

The objective of this trial was to evaluate the effects of amino acid profile and essential oil products on receiving calf growth and feed efficiency, as well as evaluate the effects of amino acid profile and essential oil products on fecal and blood Immunoglobulin A (IgA) concentrations as a marker for systemic and gastrointestinal tract immune status of receiving calves. One hundred-thirty crossbred calves (500-600 lbs) were transported to the ADM Animal Nutrition Research Facility in Mendon, IL. On d -1, cattle were weighed, dewormed, implanted, vaccinated, and a rectal temperature was recorded. An at-risk group of cattle was targeted for this trial. Based on initial BW and health status, cattle were allotted to 12 pens with 10 calves per pen so that each pen had similar weight and distribution of sick animals. Treatments were randomly assigned to pens with 2 pens per treatment. Initial body weights were similar ($P = 0.99$) among treatments. Cattle were weighed on days -1, 0, 21, 42, 63, and 64. Treatments were arranged in a 2 x 3 factorial design of AminoGain formula (AminoGain 6 or AminoGain 6 Plus) and essential oil product (no essential oil, Stay Strong EO, or Spearmint Extract). Individual animal intake was recorded from d 0-63 using the

GrowSafe[®] cattle feeding system. Stay Strong EO was targeted to deliver 1 g / 100 lb body weight. Based on calculations, 1.07 g/100 lb body weight was delivered. Preliminary analysis indicated the Spearmint Extract was composed of 13% limonene and 70% carvone. The Spearmint Extract treatment targeted to deliver 40 ppm limonene and 220 ppm carvone. Once the analysis came back, the extract actually contained 0.08% limonene and 91.73% carvone. There is a possibility that limonene was oxidized to carvone subsequent to initial analysis. Three animals from each pen were randomly selected for fecal and sample collection. Blood and fecal samples were collected on d 0, 21, and 42 for analyses of IgA concentrations. An interaction of AminoGain 6 formulation by essential oil source resulted ($P < 0.02$) for cumulative ADG. Cumulative ADG did not differ ($P > 0.05$) with the addition of Spearmint Extract or Stay Strong EO to the AminoGain 6 formula. However, the addition of Spearmint Extract worsened ($P < 0.03$) cumulative ADG when added to the AminoGain 6 Plus formula. This was likely the result of cumulative feed intake tending ($P \leq 0.06$) to be lower when the AminoGain 6 Plus x Spearmint Extract was fed. IgA concentrations in blood were not different ($P > 0.05$) as a result of treatment for any of the measured time points. The responsiveness of IgA assay to changing disease status make it an effective tool to assess gross disease state of receiving cattle. Further research is

needed to determine the effects of using essential oil as a feed additive upon health and growth of cattle.

Keywords: Amino Acids, Essential oils, IgA, GrowSafe System, Beef Cattle

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Definition of Terms/Abbreviations

Amino Acids (AA)- the building blocks from which all bodily proteins are made in the body. There are 20 known standard amino acids forming various proteins. Ten are called essential amino acids and the other ten are non-essential amino acids

Acid Detergent Fiber (ADF)- the residue remaining after boiling a forage sample in acid detergent solution. ADF contains cellulose, lignin, and silica.

Arginine (ARG)- essential amino acid that protects intestinal mucosal against E. coli/LPS-induced damage to intestinal barrier function; increases intestinal IgA concentrations; lowers inflammatory cytokines in intestine; involved in immune cell function

Ash- a measure of the total mineral content; the residue remaining after burning a sample.

Average Daily Gain (ADG)- pounds gained per day

Average Dry Matter Intake (ADMI)- $\text{Dry Matter Intake} \div \text{Number of Days}$

Bovine Respiratory Disease (BRD)- affects the upper and lower respiratory tracts and can lead to pneumonia. This disease is considered the most economically significant disease of fed cattle in the United States.

Carvone- component of caraway dill and spearmint seeds; has both antibacterial and antifungal activity

Carvacrol- primary essential oil in scotch spearmint, found in oregano, thyme, pepperwort, and bergamot

Crude Protein (CP)- the crude protein content of a feed sample represents the total nitrogen (N) in the diet. This value is 6.25 times the nitrogen content for forage or 5.7 times the nitrogen content for grain.

Corn Steep Liquor (CSL)- a by-product of wet corn milling and is a viscous liquid mixture of soluble protein, amino acids, carbohydrates, organic acids, vitamins, and minerals.

Cysteine (CYS)- non-essential amino acid that decreases oxidative stress in intestine; increased crypt cell proliferation

Hundredweight (cwt)- unit of weight equal to 100 pounds

Dry Matter (DM)- That part of feed, which is not water. Percent DM = 100% - moisture %. Feed values and nutrient requirements for ruminants are expressed on a dry matter or moisture-free basis to compensate for the large variation in moisture content of feeds commonly fed to cattle.

Dry Matter Intake (DMI)- As Fed Intake \times % Dry Matter

Electronic Identification Tag (EID)- ear tag used for identification with GrowSafe System[®] to calculate feed intakes

Essential Oils (EO)- the naturally occurring secondary metabolites that can be extracted from plant tissues.

Ether Extract- portion of dry matter extracted with ether. Used to measure crude fat.

Farm Identification Tags (FID)- ear tag used for identification at farm

Gain to Feed ratio (G:F)- a measure of an animal's efficiency in converting feed nutrients into increased body mass

Grams (g)- metric unit of mass

Grams per Day (g/d)- metric unit of mass used per day

Gram Negative Bacteria- bacteria normally found in the gastrointestinal tract that is responsible for diseases; have a thin cell wall; E. coli is an example of this bacteria. This bacteria does not stain with gram stain because of its protective layer.

Gram Positive Bacteria- good bacteria, live in the gut and help digest food; have a thick cell wall. This bacteria stains with gram stain because it doesn't have a protective membrane.

Histidine (HIS)- essential amino acid that is the precursor for histamine. Histamine is involved in the local immune response to invading proteins.

Increases permeability of capillaries to white blood cells and proteins that target against invading proteins.

Immunoglobulin A (IgA)- a major class of immunoglobulins (antibodies) found in serum and external body secretions, as well as in the gastrointestinal and respiratory mucosa.

Ionophore- any molecule, as of a drug, that increases the permeability of cell membranes to a specific ion and facilitates the transport of ions across a cell membrane

Limonene- primary essential oil in scotch spearmint, found in citrus oil and can be oxidized to carvone

Megacalorie (Mcal)- 1,000,000 calories

Methionine (MET)- essential amino acid that decreases oxidative stress in intestine; increased crypt cell proliferation

Milliliter (ml)- unit of capacity

Modified Distillers Grains plus solubles (MDGS)- a co-product from ethanol production, a modified wet product partially dried down, and a common additive to cattle feed for added protein and fat

Nanograms (ng)- unit of mass; one billionth of a gram

Net Energy for Gain (NE_g)- an estimate of the energy in a feed used for body weight gain once maintenance is achieved

Net Energy for Maintenance (NE_m)- an estimate of the energy in a feed used to keep an animal in energy equilibrium, neither gaining weight nor losing weight.

Neutral Detergent Fiber (NDF)- the residue left after boiling a sample in neutral detergent solution. The NDF in forages represents the indigestible and slowly digestible components in plant cell walls.

Parts per Million (ppm)- unit of concentration for small values

Ryelage- forage that is chopped at a higher moisture content, then sealed in a plastic wrap.

Threonine (THR)- essential amino acid that is involved in mucin synthesis and intestinal structure.

Total Digestible Nutrients (TDN)- a measure of the energy value in a feedstuff.

Total Mixed Ration (TMR)- a homogeneous mixture of mechanically mixed ration ingredients that typically combine forages and concentrates such as grains to optimize animal performance

Chapter I

Introduction

Cattle arriving at the feedlot have experienced stress as a result of transportation, inadequate feed and water, and co-mingling with other animals. These factors result in increased risk of disease outbreak and death.

Cattle farmers strive to maintain a healthy crop of cattle and in turn spend a substantial amount of money each year to keep their herd safe from diseases. This can include the money spent on their health care, such as vaccinations, deworming, fly control, antibiotics, and implants, as well as the various feed additives like vitamins, minerals, and growth promoters. In recent years, there has been an increased public concern over antibiotic use in food animals due to the concern regarding antibiotic resistance and adverse effects upon human health due to antibiotic residues in meat. Consequently, microbiologists and nutritionists have begun researching alternative products, which can be used instead of antibiotics and growth promoters, promoting optimal patterns of rumen fermentation, improving feed efficiency and animal productivity. Among these alternatives is a renewed interest in essential oils, as well as amino acids (Benchaar et al., 2008).

The amino acids (AA) that were focused on in this trial were methionine, arginine, histidine, threonine, and cysteine. These AA have been implicated in unique paths that enable the animals to initiate and then manage an immune response (Ruth & Field, 2013). The differences in AA profile between AminoGain 6 and AminoGain 6 Plus were achieved by adding fishmeal, blood meal, and bypass soybean meal.

The essential oils studied in the trial were Stay Strong Essential Oils (SS EO; Ralco Inc., Marshall, MN) and Scotch Spearmint Extract. The SS EO was fed at the manufacture's recommendation of 1 g/100 lb BW. It is an oregano based product which contains essential oils. Carvacrol, the oil of oregano which is derived from oregano seeds, can also be generated from carvone, the oil of spearmint, through acid-catalyzed isomerization (Kjonaas & Mattingly, 2005 and Baser, 2008). Carvacrol can also be found in thyme, pepperwort, and wild bergamot. Carvacrol offers a wide range of health benefits, including antimicrobial, antitumor, analgesic, anti-inflammatory, anti-parasitic, and aid in gastrointestinal diseases (Baser, 2008).

The Scotch Spearmint Extract that was used is a recycled mint oil which was accumulated through the second distillation; a process farmers do to clean their pipes; at peppermint and spearmint farms in Indiana. Due to this being a

recycled mint oil, it comes with a low cost, as opposed to the mint oil that is sold to gum and toothpaste companies. The farm that the scotch spearmint oil was acquired from, also produces spearmint and peppermint. The scotch spearmint oil used in this trial was selected because after initial lab analysis, it contained the highest amount of limonene (13%) and contained 70% carvone.

Limonene is a hydrocarbon cyclic terpene that is abundant in lemons, oranges, and grapefruit and is active against gram-negative bacteria (Castillejos et al., 2006). For the trial, it was determined to target 40 ppm limonene, which in turn provided 220 ppm carvone. The amount of 40 ppm limonene was targeted because of the trial done by Elwakeel et al. (2013), where the effect of limonene on *F. necrophorum* (bacteria found in foot rot and liver abscesses) growth *in vitro* was studied. These results suggested that 20-100 ppm limonene can be a promising tool to naturally reduce *F. necrophorum* and subsequently control liver abscesses. Therefore, it was chosen to start at 40 ppm, as that was somewhat intermediate.

Immunoglobulins (Ig), also known as antibodies, are proteins produced by plasma cells and lymphocytes (Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, 2003). There are five classes of immunoglobulins found in mammals: IgA, IgD, IgE, IgG, and IgM. In cattle, the

Ig classes that have been recognized are IgM, IgA, IgG1, IgG2 and IgE (Naessens, 1997). Immunoglobulin A (IgA) was chosen as the metric to measure in this trial because it is found in both serum and intestinal mucosa. While IgA is naturally present in the serum, elevated IgA concentrations are associated with inflammatory responses. Immunoglobulin A is the most abundant Ig in the respiratory and intestinal mucosa and it serves as a first line of defense against bacterial and viral invasion from the environment. Immunoglobulin A can also be found in secretory fluids such as saliva, tears, and nasal secretions (Saunders Comprehensive Veterinary Dictionary, 2007). To evaluate these IgA levels a Bovine IgA ELISA Kit (Bethyl Laboratories, Inc., Montgomery, TX) was used and read through an Epoch Plate Reader (BioTek, Winooski, VT).

All collection of feed data was done through the GrowSafe System[®] (Model 6000; GrowSafe Systems Ltd., Airdrie, Alberta, CA). The GrowSafe System[®] is a great benefit to have in a feed trial because it calculates what each animal consumes per meal and then determines a total amount they consumed daily. This technology allows for each individual animal to be used as an experimental unit, rather than the whole pen be one experimental unit. The system is extremely accurate and tracks feed consumed down to the hundredth of a

pound. It can also be helpful in identifying when an animal is sick because their feed intake typically decreases.

The aim of the study was to evaluate effectiveness of feeding essential oils and targeted amino acids to alleviate decreased growth in receiving cattle. It was also sought after to determine if there was a specific link between either essential oils or amino acids and improved growth performance or immune status of receiving cattle. It is hypothesized that receiving cattle offered scotch spearmint extract and a rumen undegradable protein-amino acid profile focused on immune response will have improved growth and feed efficiency.

Statement of the problem

In-light of increased regulation of prophylactic use of antibiotics for the prevention of disease outbreak in receiving cattle, there is a need for a more accepted form of prevention.

Purpose of the Study

The purpose of this study is to evaluate the efficacy of feeding essential oils and targeted amino acids to mitigate decreased growth in receiving cattle.

Research Questions

- (1) Do amino acids and essential oil products affect receiving calf growth and feed efficiency?
- (2) Do amino acids and essential oil products affect fecal and blood IgA concentrations of receiving calves?

Conceptual Framework

Since there is a great deal of public concern over the use of antibiotics in livestock production, it is important to look for alternatives for treating disease, as well as improving feed efficiency. Essential oils provide antibacterial, antifungal, and antioxidant properties that make them useful as a natural additive in feed. Whereas, amino acids have been identified in maintaining intestinal growth, function, normalizing the inflammatory response, and secretion of IgA by mucosal cells.

Assumptions

- (1) It is assumed that the targeted amino acids and essential oils will benefit calf growth and feed efficiency.

- (2) It is assumed that the targeted amino acids and essential oils will assist the immune response of the sick cattle.

Delimitations

This study will not investigate the treatments on different breeds or ages of cattle.

Limitations

This study is limited to a 63-day trial at the ADM Research Center in Mendon, IL. This study is limited to Angus/Simmental cross cattle. This study is limited to six different feed treatments and two replicate pens per treatment.

Significance of the Study

This study is significant because of the public concern over use of antibiotics. In the past decade, the addition of antibiotics in livestock production has been common, but now there is apprehension of antibiotic resistance and possible risks to human health due to residues in the final product or excretion to environment.

Organization of the Study

The study began with research of relevant literature, followed by a 63-day trial that ran from October 11th- December 13th, 2016.

Chapter Summary

This study examined the effect of essential oils and amino acid profile products on receiving calf growth and feed efficiency. It also evaluated the effects of essential oils and amino acid profiles on IgA concentrations as a marker for systemic and gastrointestinal tract immune status of 120 crossbred calves.

Over the last decade, antibiotic use in livestock production has been common. Although, recently there has been grave public concern of their use due to the potential of antibiotic resistance and the transmission to human from livestock causing various health risks. Potential alternatives to antibiotics are essential oils and amino acids. Essential oils provide antibacterial, antifungal, and antioxidant properties that make them useful as a natural additive in feed (Castillejos et al., 2006). Whereas, amino acids have been identified in maintaining intestinal growth, function, normalizing the inflammatory response, and secretion of IgA by mucosal cells (Ruth & Field, 2013).

Chapter II

Review of Relevant Literature

Introduction

Antibiotics are used for a variety of reasons in ruminant livestock. They are used for the treatment or prevention of disease, the manipulation of rumen microbe population, and to lower gut integrity. Some antibiotics used in livestock are relevant to human medicine and others are not. Antibiotics that are used in both livestock and human medicine could be a reason of stricter guidelines on treatment of animals.

In 1928, Alexander Fleming discovered Penicillin, along with the development of techniques to produce antibiotics on an industrial scale. In the 1950s, microbiologists began to identify bacteria that were resistant to antibiotics. By the 1980s, various drug-resistant bacteria were common. Some antibiotics used in livestock production are also relevant for human medicine. Presently, there is dispute concerning the causes of antibiotic resistance and what should be done about it. (Russell & Houlihan, 2003). This, as well as the increased regulation of antibiotics for the prevention of disease in cattle, are the reasons to evaluate a more accepted form of treatment.

Research documents have shown that a deficiency of protein or amino acids can weaken the immune function and increase the susceptibility of animals to disease (Peng et al., 2007) Although, more recently, studies have reported that more important than protein, is the composition of certain amino acids for the animals experiencing an immune challenge (Ruth & Field, 2013). The dietary supplementation of essential amino acids to animals experiencing malnutrition or infectious disease enhances the immune status, therefore reducing morbidity and mortality rates in cattle (Peng et al., 2007).

Two-thirds of the decrease in growth in livestock facing an immune challenge is associated with a decrease in feed intake associated with diseased animals. The other one-third of decreased growth has been credited to nutrients being separated away from growth and toward initiation of the immune response for fever mitigation, altered metabolism, recovering of damaged tissue, and the inability to absorb nutrients from the gastrointestinal tract (Klasing, 2016).

One of the main reasons that farmers and ranchers strive to have healthy cattle, is to help prevent Bovine Respiratory Disease (BRD) and also known as ‘shipping fever’. Bovine Respiratory Disease affects the upper and lower respiratory tracts and can be associated with lung infection, which may ultimately lead to pneumonia. This disease is considered the most economically significant

disease of fed cattle in the United States. The North America Bovine Respiratory Disease Treatment Market was estimated at \$24.5 million in 2016 and is expected to reach \$67.31 million by 2021. The U.S. feedlot industry approximates the loss related to BRD is due in part to loss of production, increase in labor expenses, pharmaceutical costs, and death from BRD (BRD Treatment Market, 2016). The disease is said to be responsible for 65-80% of morbidity and 45-75% mortality in feedlot cattle (Jelinski & Janzen, 2016). There are various factors that can cause BRD, including marketing, weaning shipping, co-mingling, a weather change, nutrition, and various respiratory viruses (Currin & Whittier, 2009). Signs of BRD include lack of appetite, nasal discharge, difficulty breathing, depression and fever of 104°F to 108°F (Jelinski & Janzen, 2016). Calves often start showing signs of illness between 7-21 days after being purchased, but can occur anywhere from 2-30 days after being purchased (Currin & Whittier, 2009).

One of the most popular ways to treat BRD, is with antibiotics, such as these injectibles: Micotil®, Nuflor®, and Baytril 100® (Currin & Whittier, 2009). Although, in the recent years, there has been an increase in public concern regarding the use of antibiotics in livestock production. This is mostly due in part to their possible contribution to the development of antibiotic resistant bacteria

and the transmission of antibiotics from livestock to humans (Benchaar et al., 2008).

Typically, beef cattle in feedlots are fed a class of antibiotics known as ionophores. Although there are some exceptions, gram-positive bacteria in the rumen are classically more sensitive to ionophores than gram-negative bacteria are. Through their antimicrobial activities, ionophores are capable of increasing feed efficiency by as much as 10%. A well-known and widely used ionophore is monensin, which was approved by the FDA in the 1970s. Monensin increases feed efficiency in ruminants by altering their ruminal fermentation, but is not in the class of medically-important antibiotics (Russell & Houlihan, 2003).

One of the possibilities of natural alternatives to antibiotics is essential oils. There are several uses of essential oils as feed additives for livestock because they can improve feed efficiency and animal productivity due to their antimicrobial, anti-inflammatory, anti-oxidant, and digestive effects on rumen metabolism (Rivaroli et al., 2016). Essential oils are naturally occurring secondary metabolites that are extracted from plant tissues through a steam distillation process (Benchaar et al., 2006). Essential oils are a potential substitute to antibiotics because they have been reported to possess strong antimicrobial

effects against a wide range of microorganisms including bacteria, fungi, viruses, and protozoa (Benchaar et al., 2006).

Essential oils are responsible for the odor and color of both plants and spices. They possess antibacterial, antifungal, antimicrobial and antioxidant properties which make them beneficial in animal feeds as natural additives. Essential oils can be composed of more than one-hundred individual components. (Castillejos et al., 2006). In scotch spearmint, the primary components are carvacrol and limonene. Limonene also can be found in lemons, oranges, grapefruit, peppermint, spearmint and other oils (Castillejos et al., 2006). Carvacrol can be found in oregano, thyme, pepperwort, and wild bergamot and is responsible for the antibacterial properties of many essential oils. The main active ingredient in oregano is Carvacrol, which is derived from oregano seeds. It offers a wide range of health benefits, including antimicrobial, antitumor, analgesic, anti-inflammatory, anti-parasitic, and in gastrointestinal diseases (Baser, 2008). Carvacrol, the oil of oregano, can also be generated from carvone, the oil of spearmint, through acid-catalyzed isomerization (Kjonaas & Mattingly, 2005).

One of the most important undertakings of compounds in essential oils are their ability to have a wide spectrum of antimicrobial activity. Both gram positive and gram negative bacteria exist in the rumen. Generally, gram-positive bacteria

are more sensitive to essential oils than gram-negative bacteria (Castillejos et al., 2006). This is because most components of essential oils are lipophilic, meaning they cannot penetrate in the membrane of gram-negative bacteria. Carvacrol, since it is an aromatic hydrocarbon and has a low molecular weight, is able to interact with water, cross the cell wall of gram-negative bacteria by diffusion, and interact with the lipid bilayer of cells. While the addition of essential oils may increase weight gain similar to that of synthetic antibiotics, there is little scientific evidence of their effect on rumen microbial fermentation (Calsamiglia et al., 2007). One of the purposes of this research was to determine if certain essential oils would have a positive impact on growth, feed efficiency, or perhaps an indirect indication of impact rumen fermentations, as well as fecal and blood IgA as markers of gastrointestinal or systemic immunity.

Essential oils are made up of more than one-hundred individual components. Major components can constitute up to 95% of the essential oil, while others may be present only in traces (Castillejos et al., 2006). Spearmint oil has a minimum of 51% carvone, but typically has a content of 60-70% carvone and 8-15% of limonene. Carvone is produced through both extraction and purification of essential oils from caraway, dill, and spearmint seed. Carvone has

the properties to be both antibacterial and antifungal (De Carvalho & Fonseca, 2006).

Another aspect that this trial observed was whether or not an increase in amino acids (AA) had effects receiving calf growth and feed efficiency. In addition to essential oils, the trial also evaluated the effects of AA profile within a targeted group of sick cattle. AA are the building blocks of protein and are used for the development of tissues and muscle production. It takes at least 50 amino acids to make up a protein and there are twenty different amino acids. There are ten essential amino acids, including phenylalanine, valine, threonine, tryptophane, isoleucine, methionine, histidine, arginine, leucine, and lysine. The term "essential" amino acid refers to the amino acids that cattle cannot produce themselves. Each essential amino acid must be supplied through the diet or from rumen microbes. Cattle also require "nonessential" amino acids, but they are able to synthesize them in the adequate amounts that are needed. The rumen microbes, degraded dietary protein and amino acids, and endogenous amino acids all contribute to the amount of amino acids exiting the rumen and available to be absorbed in the small intestine of the animal. Rumen microbial amino acids contribute the majority of the total amino acids, about 50-75%. (Ondarza, 2004).

There is a possibility that an increase in targeted amino acids in the rumen undegradable protein could be greatly beneficial to sick cattle. During an immune challenge, the liver shifts from being a metabolic organ directing nutrients, predominantly amino acids, toward growth to be an immune organ directing nutrients to combat the immune challenge (Klasing, 2016). Amino acids arginine (ARG), cysteine (CYS), methionine (MET), histidine (HIS), and threonine (THR) have been implicated to enable the animal to initiate and then manage the immune response (Ruth & Field, 2016; Jutel et al., 2006). The dietary amino acid profile that is desired during an immune challenge differs from a healthy growing animal as animal metabolism has shifted (Klasing, 2016). The amino acids previously identified have been implicated in maintaining intestinal growth and function, normalizing the inflammatory response, and secretion of IgA by mucosal cells (Ruth & Field, 2013).

The AA that were focused on in this trial were methionine, arginine, histidine, threonine, and cysteine. These AA have been implicated in unique paths that enable the animals to initiate and then manage an immune response. They each have unique properties such as, maintaining the integrity, growth and function of the intestine, and the secretion of IgA. Methionine and cysteine help reduce intestinal oxidative stress and increase crypt cell proliferation. Arginine protects intestinal mucosa against E. Coli, increases intestinal IgA concentrations,

lowers inflammatory cytokines in the intestine, and is involved in immune cell function. Threonine is involved in mucin synthesis, as well as intestinal structure. Histidine is a precursor for histamine, which is involved in local immune response to invading proteins. Histidine is also a vasodilator, which means it helps increase permeability of capillaries to white blood cells and proteins that target invading proteins (Ruth & Field, 2013). A deficiency in AA has been reported to impair immune function and increase susceptibility of animals to infectious disease. Therefore, dietary supplementation of specific AA to animals with malnutrition and infectious disease challenges may enhance the immune status, thus reducing morbidity and mortality rates (Peng et al., 2007). An increase in AA was accomplished by adding fishmeal, blood meal, and bypass soybean meal which are all desirable sources of AA and bypass rumen degradation.

Methionine is often the first limiting amino acid for growing cattle and is also a part of a wide variety of metabolic pathways. It can even serve as a precursor to other amino acids, like cysteine. Both methionine and cysteine can help protect proteins and cells from oxidation. It has been found that growing beef cattle can benefit from 5-10 g/d of additional methionine because it aids in improving performance in beef cows, including increased average daily gain, improved reproductive tract scores and reproductive efficiency. Since methionine

plays several roles in an animal's metabolism and it cannot produce this essential amino acid on its own, it is important to supplement the diet (Nuzback, 2013).

Immunoglobulins are major components of the humoral immune response system, meaning it is the body's defense in the immune system that protect against infection. Immunoglobulins are made up of lymphocytes and plasma cells and are found in the serum, as well as other body fluids and tissues, including urine, spinal fluid, lymph nodes, and in the digestive tract. Each immunoglobulin molecule consists of four polypeptide chains; two heavy chains (H chains) and two light chains (L chains). There are five different kinds of H chains that differentiate immunoglobulins. The five major immunoglobulins are IgA, IgD, IgE, IgG, and IgM. Two types of IgA have been identified. They are serum IgA and secretory IgA (sIgA). Secretory IgA is present in saliva, bile, synovial fluid, as well as intestinal and respiratory tract secretions. Both IgA types are known to have antiviral properties (Saunders Comprehensive Veterinary Dictionary, 2007). IgA was chosen as the metric to measure in this trial because it is found in both serum and intestinal mucosa. While IgA is present in the serum, it is mostly only detected during an inflammatory response, this was beneficial since immune challenged cattle were targeted for this trial (Saunders Comprehensive Veterinary Dictionary, 2007).

Summary

The reasoning of further research into natural alternatives of antibiotics is due to the dispute of concern of antibiotic resistance and the increased regulation of antibiotics in livestock production. Bovine Respiratory Disease is just one example of the diseases that affect cattle population and the impact that disease alone makes on the cattle industry proves the importance of finding alternatives to antibiotics. Potential options to help combat these issues are essential oils and amino acids.

There are several capabilities of essential oils as feed additives. They can improve feed efficiency, as well as animal productivity due to their antimicrobial, anti-inflammatory, anti-oxidant, and digestive effects on rumen metabolism (Rivaroli et al., 2016). While AA have been implicated in unique paths that enable the animals to initiate and then manage an immune response. The AA focused on in this trial each have unique properties of maintaining the integrity, growth and function of the intestine, and the secretion of IgA (Ruth and Field, 2013).

Immunoglobulins are a defense system in the immune system that helps protect against infection (Saunders Comprehensive Veterinary Dictionary, 2007). IgA was chosen as the metric to measure in this trial because it is found in both

serum and intestinal mucosa. While IgA is present in the serum, it is mostly only detected during an inflammatory response, this was beneficial since immune challenged cattle were targeted for this trial (Saunders Comprehensive Veterinary Dictionary, 2007).

Chapter III: Methodology

Introduction

Each year, farmers strive to maintain a healthy crop of cattle and in turn spend a substantial amount of money to keep their herd safe from diseases. This can include, but not limited to, the money spent on vaccinations, antibiotics, deworming, vitamins and minerals, and various other feed additives. On the contrary though, in recent years there has been an increase in public concern over the use of antibiotics. As well as regulation of prophylactic use of antibiotics for the prevention of disease outbreak in receiving cattle. So, in turn, a more accepted form of treatment is preferred, which will also hopefully be cost efficient.

This study took place at the ADM Research Center in Mendon, Illinois. The objectives of this trial were to evaluate: 1) effects of amino acid profile and essential oil products on receiving calf growth and feed efficiency and 2) effects of amino acid profile and essential oil products on fecal and blood Immunoglobulin A (IgA) concentrations as a marker for systemic and gastrointestinal tract immune status of receiving calves. All animal care and protocols used in this experiment were approved by the ADM Research Center's Animal Care and Use Committee.

Rationale

Quantitative Research was used in this study. The examples of quantitative research used include: weight, Average Daily Gain (ADG), Average Feed Intake (AFI), Dry Matter Intake (DMI), Average Dry Matter Intake (ADMI), Gain to Feed Ratio (G:F), as well as ELISA testing for IgA concentration. Experimental research was used since an experiment was conducted in order to give evidence to the experimental hypothesis.

Research Questions

- (1) Do amino acids and essential oil products affect receiving calf growth and feed efficiency?
- (2) Do amino acids and essential oil products affect fecal and blood IgA concentrations of receiving calves?

Context of the Study

This study took place at the ADM Research Center in Mendon, IL.

Data Collection Methods

In this study, 130 crossbred calves (570.1 ± 4.49 lbs) were brought in for a 63-d trial. Upon arrival to the facility, cattle were weighed, dewormed with an Vetrimec™ pour-on (Norbrook Laboratories Ltd., Overland Park, KS), implanted with Synovex-S® (Zoetis, Kalamazoo, MI), vaccinated with Express® 5 (Boehringer Ingelheim, St. Joseph, MO) and a rectal temperature was recorded.

Upon arrival and initial observation, the animals were classified as healthy or sick. To be classified as sick, calves had to exhibit at least one of the following: rectal temperature greater than 102.5°F, nasal discharge, respiratory distress, or lethargy. The animals were weighed on day -1 and day 0 (for an average start weight), d 21, d 42, and then on days 63 and 64 (for an average end weight). Consecutive-day weights at the beginning and end of the trial were averaged to minimize variation resulting from rumen fill. Calves were then allotted to one of twelve pens based on BW and health status, with ten animals in each pen. In each pen, approximately 70-75% of the animals were classified as sick, leaving 25-30% healthy animals. Treatments were then randomly assigned to pen.

Three animals from each pen were randomly selected for fecal and blood sample collection. Using the same designated animals each time, blood and fecal

samples were collected on d 0, 21, and 42. Blood samples were allowed to coagulate for 1 hour at room temperature and then centrifuged for collection of serum. Serum and feces were stored at -4 °F for subsequent analyses of IgA. The samples were evaluated for IgA using an Epoch Plate Reader (BioTek, Winooski, VT) and a Bovine IgA ELISA kit (E11-131, Bethyl Laboratories, Inc., Montgomery, TX).

Table 1 below illustrates a timeline of events for this trial.

Table 1. Experimental Timeline	
Day	Event
Arrival to test start	Grass hay and water <i>ad libitum</i>
October 10 th d -1	- Weigh am pre-feeding
October 11 th d 0	- Weigh am pre-feeding - Collect fecal and blood samples on subset - Allot to pens - Start test diets
November 1 st d 21	- Weigh am pre-feeding - Collect fecal and blood samples on subset
November 22 nd d 42	- Weigh am pre-feeding - Collect fecal and blood samples on subset
December 13 th d 63	- Weigh am pre-feeding
December 14 th d 64	- Weigh am pre-feeding

There were six different diets for this trial. The diets were as follows:

1. AminoGain 6- Control
2. AminoGain 6- Stay Strong Essential Oils
3. AminoGain 6- Scotch Spearmint Extract
4. AminoGain 6 Plus- Control
5. AminoGain 6 Plus- Stay Strong Essential Oils
6. AminoGain 6 Plus- Scotch Spearmint Extract

To help analyze the results of this study, quantitative statistics were used. When allotting animals to pens, cattle were stratified by weight and sickness to make each pen a replicate, with regard to initial weight and degree of sickness, as well as an effort to ensure the degree of sickness the same across all treatments so there would not be a statistical difference.

Sub-samples of each ingredient and the total mixed rations (TMR), were taken each week and pooled at the end of the trial for analysis.

The collection of feed data was done through the GrowSafe System[®] (Model 6000; GrowSafe Systems Ltd., Airdrie, Alberta, CA). Each individual animal had an electronic tag placed in their ear at initial processing. The GrowSafe System[®] tracks every time the animals reached their head in the bunks,

the amount they consumed, how many times they ate, and for what length of time. These recordings were taken twenty-four hours a day.

Tables 4.2 and 4.3 depict that the treatments were designed to have a similar ingredient composition and deliver similar energy, crude protein, vitamins and mineral. Treatments were arranged in a 2 x 3 factorial design of AminoGain formula (AminoGain 6 or AminoGain 6 Plus) and essential oil product (no essential oil, Stay Strong EO (SS EO), or Scotch Spearmint Extract). Stay Strong EO was included in the supplement to deliver 1 g / 100 lb BW. On average each animal consumed 7.38 g SS EO each day, consequently delivering 1.07 g/100 lb BW. Preliminary analysis indicated the Spearmint Extract was composed of 13% limonene and 70% carvone. Calculations for the initial amounts of supplement to be added to the treatment diets were figured using these numbers. Therefore, Spearmint Extract was included in the supplement to deliver 40 ppm limonene and 220 ppm carvone. When the actual analysis came back, the extract contained 0.08% limonene and 91.73% carvone. A limonene concentration of 40 ppm was chosen based on the trial done by Elwakeel et al. (2013), where 20-100 ppm limonene decreased *F. necrophorum* (bacteria found in foot rot) growth *in vitro* and subsequently controlled liver abscesses. However, only limonene was used in

the Elwakeel et al. (2013) trial, the potential exists for carvone to have similar effects because of the similarities in the compounds.

Data Analysis

At the end of the trial, all of the samples of the ingredients were pooled together. The pooled samples were analyzed in our lab for the following: Dry Matter, Crude Protein, Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Ether Extract (EE), Ash, minerals, as well as Total Digestible Nutrients, NEg (Net Energy for Gain), and NEm (Net Energy for Maintenance). The TMR composites was retained in the freezer pending results on individual ingredients.

The IgA in the blood and fecal samples were evaluated using the Epoch Plate Reader (BioTek, Winooski, VT) and a Bovine IgA ELISA kit (E11-131, Bethyl Laboratories, Inc., Montgomery, TX). Since this was the first time using this plate reader, it was determined to run the samples in triplicates to better the chances of a repeatable assays. Seven different standards ranging from 15.75-1,000 ng/ml were used. The process for serum IgA analyzation is as follows:

- A. Dilute 10 microliters of serum with 7.5 ml of Buffer C. Mix well.
- B. Add 100 microliters of each sample and standard to the wells. Do in triplicate. Cover the wells with adhesive sheet and let sit for one hour at room temperature.
- C. Remove adhesive sheet and rinse 4 times with wash buffer.
- D. Pour Detection Antibody reagent into V shaped tray. Add 100 microliters to each well using multichannel pippeter. Mix well by gently tapping side several times.
- E. Add adhesive cover and incubate for one hour at room temperature. Follow with rinsing with wash buffer 4 times.
- F. Pour HRP Solution C into clean V shaped tray. Add 100 microliters using multichannel pippeter. Incubate at room temperature for 30 minutes covered with adhesive cover.
- G. Pour TMB Substrate Solution into clean V shaped tray. Add 100 microliters using multichannel pippeter. Place in dark drawer for 40 minutes uncovered.
- H. Pour Stop Solution into clean V shaped tray. Add 100 microliters using multichannel pippeter. Tap plate to mix. The solution in the wells should change from yellow to blue. Place within Epoch Plate Reader for a reading within 30 minutes.

Once the results were achieved, the mean, standard deviation, and percent coefficient variation were calculated using Microsoft Excel[®]. The mean was calculated using the ‘=average’ function and the standard deviation was calculated using the ‘=stdev’ function. The percent coefficient variation was calculated by dividing the standard deviation by the mean and then multiplying by 100. The percent coefficient variation gives the variation of results obtained from repeated experiment and helps determine the outliers in the results. If the result was above 10, the sample was rerun.

For the fecal IgA analysis, two different protocols were attempted to develop an assay. The first involved thawing the frozen feces, bringing it to room temperature, and then placing it in purified water at a concentration of 10% by weighing up 1 gram of feces and adding 9 ml of water. The samples were then vortexed and incubated overnight before being spun at 2,000 x g for 15 minutes. Afterwards, it was diluted with 10 microliters of the supernatant with 7.5 ml of Buffer C and mixed. The same Bovine IgA ELISA kit used in the serum IgA process was used for the fecal IgA as well, therefore the steps were the same as listed above. As with the serum IgA analysis, the fecal samples were also ran in triplicates and seven standards were used running from 15.75-1,000 ng/ml. After achieving low results using the first protocol, 5 g of the fecal material was used to

increase sensitivity. With an increase in feces, higher concentrations in the results were achieved. However, the results varied, this may be due in part to the animals not being as sick as we had assumed. The assay was determined to effectively detect IgA in bovine fecal material, but results were lower than expected, so fecal testing ceased and is not reported for these animals.

Reliability, Validity, Generalizability, Triangulation

Data were analyzed using SAS software (SAS Inst. Inc., Cary, NC) with individual animals as the experimental unit. Treatment, pen, and animals were independent fixed effects in the model. Differences in dependent variable means that result from AminoGain formula or essential oil product or their interaction (for individual intake, average daily gain, and feed conversion efficiency) were separated by least square means using the p-diff option. Fecal and serum IgA concentrations were evaluated as repeated measures.

Advantages and Disadvantages of Strategy and Methods

The advantages of this strategy and methods are the two different types of samples (fecal and serum) that were taken from the animals, as well as the

randomization of animals chosen to be sampled. Another advantage was that the GrowSafe System[®] was used. Using this system allowed the possibility to have 120 experimental units (animals) rather than 12 experimental units (pens) because the precise amount of what each animal consumed was known. Other advantages include as well as the same mixer used for all of the different diets and the same individual mixed the diets each time. The disadvantages of the methods is that only three samples from each pen were taken pen, instead of sampling every animal. Another disadvantage is the length of the trial, there may have been different results had the trial been over a longer period of time.

Chapter Summary

There was a significant amount of data and variables to decipher in this study. To help analyze the results, multiple types of research were used. Quantitative Research was used in this study. The examples of quantitative research used include: weight, ADG, AFI, DMI, ADMI, G:F ratio, and ELISA testing for IgA concentration. Experimental research was used because an experiment was conducted in order to give evidence to the experimental hypothesis.

It is hypothesized that receiving cattle offered scotch spearmint extract and a rumen undegradable protein-amino acid profile focused on immune response will have improved growth and feed efficiency.

The collection of feed data was done through the GrowSafe System[®]. Every animal had an electronic tag in their ear to calculate the amount of feed they consumed and for what length of time. These recordings were taken twenty-four hours a day. All six of the treatments were mixed with the same mixer and each week, samples of the ingredients, as well as the TMR, were taken and pooled at the end of the trial. The pooled samples were analyzed in our lab for: Dry Matter, Crude Protein, Acid Detergent Fiber, Neutral Detergent Fiber, Ether Extract, Ash, minerals, Total Digestible Nutrients, NEg, and NEm.

The animals were weighed on day -1, 0, 21, 42, 63, and 64. On days 0, 21, and 42 a grab fecal sample was taken, as well as a blood sample taken from the jugular vein of three animals designated in each pen. These samples were extracted to test for the animal's level of IgA. The IgA values in the blood and fecal samples were evaluated using the Epoch Plate Reader (BioTek, Winooski, VT) and a Bovine IgA ELISA kit (E11-131, Bethyl Laboratories, Inc., Montgomery, TX). All samples were kept frozen until analyzation, ran in triplicates, and the same standards were used in each test.

Chapter IV: Analysis

Table 4.1 gives the nutrient composition of the dietary treatments. Tables 4.2 and 4.3 depict that the treatments were designed to have a similar ingredient composition and deliver similar energy, crude protein, vitamins and mineral. Table 4.4 shows the effects of amino acid profile and essential oil product on receiving calf growth and feed efficiency. Table 4.5 illustrates intake and growth data comparisons between the AminoGain 6 and the AminoGain 6 Plus treatments. Table 4.6 represents the effects of treatments on serum IgA concentration of receiving cattle.

Table 4.1 Nutrient Composition of Dietary Treatments

	Supplement					
	1	2	3	4	5	6
	AminoGain 6			AminoGain 6 Plus		
	Control	Stay Strong EO	Spearmint Ext	Control	Stay Strong EO	Spearmint Ext
% DM	90.80	91.04	91.02	90.59	90.94	90.11
% CP	25.58	27.94	27.46	27.74	27.59	27.82
% ADF	8.48	8.69	8.98	9.15	9.23	9.29
% NDF	26.44	23.50	25.03	24.96	24.04	23.73
% ASH	11.43	11.70	11.66	12.50	12.34	14.18
% EE	4.42	4.53	4.48	4.85	4.62	4.43
NEM (Mcal/cwt)	94.75	94.45	94.06	93.82	93.71	93.62
NEG (Mcal/cwt)	64.52	64.27	63.93	63.73	63.64	63.56
% TDN	84.79	84.58	84.29	84.10	84.02	83.96

Table 4.2 Dietary Ingredient Composition on a DMB for Mixing and Nutrient Composition

	1	2	3	4	5	6
	AminoGain 6			AminoGain 6 Plus		
	Control	Stay Strong EO	Spearmint Ext	Control	Stay Strong EO	Spearmint Ext
Ingredient	% Dry Matter Basis					
Corn	38.2	38.2	38.2	40.4	40.4	40.4
Grass hay	18.3	18.3	18.3	15.6	15.6	15.6
MDGS ¹	7.3	7.3	7.3	7.0	7.0	7.0
Ryelage	3.5	3.5	3.5	3.9	3.9	3.9
CSL ²	4.6	4.6	4.6	5.0	5.0	5.0
Suppl 1	28.0	----	----	----	----	----
Suppl 2	----	28.0	----	----	----	----
Suppl 3	----	----	28.0	----	----	----
Suppl 4	----	----	----	28.0	----	----
Suppl 5	----	----	----	----	28.0	----
Suppl 6	----	----	----	----	----	28.0
Total	100.0	100.0	100.0	100.0	100.0	100.0

¹ MDGS= Modified Distillers Grains plus solubles

² CSL= Corn Steep Liquor

Table 4.3 Nutrient Profile on Dry Matter Basis

	1	2	3	4	5	6
	AminoGain 6			AminoGain 6 Plus		
	Control	Stay Strong EO	Spearmint Ext	Control	Stay Strong EO	Spearmint Ext
DM, %	74.8	74.8	74.8	75.0	75.0	75.0
NEg, Mcal/lb	0.53	0.53	0.53	0.53	0.53	0.53
CP, %	15.9	15.9	15.9	16.0	16.0	16.0
RDP, %	9.9	9.9	9.9	9.8	9.8	9.8
RUP, %	6.0	6.0	6.0	6.2	6.2	6.2
RUP, %CP	37.8	37.8	37.8	38.5	38.5	38.5
Stay Strong EO	---	+++	---	---	+++	---
Scotch Spearmint Ext	---	---	+++	---	---	+++

Table 4.4 Effects of Amino Acid Profile and Essential Oil Product on Receiving Calf Growth and Feed Efficiency

	1	2	3	4	5	6		
	AminoGain 6			AminoGain 6 Plus				
	Control	Stay Strong EO	Spearmint Ext	Control	Stay Strong EO	Spearmint Ext	SE	P value
Pens	2	2	2	2	2	2	-	-
Observations	20	20	20	20	20	20	-	-
BW, lb								
Initial	574.3	572.8	570.1	567.6	568.8	566.8	11.21	0.99
d-21	661.0	660.0	658.0	670.0	665.0	648.0	13.39	0.77
d-42	739.0	731.0	740.0	746.0	747.0	722.0	13.80	0.45
d-63	810.1	807.0	819.3	823.5	825.8	796.8	14.90	0.33
ADG, lbs/d								
d 0-21	4.12	4.17	4.18	4.85	4.56	3.87	0.32	0.26
d 21-42	3.74	3.38	3.89	3.64	3.94	3.51	0.22	0.09
d 42-63	3.38	3.61	3.79	3.69	3.74	3.57	0.22	0.46
d 0-63	3.74 ^{ab}	3.72 ^{ab}	3.95 ^{ab}	4.06 ^a	4.08 ^a	3.65 ^b	0.13	0.02
ADMI, lbs/d								
d 0-21	14.69	14.31	14.40	14.94	15.46	13.65	0.57	0.25
d 21-42	19.95	18.88	18.97	19.99	20.32	18.14	0.50	0.07
d 42-63	23.09	22.66	23.53	24.11	24.56	22.83	0.59	0.08
d 0-63	19.24 ^{ab}	18.61 ^b	18.97 ^{ab}	19.68 ^a	20.11 ^a	18.21 ^b	0.47	0.06
G:F								
d 0-21	0.2755	0.2908	0.2815	0.3228	0.2966	0.2751	0.019	0.321
d 21-42	0.1906	0.1811	0.2093	0.1831	0.1945	0.1926	0.012	0.443
d 42-63	0.1439	0.1620	0.1612	0.1535	0.1525	0.1559	0.009	0.535
d 0-63	0.1944	0.2010	0.2086	0.2065	0.2034	0.1988	0.005	0.131

^{ab} Means within a row with different superscripts differ $P \leq 0.05$ as a result of treatment.

Table 4.5 Comparison of AminoGain 6 and AminoGain 6 Plus Formulas

	AminoGain 6 (Trmts 1-3)	AminoGain 6 Plus (Trmts 4-6)	SE	P Value
Small Pens	6	6	---	---
Calves	60	60	---	---
d 0-63 ADG lb/d	3.80	3.93	0.09	0.36
d 0-63 ADMI lb/d	18.94	19.33	0.46	0.56
d 0-63 G:F	0.201	0.203	0.005	0.82

Table 4.6 Effects of Amino Acid Profile and Essential Oil Products on Serum IgA Concentration of Receiving Cattle

	Treatment						SE	P value
	1	2	3	4	5	6		
	AminoGain 6			AminoGain 6 Plus				
	Control	Stay Strong EO	Spearmint Ext	Control	Stay Strong EO	Spearmint Ext		
Pens	2	2	2	2	2	2	-	-
Observations	20	20	20	20	20	20	-	-
IgA, ng/ml								
d-0	139.62	179.68	200.73	187.67	241.89	254.75	36.13	0.98
d-21	248.95	377.32	287.25	266.37	291.06	403.37	89.45	0.53
d-42	145.30	311.45	272.19	196.67	301.03	329.24	47.84	0.74
d0-d21 Δ	109.33	197.65	86.52	78.70	49.17	148.62	80.31	0.43
d21-d42 Δ	-103.66	-65.87	-15.06	-69.70	9.96	-74.13	75.45	0.66
d0-d42 Δ	5.67	131.77	71.46	9.01	59.13	74.49	40.00	0.56

Chapter V: Results and Discussion

In conclusion, results from the growth and feed intake data indicate that the effects of essential oils differ depending on the AminoGain formula in which they are paired with (Table 4.4). Intake may be inhibited by the inclusion of scotch spearmint extract or the addition of fishmeal to accomplish an increase in amino acids in the AminoGain 6 Plus formulas. Initial body weights were similar ($P=0.99$) among treatments. An interaction of AminoGain 6 formulation by essential oil source resulted ($P<0.02$) for cumulative ADG. Cumulative ADG did not differ ($P\geq 0.05$) with the addition of scotch spearmint or Stay Strong EO to the AminoGain 6 formula. However, the addition of scotch spearmint worsened ($P<0.03$) cumulative ADG when added to the AminoGain 6 Plus formula. This was likely the result of the cumulative feed intake which tended to worsen with the combination of scotch spearmint extract and AminoGain 6 Plus Formula ($P\leq 0.06$). Table 4.5 illustrates intake and growth data comparisons between the AminoGain 6 and the AminoGain 6 Plus treatments.

Preliminary analysis indicated the Scotch Spearmint Extract was to be composed of 13% limonene and 70% carvone. Calculations for the initial amounts of supplement to be added to treatments were done using these figures. When the actual analysis came back, the extract contained .08% limonene and 91.73%

carvone. Because limonene was believed to have oxidized to carvone during storage, the quantity of active ingredients for the Scotch Spearmint Extract treatments differed from that in the plan of experiment. Although, carvone is reported to have similar activity as limonene, therefore an active component still existed for this trial. Using the preliminary analysis numbers, a target of 40 ppm limonene was chosen based on the trial done by Elwakeel et al. (2013), where they studied the effect of limonene on *F. necrophorum* (bacteria found in foot rot and liver abscesses) growth *in vitro*. These results suggested that 20-100 ppm limonene can be a promising tool to naturally reduce *F. necrophorum* and subsequently control liver abscesses. Therefore, it was determined the Scotch Spearmint extract treatments would contain 40 ppm, as that was somewhat intermediate. Although, Elwakeel's et al. (2013) trial, only limonene was used. In this trial, scotch spearmint extract was fed which contained both limonene and carvone. There is a probability that what was fed was a much higher dose of active ingredients with the limonene and carvone combined. Because this dose was based on targeting a particular limonene concentration without regard to other active ingredients, the quantity of carvone in the diets may have been overdone. While adding essential oils to a diet may be able to increase weight gain, the scientific evidence on the effect in rumen microbial fermentation is limited. To be able to understand its influence, further studies are required, potentially *in vitro* or

in situ. Based on calculations using final lab analyses, each animal daily, on average, consumed 2.5 mg limonene, 2,821 mg carvone, and 5.5 mg eugenol. Concluding, a small amount of the oil has many positive effects, too much of the oil may have negative effects. This is definitely something to consider in the future when proceeding with trials containing essential oils.

Stay Strong Essential Oils (SS EO; Ralco Inc., Marshall, MN) was targeted to deliver 1 g/100 lb body weight. This was dosed according to label instructions. It is an oregano based product which contains essential oils, including carvone. Based on calculations, on average each animal consumed 7.38 g SS EO each day, consequently delivering 1.07 g/100 lb body weight. Therefore, our targeted 1g/100 lb bw was met.

For this trial, a high health–risk group of cattle was targeted. With the exception of when Spearmint Extract was included, cattle fed the AminoGain 6 Plus formula, in which the amino acid profile was designed for animals facing an immune challenge, gained 9% faster than cattle assigned to the AminoGain 6 formula. Although this is only a numeric observation, evaluation of the AminoGain 6 Plus formula warrants further investigation because there are a number of unknowns related to Spearmint Extract and its chemical reactivity with other dietary components. The differences in the amino acids in the AminoGain 6

Plus formula was achieved by adding fishmeal. There is a possibility that the addition of fishmeal in combination with spearmint may have caused a palatability issue for the cattle. Since the inclusion of fishmeal numerically improved ADG when scotch spearmint was not included, the nutritional value of fishmeal or a fishmeal substitute, that still allows the opportunity to achieve the desired amount of amino acids, warrants further investigation.

Due to its ability to increase feed efficiency and in order to avoid any skewing of results testing other products, monensin was not included in the diets in this trial. While monensin may not be a medically-important antibiotic, Tylan is. Tylan is used for the prevention of liver abscesses and was also not included in the test diets to avoid the possible skewing of results.

The IgA results can be found in Table 4.6. Plasma IgA was measured because it's found in both respiratory and intestinal mucosa with infection which may result with receiving calves. The difference in IgA concentration between d 0 and d 42 tends to be higher. This observation warrants further investigation into the role of these compounds on the immune response. IgA concentrations in blood were not different ($P > 0.05$) as a result of treatment for any of the measured time points. Blood IgA concentrations were higher at d 21 and by d 42 had returned to similar to that of d 0. This time series progression is expected as 21 d after arrival

should be peak for animal experiencing symptoms of respiratory distress as a result of shipping and co-mingling, and by d 42 animals should be recovered. The samples were evaluated for IgA using the Epoch Plate Reader (BioTek, Winooski, VT) and a Bovine IgA ELISA kit (E11-131, Bethyl Laboratories, Inc., Montgomery, TX). They were ran in triplicates to increase the chances of a repeatable assay. The responsiveness of IgA assay to changing disease status make it an effective tool to assess gross disease state of receiving cattle. However, the dietary treatments in this trial were not effective in altering that disease state.

For the fecal IgA analysis, two different protocols were attempted to develop an assay. The first protocol involved placing 1 gram of feces into 9 ml of purified water. The same Bovine IgA ELISA kit used in the serum IgA process was used for the fecal IgA. After achieving low results using the first protocol, it was decided to increase the amount of fecal used. In the next attempt, all other steps were identical besides 5 grams of the fecal sample was placed into 9 ml of water. With an increase in feces, higher concentrations in the results were achieved. However, the results were low, this may be due in part to the animals not suffering from disease challenge in the gastrointestinal tract. Therefore, fecal testing ceased and was not reported for these animals. While there was a large amount of variation in the results, the ability to evaluate IgA response to dietary

treatment provides a novel opportunity to further evaluate the efficacy of receiving calf treatments.

Appendix I: IACUC Approval Letter



ANIMAL CARE AND USE PROTOCOL

Project Title: Effects of essential oils and amino acid profile on the growth and health of receiving cattle

Protocol #: B16102

Principal Investigator: Dr. Aimee Wertz-Lutz

Co-Investigator: - Jim Dunn

For AACUC Use only

Type: NEW

AACUC Number: B16102

Date received: 9/9/2016

Pain/distress rating: 1

Full Committee review: NO

Designated member review: Julie Sampson

Reviewer recommendations: APPROVE

Date: 9/26/2016

A handwritten signature in black ink on a light green rectangular background. The signature is cursive and reads "Julie Sampson".

Chair Signature: _____ Date: 9/26/2016

A. ANIMAL DESCRIPTION

1. No. Animals used: 150 (120 on-test + 30 extra)

2. Species: Cattle

3. Strain/Stock: Angus Crossbred

4. Age/Weight: 8 mo / 500 lbs.

5. Source: Kansas Flinthills Region

6. Dates of Proposed Animal Use:
FROM: 9/30/2016
THROUGH: 2/1/2017

7. Have the animals been use for a previous study NO

B. DESCRIBE IN LAY TERMS:

1. The purpose and importance of the study:

To evaluate the health, growth, and feed intake of feedlot calves when fed essential oil supplements and diets of various amino acid formulations

. JUSTIFY:

1. The use of animals:

Growth performance, disease out-break, and feeding behavior cannot be simulated in a lab environment.

2. The choice of species:

The specific purpose is to evaluate the response to essential oil and amino acid addition in beef cattle receiving programs.

C. PROJECT INFORMATION

1. Identification and means for contacting personnel involved with this project:

Role/NAME	ADDRESSES	PHONE NUMBERS
Principal Investigator (PI) Aimee Wertz-Lutz	OFFICE: 1000 N 30th St. Quincy, IL 62305 E-MAIL: aimee.wertz-lutz@adm.com	OFFICE: 217-231-2316 CELL: 217-653-3351 HOME: Click here to enter number.
Co-Investigator Jim Dunn	OFFICE: 1000 N. 30th St. Quincy, IL 62305 E-MAIL: jim.dunn@adm.com	OFFICE: 217-231-2242 CELL: 260-223-0098 HOME: Click here to enter number.
Role: Research Center Manager Curt Nisbet	OFFICE: 1320 Hwy. 61 Mendon, IL 62351 E-MAIL: curt.nisbet@adm.com	OFFICE: 217-936-2297 CELL: 217-257-3995 HOME: Click here to enter number.
Role: Beef Research Coordinator Dennis Miller	OFFICE: 1320 Hwy. 61 Mendon, IL 62351 E-MAIL: dennis.miller@adm.com	OFFICE: 217-936-2250 CELL: 217-430-4231 HOME: Click here to enter number.

C. PROJECT INFORMATION (cont.)

2. Personnel Qualifications: For the PI and each person with responsibility for any animal experimental manipulations, list prior experience and training in sufficient detail to allow the AACUC to determine that all personnel are qualified to perform animal related duties applicable to this project:

Aimee Wertz-Lutz: PhD, 8 yrs. experience PI South Dakota State University; 20+ yrs. production animal research

Jim Dunn: 20+yrs. experience with animal production and domestic animal research.

Curt Nisbet: 20+ yrs. experience with animal production and animal research.

Dennis Miller: 20+ yrs. experience with animal production and animal research.

Mike Finlay: B.S. and 10+ yrs. Experience in swine and poultry production research.

Julie Sampson: 20+ yrs. experience with dairy cattle and 10+ yrs. experience with animal research.

John Swanson: 20+ experience in animal production and research.

Alex Ippensen: 5+ yrs. experience Alliance Nutrition research Center.

Laura Chockley: 3+ yrs. Vet technician; +10 yrs. Animal husbandry experience

Terry Barry: Over 20 yrs. experience in animal production.

Kelsey Machens: B.S. and 10+ yrs animal husbandry, 5+ yrs. Experience in animal production research.

3. Who will be the regularly attending veterinarian for these animals?

Name: Dr. Adam Gundel

Address: Western Illinois Veterinary Center; 3910 Wisman Lane, Quincy, IL
62301

Phone #(s): 217-228-0414

4. Are WILD ANIMALS to be used or studied? NO

D. ANIMAL HUSBANDRY

1. In which animal facility will animals be housed? (Give specific building(s) and room number(s) if known):

Mendon Beef Research Center

Twelve dry lot pens equipped with concrete fence line bunks and GrowSafe feeding bunks.

2. Will animals be housed anywhere other than a designated animal housing facility for more than 12 hours (e.g., a laboratory)?

NO

3. Explain any nonstandard husbandry requirements (feeding, housing, environment):

None

D. ANIMAL HUSBANDRY (cont.)

4. Give a brief description or timeline of animal manipulations/procedures for study purposes:

This trial is intended to be a 63-d receiving calf trial in which the growth, intake, and health of newly arrived calves is evaluated. The trial will be designed as a 2x3 factorial of amino acid formulation AminoGain 6 or AminoGain 6 Plus and essential oil product control which delivers no compound, Stay Strong EO at 1g/100 lb body weight, or scotch spearmint extract at 3.27 g/hd/d. The receiving product formulas will not have other antibiotics included. All treatments will be mixed into a complete feed. Water will be provided ad libitum.

- 1) AminoGain 6 - Control
- 2) AminoGain 6 – Stay Strong EO
- 3) AminoGain 6 – Scotch Spearmint Extract
- 4) AminoGain 6 Plus - Control
- 5) AminoGain 6 Plus - Stay Strong EO
- 6) AminoGain 6 Plus – Scotch Spearmint Extract

Cattle will be implanted prior to initiation of the experiment with Synovex-S, vaccinated for respiratory disease, and treated for internal and external parasites.

Cattle will be weighed on 2 consecutive days at trial initiation (d -1, and 0) and termination (d 63 and 64) and the weights averaged for initial and final weight. Single intermediate weights will be recorded at d 21 and 42.

Rectal temperatures will be recorded at trial initiation. Cattle will be observed for respiratory and gastrointestinal disease outbreak. Rectal temperature may be recorded when other symptoms of respiratory disease are observed.

Intake of the complete feed mix will be monitored daily using the GrowSafe individual cattle feeding system.

Fecal and blood samples will be collected from 36 animals at d 0, 21, and 42.

E. DESCRIPTION OF NONSURGICAL PROCEDURES (answer all items below)

NO TISSUE COLLECTION from euthanatized animals

YES SAMPLE COLLECTION from live animals

a. Type of sample(s): Fecal and blood

b. Volume of sample(s): Fecal hand grab; blood 7-10 mL

c. Frequency of collection: Days 0, 21, 42

d. Method of collection: Fecal – grab sample; blood venipuncture

NO INDUCED OR SPONTANEOUS NEOPLASIA in live animals.

NO Use of INFECTIOUS AGENT(S) in live animals

NO Use of RECOMBINANT DNA in live animals.

NO Use of RADIOISOTOPE(S) in live animals

NO Use of CARCINOGEN(S) in live animals

NO ANIMAL RESTRAINT lasting more than 30 minutes.

NO Will any nonsurgical procedure(s) INFLICT PAIN at a level or duration greater than a needle stick:

F. DESCRIPTION OF SURGICAL PROCEDURES

1. Will there be any surgical procedures? NO If "No", go to Section G.
2. Name of procedure(s) to be performed: Click here to enter text.
3. Justification of procedure(s): Click here to enter text.
4. Person(s) responsible for performing surgery and postoperative care:
5. What surgery facility will be used?
6. Will any anesthetics, preanesthetics, or tranquilizers be used? YES or NO
If "No", go to F.10.
7. **Anesthesia**
 - a. Preanesthetic agent(s): Click here to enter agent. Dosage: Click here to enter dosage. Route: Click here to enter route.
 - b. Anesthetic agent(s): Click here to enter agent. Dosage: Click here to enter dosage. Route: Click here to enter route.
 - c. Other agent(s): Click here to enter agent. Dosage: Click here to enter dosage. Route: Click here to enter route.
 - d. Monitoring and life support systems to be utilized to ensure adequate depth of analgesia or anesthesia and to prevent overdose:
8. Complete description of post-anesthetic recovery monitoring and care:
9. YES or NO Animals will be euthanatized under anesthesia when surgery is completed.

YES or NO Animals will recover from anesthesia following surgery. If "yes", answer a. and b. below:
 - a. YES or NO Aseptic technique will be followed.
 - b. YES or NO Post-operative care will be provided and proper records will be maintained, including medications given.

F. DESCRIPTION OF SURGICAL PROCEDURES (cont.)

10. Describe post-operative care (include both short- and long-term care; monitoring, surgical wound care including suture removal, and list drugs and doses anticipated to be used)

11. Special needs of the animals following surgery:

12. Length of time animals will be kept alive following surgery:

13. Will animals be subjected to more than one major survival surgical (operative) procedure? A major operative procedure is defined in Animal Welfare Act Regulations as any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions.

YES or NO

14. Have the animals to be used previously been subjected to a major survival surgical procedure on another protocol?

YES or NO

G. EUTHANASIA

1. Will animals be euthanized at the end of study? NO

If "No", go to G.5.

2. List possible reasons for euthanasia:

3. Euthanasia agent/method:Click here to enter agent. Dose:Click here to enter dose.

4. If this method is not approved by the AVMA Panel on Euthanasia (2000) , provide scientific justification for its use:

URL: <http://www.avma.org/resources/euthanasia.pdf>

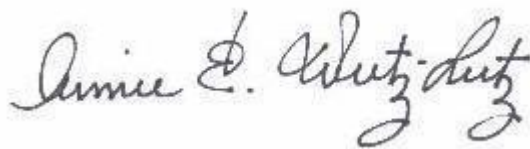
(Note: After euthanasia is performed, death should be assured by bilateral pneumothorax, aortic transection, cervical dislocation, or some other certain physical means, as appropriate.)

5. How will the animals be disposed of at the end of the study?

Cattle sold or shipped to an alternate location for finishing.

H. INVESTIGATOR ASSURANCES

1. The information provided herein is accurate to the best of my knowledge.
2. Procedures involving vertebrate animals will be performed only by trained or experienced personnel, or under the direct supervision of trained or experienced persons.
3. Accurate records of animal health and removal will be kept on file for AACUC inspections.
4. Any change in the care and use of vertebrate animals involved in this protocol, which would affect the health and welfare of the animals, will be promptly forwarded to the AACUC for review.
5. The number of animals proposed is the minimum necessary to conduct valid experimentation.
6. I have conducted a literature search to ensure that I am not unnecessarily duplicating previous experiments.
7. I have considered alternative methods to using animals.



9/22/2016

Signature of Principal Investigator or Co-Investigator
Date

Please note: An electronic (Microsoft Word) copy of the protocol is required for processing. Your email is considered an official, electronic signature, and will be kept on file for the duration of the protocol approval.

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