DISSERTATION

# CHLORELA SP.: LIPID EXTRACTED ALGAE

# UTILIZATION OF ALGAE BIODIESEL CO-PRODUCTS AS AN ALTERNATIVE PROTEIN FEED IN ANIMAL PRODUCTION

Submitted by

Marco Dib

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2012

Doctoral Committee:

Advisor: Shawn Archibeque

Terry Engle
Noa Roman-Muniz
David Van Metre
John Wagner

ABSTRACT

UTILIZATION OF ALGAE BIODIESEL CO-PRODUCTS AS AN ALTERNATIVE PROTEIN FEED IN ANIMAL PRODUCTION

Animal nutrition systems are considered the most costly component in modern animal production (ARS-USDA, 2012). To be biologically, economically and environmentally sustainable, the development of new technologies and alternative feedstuffs is necessary to enhance performance of livestock and the quality of animal protein products for the future. With grain commodities and fuel prices increasing, and the depletion of fossil fuel resources (ERS-USDA, 2012), the development of alternative sources of fuels such as corn ethanol and biofuels is of the utmost importance to meet the energy demands in the future.

With the ongoing desire to identify long term, affordable and sustainable alternatives to fossil fuels that diminish American dependence on crude oil from Middle Eastern countries, the development of alternative sources of energy has received a renewed and heightened interest recently. Additionally, crude oil prices have been steadily increasing, stimulating even greater funding, research and interest in alternative sources of energy (International Business Times, 2012). The utilization of grains as a biofuel resource is a reality in the USA today; however the use of algae over grains for the production of biofuels may be a better option due to production prices, environmental advantages, such as less requirement of water and land area than crop farms, and reduced competition for food commodities (Mata et al., 2010). Many algae species are being studied as potential alternative energy producers and some of them may be able to produce more than 10,000 gallons of oil per acre (Mata et al., 2010). When compared to the traditional ethanol production, which can be estimated to produce around 20 gallons of ethanol

per acre, algae sources raises the question of possibly replacing ethanol production with a more efficient biodiesel production in the future. The National Renewable Energy Lab (NREL) in Golden, Colorado, has been working diligently to utilize algae as an energy source. In 2010, NREL explained that replacing all the gasoline in the United States with corn ethanol would require a corn field 1600 $km^2$, while replacing all the gasoline in the United States with algae oil would (theoretically) require only 176 $km^2$. One of the algae species that is being closely investigated as a source of oil are the *Chlorella sp.* species, from the phylum Heterokontophyta, a species with an oil content greater than 20% of the DM basis (Chistis, 2007). A secondary benefit to utilizing algae as a source of biofuel is the high protein (>20% DM basis), mineral rich co-product that is produced after the oil is extracted.

In order to further explore some of the potential uses of algae co-products for animal nutrition, a project was designed to determine the effects of the lipid-extracted algal meal as a component of finishing rations for ruminant and monogastric livestock. The main project consisted of two studies that were conducted at the Colorado State University Agriculture Research, Development, and Education Center (ARDEC) facilities. Both studies were conducted for 28 days, with 8 animals per treatment. Both studies had similar diet nutritional profile to a diet of a traditional commercial finishing facility.

The first study used crossbred wethers (n = 40; initial BW = 45.3 kg $\pm$ 3.5) in a randomized complete block design to evaluate the effects of titrated concentrations of algal meal as a protein supplement on live performance, live health status and carcass characteristics. Wethers were randomly assigned to one of the 5 treatments and blocked by time that they began the trial (ten animals per block). Treatments included: 1.) a control diet with soybean meal and rice meal as protein supplementation sources (CON); 2.) 5% of algae meal on a DM basis

(5%AM); 3.) 10% of algae meal on a DM basis (10%AM); 4.) 15% of algae meal on a DM basis (15%AM); and 5.) 20% of algae meal on a DM (20%AM); All diets were formulated to be isocaloric and isonitrogenous. All wethers were fed a high concentrate finishing diet once daily in individual stalls. Wethers were individually weighed on d -1, 0, 21, and 28. On d 22, wethers were transported to metabolic crates for determination of nutrient digestibility and retention. On day 28, animals were transported to a commercial abattoir for harvest. The null hypothesis is that the algae meal may be included in finishing rations at varying concentrations, without altering the performance of finishing wethers. Initial average (45.4 kg) and final average (44.5 kg) BW, average daily gain (ADG) for the adjustment period (0.24 kg/d), ADG for metabolism period (-0.84 kg/d), DMI (1.38 kg/d), and gain-to-feed (0.187) were similar ($P > 0.55$) across treatments. Furthermore, hot carcass weight, subcutaneous adipose depth, *Longissimus* muscle area, calculated YG, marbling score, dressing percentage, muscle percentage, body wall thickness, leg circumference, flank streaking, quality grade, carcass conformation and carcass length were also similar ($P > 0.27$) across treatments.

Research results suggest that feeding up to 20% of algae co-product meal as a replacement protein source to finishing wethers is feasible with limited impact on performance and carcass characteristics as compared to the standard protein sources that have been used by the industry. Further research may be necessary to determine the response of different levels of supplementation of algal meal for sheep, effects on animals in a different physiological stage or effects on other ruminants in the finishing diet on performance and carcass merit.

The second study used crossbred barrows (n = 40; initial BW = 42.3 kg $\pm$ 3.4) in a randomized complete block design to evaluate the effect of different levels of protein supplementation on live performance, live health status and carcass characteristics. Barrows

were blocked by time of start on treatments and randomly assigned to one of the 5 treatments. Treatments included: 1.) a control diet with soybean meal as protein supplementation sources (CON); 2.) 5% of algae meal on a DM basis (5% AM); 3.) 10% of algae meal on a DM basis (10% AM); 4.) 15% of algae meal on a DM (15% AM); and 5.) 20% of algae meal on a DM basis (20% AM); All diets were formulated to be isocaloric and isonitrogenous. All pigs were fed a typical high concentrate dried whole corn based finishing diet once daily in individual pens at to the Colorado State University Agricultural Research, Development and Education Center (ARDEC). Pigs were individually weighed on d -1, 0, 21, and 28. On d 21, pigs were transported to the ARDEC metabolism facility for the metabolic phase of the study. A lumbar spinal tumor was discovered in one of the animals fed the 15% AM treatment and it had to be removed from the study and euthanized. On day 28, barrows were transported to a commercial abattoir for harvest. Initial and final BW, average daily gain (ADG) for feedlot period, ADG for metabolism period, and gain:feed were significantly different ($P \leq 0.01$) across treatments, with greater performance values encountered in the CON group and lesser values in the 5%, 10%, 15% and 20% AM. Furthermore, hot carcass weight, unribbed carcass weight, ham, loin, belly, butt, shoulder, feet, total parts, carcass length, ham circumference, and lion eye area were also decreased ($P < 0.01$) as the concentration of AM in the ration increased above %% of the ration DM. Last rib fat, last lumbar vertebrae fat, first rib fat, belly thickness, $10^{th}$ rib fat, LE L, LE a*, LE b*, marbling score and color score were similar ($P \geq 0.17$) across treatments. These data suggest that feeding up to 20% of algae co-product meal to finishing pigs has a negative impact on performance and carcass characteristics as compared to the standard protein sources that have been used by the industry. Further research may be necessary to determine the response of different levels of supplementation of algal meal for pigs and other monogastric species, effects

on animals in a different physiological stage and maximal tolerable levels of algae co-product supplementation.

Utilizing the algal meal from *Chlorella sp* as a source of protein in growing and finishing livestock rations is a possibility when the algal meal is priced the same or less than DDGS. At the same market price, the cost per kilogram of protein can be very competitive. The algal meal could also be considered a potentially competitive source of energy compared to DDGS when fed to ruminants, but likely not for non-ruminant livestock.

In summary, the utilization of lipid extracted algal meal from *Chlorella sp* may be a safe alternative feedstuff to be fed as a protein source for growing and finishing livestock animals. For further understanding of other algal characteristics, effects and economics, additional research is needed especially in large-scale experiments.

# ACKNOWLEDGEMENTS

It is hard to thank everybody who has helped me on my life journey thus far, but I will try to express my appreciation to the best of my ability. Many people must be thanked for their part in my quest of education and a Ph. D. in Ruminant Nutrition. Deciding to pursue higher education degrees far away from my home country (Brazil) was not an easy decision. Fortunately my parents once told me that you have to reach out and grab the things you want most in life, so I listened to them and my decision to study abroad was one of the best I have made thus far. I really need to thank God and my family for all the opportunities I had and still have. I would like to thank my parents for loving me as much as they do, for the education they have provided, for the values, ethics, and honesty they have taught me, and also for the wise advice given throughout the different stages of my life. Additionally many thanks must be given to my grandparents and relatives that have always supported me in all of my decisions. Thanks to all my friends that had faith in me and were present both physically and spiritually during moments of difficulty and greatness like Pedro, Paul and Emily, Leonardo, Jhones, Matt, Riley, and many many others.

Special thanks to Jake Fisher and his family for “adopting” me and making me a part of their family since I moved to the USA.

I would like to thank Dr. Shawn Archibeque and Terry Engle who gave me the opportunity to study as a Ph. D. researcher at the Colorado State University and introduced me to several extraordinary people such as Bill Wailes, Shannon Archibeque-Engle, Dr. Kevin Pond, among other faculty and staff members from the College of Agriculture at Colorado State

University. Thank you all for treating me the best way possible and I really appreciate your guidance and kindness throughout the past two years.

I would like to thank Drs. Shawn Archibeque and Terry Engle for all the knowledge, advice, interaction and dedication to me as a student and to all other graduate students that are part of our program. Thanks for all the experiences shared, for being so professional, and for challenging me to rethink and think again the greater science of animals. Thanks to John Wagner who helped me not only with statistical challenges but also with my feedlot practical skills. I would like to thank all of the other professors who somehow helped me to complete my program.

And last but not least, I would like to thank all the staff and students that helped me to accomplish my academic objectives, such as Melissa Weiss, Nancy Weiss, Laura Bonner, Kelley Neuhold, Emmanuel Caldera, Ashley Adams, Marcy Kappen, Crystal Warner, Maria Meinen, among others.

DEDICATION

I dedicate this thesis to my family and especially to my brother,

***Osmanio Dib Junior***,

whom always gives me the strength to keep moving, always has a smile on his face to remind me that life is a great gift and everybody should make the most of it. He taught me that nothing is impossible and happiness is only true if shared.

TABLE OF CONTENTS

# CHAPTER I

## INTRODUCTION

Humankind has a long and storied history intertwined with the domestication of animals that is estimated to have existed for at least 10,000 years. Humans have changed their behavior throughout the years and the domestication of a large number of animals has become potential resources for humanity such as food, work, and pleasure. Animals have provided a huge variety of products that facilitate human life quality such as wool, skin, meat, milk, and eggs, among others. Animals also are used for transportation, labor and traction, companionship and hunting, along with other activities for necessity or recreation of human life (Van Soest, 1982).

Swine and cattle are among numerous different types of animals domesticated throughout various times in history. Cattle are ruminants and are classified in the order *Arteriodactyla* and suborder *ruminantia,*. Approximately 200 species of ruminants can be found around the globe but only 9 of them have been domesticated during the last 10,000 years, European cattle, Zebu cattle, Bali cattle, sheep, goats, water buffaloes, reindeer, mithans, and yaks (Hackmann and Spain, 2010). Ruminants are different from all other mammals because of their digestive anatomy composed by four stomach compartments (reticulum, rumen, omasum and abomasum). Another unique characteristic is the interaction between animals, plants and microorganisms present inside the gastrointestinal tract resulting in a symbiotic relationship through gastroenteric microbial fermentation. Plants consumed by ruminants are utilized as substrates by the microorganisms and the products from fermentation and microorganisms provide energy, protein, and other nutrients to the host animal. Pigs are animals classified also in the order Artiodactyla, and then family *Suidae*, subfamily *Suinae* and genus *Sus.* Pigs differ from ruminants in that pigs are omnivores, which mean that they consume both plant and animal

tissues in nature. However, in modern U. S. production systems pigs are fed diets that primarily consist of corn and soybean meal products along with other vegetable products, mineral and vitamin supplements.

Animal products such as milk and meat have always been an important component of human diets; therefore technologies to enhance production efficiency and increase economic return for producers are also important. Scientific development and creation of new technologies to supply the food due to an enlarging human population is one of the concerns for the future. Innovations and solutions to improve agriculture production systems have become necessary to meet this problem. Use of alternative nutrient sources, feed strategies, additives and implants, genetic selection programs, among other options may be important to fulfill the objectives of meat producers and consumers.

Another alternative to reduce costs in production in the livestock industry is the use of co-products from other industries such as biodiesel, ethanol, beverage, food, forestry, cotton and citrus. Recently, the utilization of grain ethanol co-products has increased considerably for livestock feed and it is still a growing area to be explored. Therefore the utilization of those feedstuffs may be beneficial in terms of economics and nutrition. Since animals have the potential of digesting and utilizing the majority of the nutrient content of by-products, it is more rational to feed livestock rather than disposing of the material into landfills or utilizing them as fertilizer, where only a few nutrients may be used by the crops grown in these lands.

# CHAPTER II

# LITERATURE REVIEW

## *Microalgae Characteristics*

The origins of taxonomy relied on Carl Linneaus assumption that originally there were only two kingdoms on earth. The two of them were the Animal and the Plant kingdoms. With the help of numerous biologists modern taxonomy has evolved to include three more kingdoms: Monera, Protista, and Fungi. Further, live organisms have been categorized in two domains: Eukaryotes and Prokaryotes. The latest taxonomy structure currently used was proposed in 1998 by Cavalier-Smith, which contained 6 Kingdoms: Animal, Protozoa, Fungi, Plant (that includes red and green algae), Chromista (all algae with chlorophyll a and c, includes Heterokonts), and Bacteria. Subsequently, classification continues into Phylum, Class, Order, Family, Genus and Species.

Since there are many controversies about taxonomic algae classification, the majority of species found today are classified either under the Kingdom Plant or Chromista and then divided within numerous phyla. Microalgae organisms can be considered part of Prokaryote or Eukaryote groups of living creatures (Richmond, 2004), and true algae are generally considered Eukaryotic organisms because of their structure, which is composed by a nucleus inside a membrane, and chloroplasts in the cytosol. According to Richmond (2004), more than 50,000 species of microalgae exist and around 30,000 have been studied and analyzed. Because of the variety of species and strains and the abundance of biomass, microalgae may be utilized more frequently in the future on pharmaceutical products, energy, and food for human consumption (Mata et al., 2010). The main process to produce energy in algae organisms is photosynthesis and

the most important co-product of this process is the oxygen that will be utilized by other living organisms for respiration. Structurally, algae organisms are much simpler organisms than land plants. Some examples of prokaryotic algae are Cyanobacteria (Cyanophyceae) and eukaryotic algae are the more commonly used green algae (Chlorophyta). To provide perspective, the Goettingen University in Germany started collecting different strains and algae species since 1920's and 77% of the strains gathered were green algae and 8% were cyanobacteria (Mata et a., 2010).

*Phylum Cyanobacteria*

The importance of cyanobacteria for the modern world comes with their role in the human food chain, where different cyanobacteria are used to enhance the nutritional profile of some foods. One cyanobacteria well known and vastly utilized come from the genus Spirulina, which has large amounts of high quality protein (55-77% on a DM basis). According to Ciferri et al. (1983), another characteristic of cyanobacteria is their unique fatty acid profile, which typically contains alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachadonic acid (AA), among others, in relatively greater amounts than many other plant materials. (Ciferri et al. 1983). These characteristics raise the question of potential microalgae use in the future for numerous possibilities that ranges from food enhancement to biodiesel production.

*Phylum Rhodophyta*

Rhodophyta algae are known as Red Algae and consist of around 6000 species. Its unique characteristic is located on the cell wall that contains two membranes instead of one. Cole et al.

(1990) described that the exterior membrane is comprised of a component similar structurally to the carbohydrate pectin and its interior membrane is rich in cellulose for photosynthesis. Like cyanobacteria, red algae are also used in food manufacturing processes, mainly in the production of agar.

*Phylum Heterokonts: Nannochloropsis and Schizochytrium*

Heterokonts algae are abundant and have more than 10,000 species documented from minuscular sizes to gigantic kelps seen in the ocean. This phylum incorporates many of the recent species under study for nutritional and biofuel production. The two main species from this phylum under research are *Nannochloropsis oculata,* and *Schizochytrium* (a golden algae). Other organisms in this group are also important on the scientific world such as brown algae, yellow-green algae and parasitic *Phytophthora* (Howe, 2011).

*Phylum Chlorophyta*

Chlorophyta algae are also very important and studied for different purposes. For example, *Dunaliella sp.* and *Chlorella sp.* are Chlorophyta species that have shown positive capacity for production of β-carotene to act as an anticancer agent (Mokady et al. 1991), a prophylactic treatment in Crone's disease (Lavy et al. 2003), an in-vivo antioxidant (Murthy et al. 2004), and a possible food supplement treatment for insulin resistance (Lee et al. 2009). To date algae research has focused on looking at the health benefits and potential natural feeding supplement to humans. The majority of that research mainly examined ingestion and the effects of whole microalgae by animals so the results could be applied to humans.

*Chlorella sp.*

The taxonomic classification of *Chlorella sp.* according to Bold and Wyne (1978) is: Division – Chlorophycophyta; Class – Chlorophyceae; Order - Chlorellales; Family – Chlorellaceae; and Genus – *Chlorella*.

*Chlorella sp*. was described by Chapman and Chapman (1973) as green spherical single-celled organisms that can occur in both fresh and marine water. The genus *Chlorella sp* is only represented by eight species. Their shapes consist of spherical (globular or ellipsoidal) with micro cellulosic wall membrane, ranging from 2.0 to 10.0 μm. Oil content in microalgae can range from 10 up to 80% in its dried biomass (Spolaore et al., 2006), and *Chlorella sp* was reported ranging from 20-35% oil content itself on a DM basis (Chisti, 2007; Spolaore et al., 2006).

*Chlorella sp* may have a variable nutritional composition due to numerous factors. Kay (1991) reported an average composition of about 20% fat, 45% protein, 20% carbohydrate, 10% various minerals and vitamins in a dry basis. It is important to remember that species and methods of cultivation, and water quality from the production system will influence the microalgae composition.

*Chlorella sp* can be found spread out in many different locations such as fresh water, air and soil (Wu, 2001). Its potential to accumulate high levels of lipids, such as C 18:1, C 16:0 and C 18:3 has been target of different studies lately.

*Microalgae in Biodiesel Production*

Microalgae have been reported as a potential source of many different renewable biofuels such as methane (Spolaore et al., 2006), biohydrogen (Fedorov et al., 2005; Kapdan and Kargi,

2006). biodiesel produced from the microalgal oil (Banerjee et al., 2002; Gavrilescu and Chisti, 2005). Algal oil and biodiesel have been considered a promising alternative fuel source for the future. Since mass culture was first scaled up (Burlew, 1953) at Massachusetts Institute of Technology (MIT), various studies were designed to determine different aspects of algae and biodiesel production, including methane production (Oswalnd and Golueke, 1960; Benemann et al., 1980), algae characterization (Jarvis, ASP), genomics, and comparison between species, among other characteristics. Microalgae cultivation then became an option to optimize oil production in a more efficient and environmentally friendly way.

Petroleum supply is inversely proportional to USA dependency on crude oil. Depletion of fossil fuels and growing energy prices also added to the pool of justifications to increase biodiesel and microalgae research (Xiong et al., 2010). It is estimated that approximately 60% of the United States petroleum demand is currently dependent upon supplies from countries that are considered to be relatively economically and politically unstable, thus creating a desire to shift that demand to a more reliable source. In addition, the concern with environmental laws and greenhouse gas (GHG) emission is a hot topic where renewable biofuels will play a role not only on the economical side but also from a life quality and environmental protection perspective. The 2007 Energy Independency and Security Act signed by President Bush, is an inordinate incentive to biodiesel production since the Renewable Fuels Standard section of the law calls for 21 billion gallons of biofuels/ year to be produced by the year of 2022 (non-corn ethanol).

Currently, biodiesel is produced mainly from either animal oils and/or seed oil crops. Some examples of existing resources for biodiesel are waste cooking oil, soybean oil, palm oil, corn oil, animal fat, canola oil, among many others. Microalgae, with its extensive biomass multiplication and ability to accumulate substantial amounts of lipid, has become one of the ideal

resources for biodiesel production (Johnson and Wen, 2008). The main attractive characteristics of producing algal biofuels consist of a high productivity per area, non competition with human food sources, high solar energy yield, year-round cultivation, use of land and water of extremely low quality, and mitigation of GHG to the atmosphere (Dismukes et al., 2008; Williams et al., 2009). In addition, microalgae feedstock cultivation can be coupled not only to other industries that are responsible for high emissions of $CO_2$ but also have the potential to utilize nutrients from wastewater treatment plants. According to Yeang (2008) and Ahmed et al. (1994), pilot plant facilities have demonstrated production of approximately 46,000 L/hectare (5000 gallons/acre) compared to 2533 L/hectare of corn ethanol (271 gallons/acre) and 584 L/hectare of soybean biodiesel (62.5 gallons/acre). If these magnitudes of productivity hold true, then algal biofuels may provide a sustainable and promising for the future of biodiesel production. In conjunction with the increased production of algal biomass for biofuel production there would be the concomitant increased availability of a high volume of lipid extracted algae, or algae meal, that may be available to be used in livestock nutrition in upcoming.

Since the production of microalgae ranges from 10-20 times greater than oilseed crops on a per acre basis, the economics of production and feasibility of producing biodiesel with large amounts of microalgae biomass is being studied at the moment. The cultivation of microalgae can be done in many different ways, however the most common at this time are the open-culture systems (usually lakes or ponds) or the closed-culture systems in photobioreactors (Mata et al., 2010). For example, Sialve et al. (2009) reported biomass production of around 150 tons per hectare per year in photobioreactor.

*Biodiesel Production Process*

The process of producing biodiesel consists mainly of four parts (Fig. 1). The first part involves the choice and growth of the parental algae for lipid production. The second consists of the harvest of the algae and beginning of processing. The third part involves the extraction of the oil from the resource. During the fourth phase, the triglyceride undergoes a transesterification process, which requires 3 molecules of alcohol per triglyceride, which will produce 3 molecules of biodiesel (methyl esters) and a co-product, glycerol (Fig. 2).

Usually, the transesterification process uses acids, alkalis and lipase enzymes that catalyze the reactions, and alkali-catalyzed transesterification reactions can be up to 4000 times faster than the acid-based methods. The high costs of the lipase enzymes also intensify the usage of alkalis such as sodium and potassium hydroxide during this process (Fukuda et al., 2001, Sharma et al., 2001).

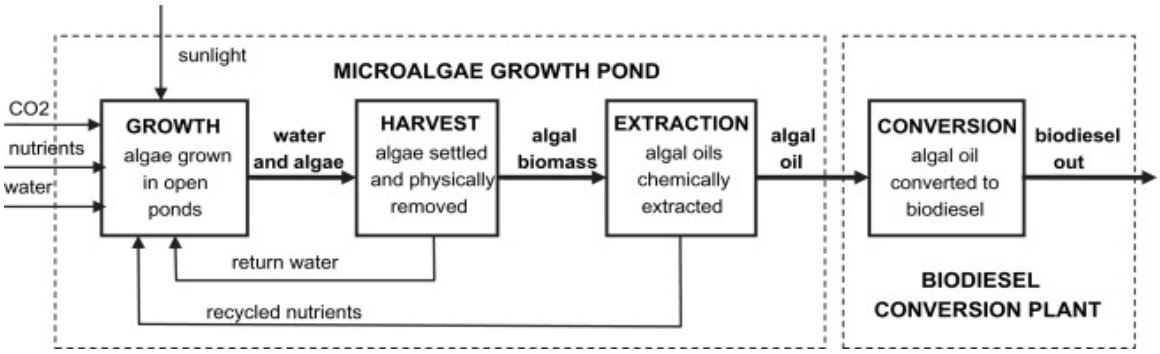


Fig. 1 – Adapted from Gallagher, 2011

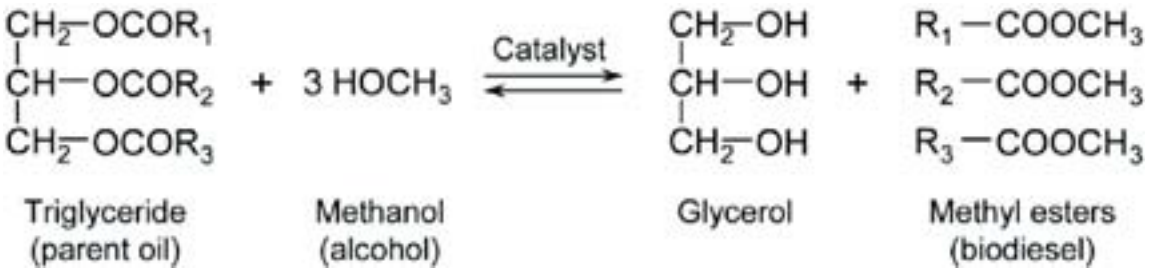


Fig. 2 – Adapted from Chisti 2007. Transesterification of oil into biodiesel

*Past Research*

The majority of microalgae research comes from its use as a pharmaceutical resource, a potential therapeutic treatment and as a nutritional supplement for humans. The expectations and possible potential effects on human health benefits from microalgae range from simple color enhancement due to its pigmentation all the way to anti-carcinogenic properties and direct impacts on glucose metabolism (Richmond, 2004; Spolaore et al., 2006). Mokady (1991) reported that *Dunaliella* sp. and *Chlorella* sp. are promising sources of β-carotene to be used as an anticancer agent. In addition, Lavy (2003) and Murthy (2004) showed in their research that the same species can also be used as a prophylactic treatment in Crone's disease and as an antioxidant. Another usage reported in past literature is the treatment for insulin resistance via micro algae food supplementation (Lee 2009).

Some of the important species of microalgae of the phyla Heterokontophyta and Chlorophyta that have been explored in a narrow context for other uses are *Nannochloropsis sp.,* and *Schizochytrium sp., and Chlorella sp.* The first two species from the phyla Heterokontophyta have been mostly used for their increased concentrations of eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) and the potential effects of these fatty acids on human health status and more recently on biodiesel production potential. Both of these algae have high levels of long chain fatty acids, which have made them quite interesting as feed supplements. *Nannochloropsis sp*. was first mentioned in 1981 by Hibberd and is characterized by its lack of chlorophyll b as well as its high levels of EPA. For this specific reason, *Nannochloropsis sp*. is currently being studied as a potential source of oil for the biofuel industry, and subsequently a source of a high protein feed supplement after the oil has been extracted. In contrast, *Schizochytrium*, a golden alga that was developed and successfully marketed into the animal and human feed supplement industry as the product DHA-Gold®

(Martek), is a whole algae product and not an algae meal. For example, this product is now fed to poultry to produce eggs with an increased concentration of DHA.

The group of organisms that are referred to as "algae" is clearly a large and complex array of many different organisms and microorganisms. This discussion is a very condensed review of a few of the "algae" groups that are in the mainstream food and research industry today. Some of the groups are much better understood than others, and there are many different species within the many phyla in existence yet to be studied. However, the research surrounding the use of the residual protein meal after the oil has been extracted from any algae is minimal, and has not been explored in detail as a potential protein supplement for animal feed.

A few pilot studies have been conducted using different species of microalgae, such as *Nannochloropsis oculata* and *Schizochytrium sp.,* which are part of the Heterokonts phyla. These specific species have been explored on the possible beneficial effects of their high levels of polyunsaturated fatty acids (PUFA) on animal and human health (EPA and or DHA). These algae species contain relatively large concentrations of fatty acids, EPA and protein with a lack of chlorophyll, which facilitates its digestion by animals. For this reason numerous research studies are being conducted to evaluate potential value for the species *Nannochloropsis sp.* as an oil source for biodiesel production and subsequently as a high protein feedstuff for animal supplementation. In the past, a golden algae specie called *Schizochytrium sp.*, was successfully introduced to the animal and human feed supplement market as the product DHA-Gold® (Martek), which now is fed to poultry to enrich DHA concentrations in eggs.

After an extensive and thorough review of the published literature it was possible to conclude that the research on lipid extracted algae as a protein meal supplement for animals is

practically nonexistent. This implies that research in this field is not only necessary but also allows research institutions to thrive for the discovery of the unknown.

Whole algae (*Nannochloropsis oculata*) has been fed to rats (Markovits et al., 1992) at concentrations of 5 and 10% of the DM for up to four weeks to determine algae safety as a dietary source. Markovits et al. (1992) reported no difference in growth, feed efficiency, or plasma triglyceride concentrations when rats were fed the diets with whole algae rather than the conventional diets. Feed intake was significantly different for weeks 2, 3 and 4 of the feeding period ($P < 0.05$), with rats fed the 10% algae consuming greater amounts than 5% algae and control for weeks 2, 3 and 4 of the trial

Another study was conducted a year later by Sukenik et al. (1993) in Israel with the main objective to determine the bioavailability of the ω3 fatty acids found in specific algal species, and compare it to the ω3 fatty acids found in capelin oil. Dietary treatments containing 10% algae from *Nannochloropsis oculata*. or *Isochrysis galbana*, or a combination of 5% *Nannochloropsis oculata*. and 5% *Isochrysis galbana* were fed in comparison to a control soybean oil based diet also to rats. After a feeding period of two weeks there was a significant reduction *(*$P < 0.05$) of arachidonic acid (AA) in plasma, red blood cells (RBC) and liver lipids, as well as a significant increase in the total amount of ω 3 PUFA in the animals fed an algae enriched diet. The ω6:ω3 also decreased in the liver lipids from 5.7 in the control group, to 2.50-2.72 in the algae enriched diets. The RBC ω6:ω3 was reduced from 17.5 to 3.6-4.4 in the control and algae diets respectively. In none of the studies were adverse health effects or toxicity were found in the animals fed the diets containing these algae products.

In 1995, another whole *Nannochloropsis* sp. study was conducted (Mokady et al.) on female pregnant and nursing rats. Treatment diet containing 20 g/kg was fed to the animals

during the whole reproductive cycle period (mating, pregnancy and lactation) and data suggested passage of the ω3 fatty acids from the mother to the developing fetuses and subsequently the newborns. No adverse health effects or toxicity were reported by the author on the dams, fetus, or newborns. Growth rates were reported as normal on growing pups.

When Werman et al. (2003) presented a study where one of the treatments contained lipid extracted algae at 65g/kg, results showed that rats presented a significant reduction in plasma cholesterol compared to the control group. This reduction could be due to NDF content in the algae residue, however this is just a speculation since the experiment was not designed to measure this matter specifically. Once more, the author reported no health issues.

Howe et al. (2010; 2011) evaluated the use of algal meal in the diets of growing rats and rabbits. Growth performance in the rats fed algae meal was inferior to the rats fed the control treatment, but no differences were determined when fed to rabbits. A potential explanation for these results may be that rabbits have a more advanced symbiosis with digestive microflora in the cecum, which allows them to more fully utilize the highly fibrous feedstuffs. Understanding the nutritional values of *Nannochloropsis oculata*. as a potential animal supplement, Archibeque et al. (2009) compared the nutrient profiles of *Nannochloropsis* biomass, *Nannochloropsis* meal (lipid extracted), soybean meal, and steam flaked corn. As a result, the comparison showed that the NDF and ADF fiber composition of *Nannochloropsis* meal was greater than soybean meal, and steam flaked corn, 25.12% vs. 11.45% and 9.59%), and 6.64 vs. 5.89, 2.92% respectively.

Fox et al. (2008) suggested that the projected need for distiller's grains as livestock feed could potentially exceed 46,000,000 MT, which would be not met by the industry. Nonetheless, comparing corn ethanol industry and possible algae biodiesel production, useful co-products from their manufacturing processes can be complementary and useful in livestock nutrition.

The use of corn (and other crops) as a source of fuel is directly competing with traditional livestock feeds, as well as the human food chain. The co-products from ethanol production can be used in animal feeds, but there are maximum levels that the animals can consume and remain healthy and productive. The feeding limitations, combined with the competition between ethanol manufacturers and livestock producers is driving the cost of grains greater, resulting in a greater priced food supply for the population.

An alternative to both of these challenges is to look beyond traditional oil crops for biofuel raw material, and the utilization of the co-products of alternative biofuel production. Algae offer a possible solution to both issues and should be researched fully to understand its full potential.

*Summary of Algal Research*

In summary, the results presented by research that has been done in the past, show no negative consequences for animal health. The experiments cited above reported that animals continued to grow as predicted, as well as their final products such as, milk, eggs and meat were normal for the scientific standards, and no negative toxicological characteristics were noted thus far.

Franklin et al. (1999) fed dietary marine algae (*Schizochytrium sp*.) to dairy cows with the objective to increase levels of desirable fatty acids in milk fat. *Schizochytrium sp*. is an algae rich in (n-3) fatty acids in its composition and the results showed a decrease in percentage of fat in milk but no differences in energy-corrected milk. Decrease in feed intake occurred in this experiment for cows on algae treatments. Milk fat composition from cows fed algae contained greater concentrations of conjugated linoleic acid and (n-3) fatty acids proving that milk fat

composition can be modified feeding whole algae to increase more favorable fatty acids for the consumers.

Nitsan et al. (1999) also fed whole algae (*Nannochloropsis sp.)* to poultry with the objective of increase levels of ω 3 fatty acids in eggs and meat. *Nannochloropsis sp.* has also some unique characteristics on its fatty acid composition such as high concentration of EPA (20:5 ω 3) and absence of other ω3 fatty acids. Results showed that the supplementation of algae increased yolk pigmentation and 1% algae also increased levels of ω3 fatty acids in the egg yolk. No difference was encountered on thigh fatty acid profile in this study probably due to small amounts of fatty acids supplementation in the study. However differences in thigh muscle fatty acid profile were previously found when higher levels were supplemented to poultry (Nitsan et al., 1999).

In general, as a nascent industry, the ultimate methodology of algal biomass and co-product production and use has not been well established and the optimal methods for using these products are still under development. The true nutritional value of lipid extracted algae is not yet established and serious differences should be considered when feeding this product for ruminants versus non-ruminant species. It is very important to remember that not only the protein contents of the co-product is elevated but also the amount of fiber is also increased relative to many more common feed components which may decrease digestion by monogastric animals due to lack of appropriate symbioses allowing cellulolytic digestion. Depending mainly on price and availability, lipid extracted algae co-product may take an important place on the animal feeding industry.

Further performance studies are needed for different livestock species and production stages, also the standardization of the co-product need to be determine before consistent results

can be presented with accuracy. Different species of algae need to be taken in consideration as well as the method of production and extraction when doing research with the algal meal. The future of fuel in this country may change quickly if the production of biodiesel increase exponentially and new feedstuff will be available from these sustainable sources of biofuel making possible to change the history of animal nutrition.

*Biofuels and Biofuels By-products in The USA – Past and Present*

The term biofuel can be defined as a fuel source derived from carbon fixation or the reduction of $CO_2$ to organic compounds catalyzed by living organisms. Fossil fuels are not usually considered a type of biofuel since its formation comes from a different nature and it takes extremely long periods of time for its formation. The most famous characters of the biofuel industry in the USA currently is the ethanol coming from cereal grains that have been fermented, and the biodiesel coming from the transesterification process between an oil source (animal or vegetal) and methanol creating glycerin and biodiesel.

Around the globe, bioethanol is an important player on the fuel industry and it is composed by an alcohol structure produced through the fermentation of carbohydrates, usually from corn and sugar cane on a large-scale scenario. The biodiesel is not as economically as important as ethanol at this point in time, however its significance is raising more and more with the need for alternative fuel sources to supply the increasing demand for transportation and energy sectors of the modern world.

When crude oil prices reached values of over US$100.00 per barrel in 2008, the interest in finding alternative fuel sources increased exponentially and ethanol was the first type of biofuel to really take off in a large-scale production in the USA. Unlike many other countries,

ethanol in the United States utilizes grain crops while other countries, like Brazil, utilize sugar cane as their main source of carbohydrates for this fermentative process. For centuries, the human population has fermented cereal grains and produced alcohol from that process and by the late 19th century some researchers were already using the co-products of that process as feedstuff (Henry, 1900). At first, the production of alcohol had the objective of human consumption as alcoholic beverages, and with time automobile fuel and other uses for it became more important to the industry.

During the initial period of ethanol utilization as automobile fuel in the mid 1800's, its popularity was not very high. For example, an extra tax was levied on industrial alcohol in 1862 to help raise money to fund the Civil War. The taxation extended until 1906, when ethanol started to be used in larger quantities as a fuel source in the United States. From 1919-1933, the use of ethanol was nonexistent due to Prohibition, when the production, commercialization and transportation of alcohol was illegal. At that time, gasoline became the most important and popular fuel source in the U.S.A and other parts of the world and remains so currently. Later on, the Solar Energy Research, Development and Demonstration Act signed in 1974, enacted and allocated funds for the development of processes and techniques to produce alternative fuel sources. And in 1992, when the Energy Policy Act was enacted, a percentage of the total number of cars produced, had to be made with the functionality of using gasoline and/or ethanol. Those engines were called flex-fuel engines. This same Act aided in the implementation of the 85% ethanol or E85 fuel type to run these new engines.

USDA and National Corn Growers Association report corn as the most important grain crop utilized around the world for different purposes. More than 4200 different products come from corn. Ethanol and ethanol co-products are only a few of them. The United States of

America produced approximately 12.4 billion bushels of corn on more than 80 million acres planted in 2011 according to the USDA Crop Production Summary (2012). That corresponds to more than 40% of the world's corn crop, and is one of the primary factors that allows the United States to currently be the largest producer of corn ethanol in the world.

The next episode that changed the production of corn ethanol in America was the Energy Tax Act of 1978 that created tax credits incentives for corn ethanol producers, resulting in a rise in production from approximately 20 million gallons to 750 million gallons from 1979 until 1986. Almost 30 years later, in 2005 the American government created the Energy Policy Act, which mandated that at least 7.5 billion gallons of ethanol per year had to be consumed by the year of 2012. Another act followed this last one in 2007 mandating now that the annual consumption of corn ethanol by 2015 had to be at least 15 billion gallons per year. In the same year (2007), the Energy Independence and Security Act was initiated to also support the increase production of corn ethanol internally. The original reasoning for these acts to be put in place was to try to reduce greenhouse gas emissions and try improving rural economy in America (USDA 2010).

The government stipulated a goal target of least 36 billion gallons of biofuels utilization by 2022 where up to 15 billion gallons could come from the corn ethanol production and hopefully the rest coming from other alternative biofuels such as biodiesel from other grains and microalgae. This is mainly the reason for the rapid increase in research and production of biodiesel in the country and around the world.

In the USA, approximately 35% of the domestic corn production is being used for ethanol production, which results not only in ethanol but also millions of tons of co-products utilized as feed sources for livestock countrywide. From the two ethanol milling processes (wet

or dry), many different co-products have been utilized in livestock nutrition. Some of these co-products are: Wet corn gluten feed (WCGF), dry, wet or modified distiller's grains with or without distiller's solubles, and corn steep liquor, among others. Since the ethanol industry only utilizes the starch portion of the corn kernel, the remaining nutrients are approximately concentrated 3 times more than the original grain, creating a valuable feedstuff for livestock. Following the same logic, the same would happen with the microalgae co-products. With the utilization of the oil from the microalgae biomass, the other nutrients would become more concentrated and the algae meal leftover and the glycerin would become a very attractive feedstuff for livestock as well.

*Biofuel Co-products for Livestock*

Many co-products from different industries have been fed to livestock for a long time. Some of the most common feedstuffs used currently in many animal rations are soybean meal, bakery waste, brewery waste, corn gluten feed, distiller's grains, beet or cane molasses, glycerin, and many others. These products are used by practically by all the livestock species such as cattle, dairy, swine, fish and poultry. The main driver for co-product utilization in animal diets is price, followed by availability. Additionally, this use of nutrients in co-products that humans either can't, or won't consume will continually reinforce the need for livestock in the sustainable production of food throughout history and into the future.

Feeding values, nutrient composition, and performance data is available for the majority of all these products in the literature. Since the most important biofuel co-product utilized presently in livestock production are different variations of distiller's grains with or without solubles added, some considerations should be addressed. According to Klopfenstein et al.

(2008), distiller's grains usage in beef cattle production is beneficial, feasible and an alternative in replacing grains as energy or protein sources depending on the diet and co-product amounts utilized; similar findings have also been described for dairy cattle ( Schingoethe et al., 2009) Co-products of the ethanol industry have shown favorable results not only in ruminant production but also in non-ruminant animals such as pigs. A review paper written by Stein et al. (2009) reviewed the use of dried distiller's grain solubles for swine production, and results shown by 40 years of studies that growing pigs at all stages can be fed rations with up to 30% distiller's grains, and lactating and gestating sows can tolerate diets including up to 50% distiller's without affecting live animal performance. Some negative impacts that may be observed when feeding distiller's grains is the higher percentage of polyunsaturated fatty acids which, while potentially good for human health, is not as favorable for the shelf life of the meat, or its color and flavor. Ruminants fed high levels of distiller's grains in their diets generally produce meat that become darker and with less flavor faster than animals that are fed low amounts of distiller's grains (Gill et al., 2008). The importance of distiller's grains in the agricultural world is notable; however, decreases in corn production and high prices caused by the second consecutive year of drought may change its utilization and availability for the near future. With that said, other co-products may be considered in livestock production to replace or complement protein sources in animal diets.

Animal nutrition systems are considered the most costly component in modern animal production (ARS-USDA, 2012). To be biologically, economically and environmentally sustainable, the development of new technologies and alternative feedstuffs are necessary to enhance performance of livestock and the quality of animal protein products for the future. With grain commodities and fuel prices rising in the past three years (ERS-USDA, 2012), the

development of alternative sources of fossil fuels such as corn ethanol and biofuels is of the utmost importance to our society.

Some co-products from the ethanol industry have been utilized in animal diets such as distiller's grains, distiller's solubles, corn gluten feed, for their efficiency which have been tested over the years with many different species (Gill et al., 2008; Klopfenstein et al., 2008; Dib et al., 2010; Schoonmaker et al., 2010; Bremer et al., 2011). The main hypothesis is is that the algae meal may be included in finishing rations at varying concentrations, without altering the performance of finishing livestock.

As a result, algae biodiesel industry rises as a potential future fuel resource by producing a high protein and fiber co-product where ruminant production systems could take advantage, utilizing it as a replacement of soybean meal, corn gluten meal, distiller's grains, and other protein feedstuffs. The use of an algal meal could be a nutritious, economic, and environmentally sustainable substitution for these animal protein agricultural crops. Since algal meal has not yet been approved for feeding livestock, there is a paucity of data regarding their use by large ruminant animals.

Therefore, research in the algae field can have enormous impact not only on the biofuels industry, but also on the beef and dairy industries in the near future throughout the USA and the world.

*Ruminant vs. Non-Ruminant Digestive System*

*Ruminant Digestive System*

Ruminants are unique animals that have many different characteristics than other mammals. They do not present upper incisor teeth in their mouth, however they possess of a robust dental pad instead. Their tongues and lips are utilized for grazing or sorting of smaller

feeds such as grains. Abundant mastication is commonly observed to form a better mixture of the feed with saliva but also to ease the formation of a bolus for facilitate the swallowing process and start break down of the particle size.

Ruminant saliva contains large amounts of alkaline substances that will mainly buffer acids produced during fermentation that drop the rumen pH, aid chewing and swallowing of dry feeds, and permit some minimal breakdown of short chain triglycerides by salivary lipase. Saliva volume produce by the animal is influenced by the feed ingested and water intake.

The most important difference between ruminants and non-ruminants is the anatomy of the ruminant stomach, which is divided into four compartments: the reticulum, the rumen, the omasum and the abomasum. The reticulum and rumen are virtually together with a fold of tissue that partially separates them. This fold is called the reticulo-rumen fold, and its main function is to keep undesired material out of the rumen in the neonatal ruminant and also to make possible the free movement of digesta between the two compartments for rumination. Rumination is the regurgitation process that these animals utilize to reduce particle size to facilitate the fermentation processes in the rumen. Ruminant animals usually spend more time ruminating and salivating than when they are actually eating and this process is more frequently observed the night on a grazing situation.

The majority of the ruminant digestion occurs in the rumen via microbial fermentation. The rumen is filled with a large microbial population that survives in a symbiotic relationship with the animal. Rumen walls contain epithelial cells, which finish in papillae. structures that can differ from animal to animal due to species, genetic and diet composition. More concentrated diets tend to stimulate the development of papillae to increase the surface area for more efficient

absorption of the nutrients. The greatest difference between ruminant animals and most non-ruminant animals can be described as the capability of the ruminant to ferment and digest substantial amounts of cellulosic material creating volatile fatty acids, methane and $CO_2$ as products. Volatile fatty acids are mainly propionic, butyric and acetic acid, along with other acids in smaller quantities. These are absorbed via ruminal wall and served as the main energy source for the animal.

The omasum is next and its structure can be described as a hard round compartment filled many longitudinal folds or leaves. The primary function of the omasum is to serve as a filter, thus limiting the ability of larger matter to pass from the rumen. In the omasum, some water absorption from the digesta also occurs.

The abomasum is the last compartment of the ruminant stomach, and is very similar structurally and functionally to the glandular stomach in humans and other non-ruminants. Acid and enzymatic digestion occurs in this compartment. This acidic blend of the ingesta then passes into the duodenum where it is mixed with bile, enterocytic and pancreatic secretions, allowing for subsequent digestion and absorption of nutrients. Some small portion of the digestion of soluble carbohydrates may occur at this time, however mainly lipids and other rumen by-pass products will be digested here. Amino acids and majority of the digested nutrients are now absorbed in the small intestine. The large intestine and cecum are considered of lesser importance in the ruminants than monogastrics due to the previous fermentation processes applied to the plant fibers in the rumen. Some water, minerals, nitrogen, volatile fatty acid, and carbohydrate absorption occurs here.

*Monogastric Digestive Systems*

In pigs and other monogastric animals, the anatomy of the mouth is a little different than ruminants where the lips, cheeks, palate, teeth and tongue are involved with apprehension, mastication and some chemical breakdown of the feed and its movement into the digestive tract. Swine can drink up slurry feeds and water more effectively than many other species and porcine saliva is produced by three primary glands in the mouth cavity. Swine also differ from ruminants in that they have only one stomach compartment and motility of this gastric stomach is very important during the mixing process of the digesta with the gastric fluids after feed was swallowed. Also, that movement helps to move this acidic blend of the ingesta into the duodenum where it is mixed with bile, enterocytic and pancreatic secretions, allowing for subsequent digestion and absorption of nutrients.

The small intestine is mainly divided in three parts: the duodenum, jejunum and the ileum. These are the locations where the majority of digestion and absorption of nutrient takes place in these animals. Coming out of the stomach, the digesta is buffered in the first part of the duodenum and intestinal, liver and pancreatic secretions are release to be mixed with the bolus. The duodenum is the site for the mixing of digesta with intestinal, hepatic, and pancreatic secretions.

In the cecum and colon, some nutrients are retrieved, primarily water and electrolytes from the digesta coming from the small intestine. The cecum is a blind sac appearing at the junction of the ileum and colon. At this site, anaerobic fermentation of fiber occurs and produces some utilizable energy in the form of volatile fatty acids like in the rumen, however in a much smaller scale. Because they lack a large-volume fermentation chamber in their gastrointestinal

tract, pigs cannot utilize the majority of the fiber ingested in the diet. That is the reason for the low performance when growing or finishing diets contain high amounts of fiber.

*Protein Supplementation to Livestock*

Protein sources utilized on livestock supplementation originate usually from plant, animal sources or urea, for ruminants. A protein supplement can be characterized by a feed containing more than 20% crude protein in its composition on a DM basis according to the International Feed Number (IFN) definitions. Usually, protein sources are the most costly constituents in the animal's ration, therefore selection of protein sources should be made considering cost, quality (as indicated by such parameters as biological value), and availability of nutrient and product at your geographic location.

The utilization of different sources of protein for supplementation depends on the specie of livestock, its production stage and nutrient requirements. The relative proportions of amino acids is important for monogastrics is very important because they lack the symbiotic gut microflora to synthesize essential amino acids.

The majority of the plant protein feeds are co-products of the vegetable oil industry, and soybean meal is the most famous and common protein supplement for livestock in the USA. Soybean meal and other co-products from the vegetable oil industry usually present a product low in the sulfur, and high in amino acids such as cysteine and methionine (usually low in lysine). Soybean meal commercialized currently has a protein level ranging from 44% to 52% depending whether or not the hulls are added back to the product. This product is a very palatable feedstuff, not only presenting highly digestible protein but also significant quantities of

energy.

Canola meal, cottonseed meal and linseed meal are also commonly used as protein supplements in the livestock industry and the importance of each varies in different regions of the country, in accordance to availability. Other very important co-products for the livestock industry are brewer's and distiller's grains. Distiller's grains contain usually around 25-30% protein, and present relatively high fiber levels in its composition. Due to the yeast presence and sulfuric acid added during the processes in the plant, a high B vitamin content and high sulfur content is also observed. The majority of the distiller’s products commercialized currently come from corn, therefore low lysine levels are observed on these products. Brewer's dried grains have considerably high fiber content (18-19%), and are therefore, most suitable and utilized as a protein supplement for ruminants (especially dairies). The protein level is also around 25-30% and low in lysine, but high in methionine.

Other sources utilized by livestock are animal protein supplements such as meat meal, blood meal, and bone meal. They are usually co-products of the meat-packing industry. They are made by waste animal tissues, and the amino acid distribution is usually similar to the animals utilized in the process. Quality of these may vary due to the different between batches and from plant to plant. Increased regulations have altered the animal protein feeding practices.

The last common source of protein supplements would be the single cell materials. Bacteria, yeast, fungi and algae are part of this group. They can ferment residues, help with food processing, alcohol production and even degradation of human or animal wastes. The protein concentrations from these products may range from 20-80% and the availability for digestion is reasonably high. Most contain moderate energy and may provide other nutrients such as the B

vitamins. Enhancement of development and production techniques of these product are expected in the near future; subsequently costs will decrease and these protein sources will become more competitive with other feedstuffs in the current market.

REFERENCES


9000 Year-Old Beer Re-Created From Chinese Recipe. National Geographic News. www.news/nationalgeographic.com/news/2005/07/0718. Accessed Oct. 20th, 2012.

Abril, R., J. Garrett, S. Zeller, W. Sander. 2003. Safety assessment of DHA-rich microalgae from *Scizochytrium* sp. Part V: target animal safety/toxicity study in growing swine. Reg. Tox. Pharm. 37:73-82.

Ahmed, A. Z., 1994. Preliminary survey of soil algal flora in Upper Egypt. Egypt. J. Bot., 34: 17-36.

Archibeque, S. A., A. Ettinger, B.D Willson. 2009. *Nannochloropsis Oculata* As a source for animal feed Acta. Agron. Hung. 57:245-248.

ARS-USDA. 2012. USDA Crop production 2011 summary. http://usda01.library.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2012.pdf Accessed on: Aug. 12, 2012.

Banerjee A, Sharma R, Chisti Y, Banerjee UC. 2002. Botryococcus braunii: a renewable source of hydrocarbons and other chemicals. Crit Rev. Biotechnol 22:245–79.

Benemann, J. R., B. L. Koopman, J. C. Weissman, D. E. Eisenberg and R. P. Goebel. 1980. Development of microalgae harvesting and high rate pond technology. In G. Shelef and C. J. Soeder, eds. Algal Biomass, 457 - 499. Elsevier Biomedical Press, Amsterdam.

Boeckaert, C., N. Boon, I. Z. Abdulsudi, W. Verstraete, V. Fievez. 2006. Accumulation of biohydrogenation intermediates and changes in the rumen protozoal population after micro algae feeding to dairy cattle. Comm. Appl. Biol. Sci. Ghent University. 71:83-86.

Boeckaert, C., B. Vlaeminck, J. Dijkstra, A. Issa-Zacharia, T. Van Nespen, W. Van Straalen, V. Fievez. 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J. Dairy Sci. 91:4714-4727.

Burlew J. S. (ed.) (1953) Algal culture from laboratory to pilot plant. Carnegie Institution of Washington, Washington DC. p. 357-358..

Ciferri, O.. 1983. Spriulina, the edible microorganism. Microbiological Rev. 47: 551-578.

Cole, K., R. Sheath. Biology of the red algae. 1990. Cambridge University Press 2:221-258.

Chisti, Y.. 2007. Review Paper: Biodiesel from Microalgae. Bio. Tech. Adv. 25:294-306.

Dismukes, G. C., C. Damian, B. Nicholas, A. M. Gennady, P. C. Matthew. 2008. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Current Opinion in Biotechnology. 19:235-240.

Forbes, R. J.. 1970. A short history of the art of distillation: From the beginning up to the death of Cellier Blumenthal. BILL., ISBN 9789004006171.

Dahms, I. F., P.A. Marone, E. Bailey-Hall, A. S. Ryan. 2011. Safety evaluation of algal oil from *Schizochytrium sp.* Food and Chem. Tox. 49:70-77.

ERS-USDA. 2012. http://www.ers.usda.gov/Briefing/ Corn/2010baseline.htm.

Fedorov A. S., Kosourov S, Ghirardi ML, Seibert M. 2005. Continuous H2 photoproduction by Chlamydomonas Reinhardtii using a novel two-stage, sulfate-limited chemostat system. Appl Biochem Biotechnol. 403–12.

Fievez, V. 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J. Dairy Sci. 91:4714-4727.

Franklin, S., K. Martin, R. Baer, D Schingoethe, A. Hippen. 1999. Dietary marine algal (*Schizochytrium sp.*) increases concentrations of conjugated Linoleic, Docosohexaenoic and Transvaccenic Acids in milk of dairy cows. J. Nutr. 129:2048-2052.

Fukuda H, Kondo A, Noda H. Biodiesel fuel production by transesterification of oils. 2001. J Biosci Bioeng 92:405–16.

Gallagher, B. J.. 2011. The economics of producing biodiesel from algae. Renewable Energy. 36:158-162.

Gavrilescu M., Chisti Y. 2005. Biotechnology—a sustainable alternative for chemical industry. Biotechnol Adv. 23:471–99.

Gill, R. K., D. L. VanOverbeke, B. Depenbusch, J. S. Drouillard and A. DiCostanzo. 2008. Impact of beef cattle diets containing corn or sorghum distillers grains on beef color, fatty acid profiles, and sensory attributes. J. Anim. Sci. 86:923-935.

Gouveia, L., A. C. Oliveira. 2009. Microalgae as a raw material for biofuels production. J. Ind Microbiol. Biotech. 36:269-274.

Hackmann, T. J. and J. N. Spain. 2010. Invited review: Ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. J. Dairy Sci. 93:1320-1334.

Hammond, B.G., DA. Mayhew, M. W. Naylor, F. A. Ruecker, R. W. Mast, W. J. Sander. 2001. Safety assessment of DHA rich microalgae from *Schizochytrium sp.*: I. subchronic rat feeding study. Reg. Tox. and Pharm. 33:192-204.

Hammond, B. G., D. A. Mayhew, J. F. Holson, M. D. Nemec, R.W. Mast, W.J. Sander. 2001. Safety assessment of DHA rich microalgae from *Schizochytrium sp.:* II. developmental toxicity evaluation in rats and rabbits. Reg. Tox. and Pharm. 33:205-217.

Hammond, B. G., D. A. Mayhew, K. Robinson, R. W. Mast, W. J. Sander. 2001. Safety assessment of DHA rich microalgae from *Schizochytrium sp.* III. Single generation rat reproduction study. Reg. Tox. and Pharm. 33:356-362.

Hammond, B. G., D. A. Mayhew, L. D. Kier, R.W. Mast, W. J. Sander. 2002. Safety assessment of DHA rich microalgae from *Schizochytrium sp.* IV. Mutagentcity studies. Reg. Tox. And Pharm. 35:255-265.

Herber-McNeill, S. M., M. E. Van Elswyki. 1998. Dietary marine algae maintains egg consumer acceptability while enhancing yolk color. Poultry Science 77:493-496.

Henry, W. A. 1900. Dried distillery grains compared with oats. Feed and Feeding 2nd Edition 421. Morrison Publishing Co.

Hibberd, D. J. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae. Bot. J. Linnean Soc. 82:93-99.

Howe, B. A., I. N. Roman-Muniz, B. D. Willson, and S. L. Archibeque. 2010. Evaluation of algae meal from Nannochloropsis oculata as a protein source for non-ruminant animals. J. Anim. Sci. 88: (Suppl 2) (Abstr.).

Howe, B. A, I. N. Roman-Muniz, B. D. Willson, and S. L. Archibeque. 2011. Nannochloropsis oculata meal did not alter nutrient usage and had no adverse health effects when fed to rabbits as a protein source. J. Anim. Sci. 89: (Suppl 2) (Abstr.).

Howe, B. 2012. Nannochloropsis Oculata: a safe protein feed for growing rats and rabbits. PhD Diss. Colorado State Univ., Fort Collins.

International Business Times. 2012. Oil prices: will crude fall substantially in 2013? http://www.ibtimes.com/oil-prices-will-crude-fall-substantially-2013-850159. Accessed Oct. 20, 2012.

Johnson MB, Wen Z. 2008. Development of an attached microalgal growth system for biofuel production. Appl Microbiol Biotechnol. 85:525–34.

Kapdan I. K., Kargi F. 2006. Bio-hydrogen production from waste materials. Enzyme Microb Technol. 38:569–82.

Klopfenstein, T. J., G. E. Erickson, V. R. Bremer. 2008. Board invited review: use of distillers co-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

Lavy, A., Y. Neveh, R. Coleman, S. Mokady, M. Werman. 2003. Dietary Dunaliella *bardawil*, a β-carotene rich algae, protects against Acetic Acid induced small bowel inflammation in rats. Inflammatory Bowel Disease 9: 372-379.

Lee, H., M. Kim. 2009. Effect of Chlorella *vulgaris* on glucose metabolism in Wistar Rats fed high fat diet. J.Med Food 12(5):1029-1037.

Markovits, A., R. Conejeros, L. Lopez,M. Lutz. 1992. Evaluation of marine microalga *Nannochloropsis sp* as a potential dietary supplement. Chemical, nutritional and short term toxicological evaluation in rats. Nutr. Res. 12:1273-1284.

Mata, T. M., A. A. Martins and N. S. Caetano. 2010. Microalgae for biodiesel production and other applications: A review. Ren.and Sus. En. Reviews 14:217-232.

Mokady, S. 1995. A marine unicellular alga in diets of pregnant and lactating rats as a source of ω3Fatty Acids for the developing brain of their progeny. J. Sci. Food Agric. 68:133-139.

Mokady, S., A. Ben-Amotz. 1991. Dietary Lipid level and the availability of β-carotene of *Dunaliella bardawili* in rats. Nutrition & Cancer, 15:47-52.

Murthy, K. N., A. Vanitha, J. Rajesha, M. Swamy, P.R. Sowmya, G. Ravishankar. 2005. In vivo antioxidant activity of Carotenoids from Dunaliella *salina*, a Green Microalgae. Life Sciences 76:1381-1390.

Nitsan, Z., S. Mokady, A. Sukenik. 1999. Enrichment of poultry products with ω3 Fatty Acids by dietary supplementation with Alga *Nannochloropsis* and Mantur Oil. J. Agric. Food Chem.. 47: 5127-5132.

Oswald, W. J., and C. G. Golueke. 1960. Biological transformation of solar energy. Adv. in Appl. Microb. 11:223–242.

Richmond, A. 2004. Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd. Roach, J.

Renewable Fuels Association (RFA). 2008. Feeding the future: The role of the U.S. ethanol industry in food and feed production. Washington DC. http://www.ethanolrfa.org/page/-/objects/documents/2278/feed_co-products.pdf?nocdn=1. Accessed Oct. 24th, 2012.

Scingoethe, D. J., K. F. Kalscheur, A. R. Hippen, A. D. Garcia. 2003. Invited Review: The use of distillers products in dairy cattle diets. J. Dairy Sci. 92:5802-5813.

Sellner, K. G., G. J. Doucette, G. J. Kirkpatrick. 2003. Harmful Algal Blooms: Causes, impacts, and detection. J. of Indust. Microb. and Biotech.. 30:383-406.

Sharma R., Y. Chisti, U. C. Banerjee. 2001. Production, purification, characterization, and applications of lipases. Biotechnol Adv.19:627–62.

Sialve B., N. Bernet, O. Bernard. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv. 27:409–16.

Singh, A., P. S. Nigam, J. D. Murphy. 2011. Renewable fuels from Algae: An answer to debateable land based fuels. Biores. Tech. 102:10-16.

Spolaore P, C. Joannis-Cassan, E. Duran, A. Isambert. 2006. Commercial applications of microalgae. J Biosci Bioeng 101:87–96.

Stein, H. H., G.C. Shurso. 2009. Board Invited Review: The use and application of distillers dried grains with solubles in swine diets. J. Anim. Sci. 87:1292-1303.

Sukenik, A., T. Hidehiro, S. Mokady. 1994. Dietary lipids from marine unicellular algae enhance the amount of liver and blood omega-3 fatty acids in rats. Ann. Nutr.Metab, 38:85-96.

USDA. 2012. Crop Production 2011 Summary. http://usda01.library.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2012.pdf. Accessed on 10/11/2012.

Werman, M., A. Sukenik, S. Mokady. 2003. Effects of the marine unicellular alga *Nannochloropsis* sp. to reduce the plasma and liver cholesterol levels in male rats fed on diets with cholesterol. Biosci. Biotechnol. Biochem., 67:2266-2268.

A USDA regional roadmap to meeting the biofuels goal of the renewable fuels standards by 2022. 2010. USDA Biofuels Strategic Production Report.

Williams, P. R. D., D. Inman, A. Aden and G. A. Heath. 2009. Environmental and Sustainability Factors Associated With Next-Generation Biofuels in the US: What Do We Really Know? Environmental Science & Technology, 43:4763-4775.

Wu H. L. 2001. Identification of Chlorella spp. isolates using ribosomal DNA sequences. Bot Bull Acad Sinica, 42:115–21.

Villar, R., M. R. Laguna, I. Cadavid, J. M. Calleja. 1994. Effects of aqueous extracts of six marine microalgae on smooth muscle contraction in rat Duodenum and Vas Deferens. Planta Med 60:521-526.

Xiong W., C. Gao, D. Yan , C. Wu, Q. Wu. 2010. Double CO2 fixation in photosynthesis–fermentation model enhances algal lipid synthesis for biodiesel production. Bioresour Technol, 101:2287–93.

Yeang, K. 2008. Biofuel from algae. Arch. Des. 78:118–119.

# CHAPTER III

# EFFECTS OF ALGAL MEAL SUPPLEMENTATION TO FINISHING WETHERS ON PERFORMANCE AND CARCASS CHARACTERISTICS

## INTRODUCTION

Animal nutrition is considered the most costly component in modern animal production (ARS-USDA, 2012). To be biologically, economically and environmentally sustainable, the development of new technologies and alternative feedstuffs are necessary to enhance performance and quality of animal protein products for the future. With grain commodity and fuel prices rising in the past three years (ERS-USDA, 2012), development of alternative sources of fossil fuels such as corn ethanol and biofuels is of the utmost importance.

Some by-products from the ethanol industry have been utilized in animal diets such as wet distiller's grains, distiller's solubles, corn gluten feed, and have been tested over the years among different species (Gill et al., 2008; Klopfenstein et al., 2008; Dib et al., 2010; Schoonmaker et al., 2010; Bremer et al., 2011).

Microalgae is now being considered as an oil source for biodiesel production. Although research on this topic has been active for the past 20 years, the interest on the cultivation of microalgae species for biodiesel and glycerin production has recently received a renewed interest. Different species of microalgae produce different amounts of oil, and some of them can produce approximately 5000 gallons of biodiesel per acre of land. Compared to the18 gallons per acre that corn produces of ethanol and 48 gallons per acre of biodiesel from soybeans (Sheehan et al. 1998), microalgae seems to be very attractive for the future of biofuel production, especially because ponds can be placed in non-fertile land. In addition to biodiesel and glycerin

production, the process creates the microalgae meal, which is a high protein supplement with nutrient specifications comparable to distiller's, and soybean meal. The main hypothesis is that algal meal would serve as a suitable protein supplement for ruminant animals with minimal effects upon live growth performance, carcass characteristics and health status.

The algae biodiesel industry rises then as a potential future fuel resource also producing a high protein and fiber by-product which ruminant production systems could take advantage of, utilizing it as a replacement of soybean meal, corn gluten meal, and distiller's grains, among others. The use of an algal meal could be a valuable, economic, and environmentally sustainable substitution for these common agricultural crops. Since algal meal has not yet been approved for feeding livestock, there is a paucity of data regarding their use by large ruminant animals.

The main hypothesis of the present study is that the algae meal may be included in finishing rations at varying concentrations, without altering the performance of finishing wethers.

Therefore, this experiment was designed to determine the effects of varying inclusions of algal meal supplementation on performance, blood chemistry and live health status, and carcass characteristics of finishing wethers.

## MATERIALS AND METHODS

This experiment was reviewed and approved by the Colorado State University Institutional Animal Care and Use Committee.

*Lipid Extracted Algae Meal*

The algal meal that was used in this study was derived from *Chlorella sp.* utilizing a production method of photosynthetic growth in fresh water open pond system. For confidentiality reasons, the brand of the algal meal could not be revealed.The harvest method used in this algal meal product was flocculation with the assistance of a flocculating agent. Flocculation method consists of aggregation of the micro algae cells, increasing their effective particle size, enhancing its sedimentation, centrifugal recovery, and filtration to facilitate the lipid extraction process. The material was then dewatered with the use of a screen and afterwards spray dried. Lipid extraction was performed using the continuous extraction method, which applies mechanical cell lysis in a beadmill with methyl pentane solvent . The microalgae meal leftover was processed and dried at $100 \pm 5^{o}$ C. The remaining dried algal mass was then packed and shipped to the Colorado State University Facilities. Upon arrival, core samples were taken from each lipid extracted algae meal package and blended for nutrient analysis. The nutrient results for the algal meal are presented in Table 3.1. These results were utilized to formulate the manufacture of the algal diets for the different treatments.

*Animals and Treatments*

The experiment was conducted to conform to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and was approved by the Institutional Animal Care and Use Committee of Colorado State University. All animals were inspected by a veterinarian before the study started and also once a week throughout the experiment.

Forty yearling crossbred wethers were purchased and transported to the research center

from a commercial feedlot. Upon arrival, animals were processed, tagged, weighed for two consecutive days, and inspected by the attending veterinarian to assess initial health status. During the first 3 days animals received alfalfa hay and a commercial supplement for finishing sheep. On the third day, animals were randomized and allocated to individual pens where they stayed for 2 weeks to adapt to the pen and a common high concentrate diet.

Animals were evaluated in a generalized randomized complete block design for the data collection of DMI, feed efficiency, ADG, blood metabolites chemistry, live health status, live final BW, carcass characteristics, fatty acid composition of the *Longissimus dorsi*, digestibility and nutrient and mineral balance. These characteristics of measurement were chosen to measure the effects of titrated concentrations of algal meal. The animals were blocked by week with each block consisting of 10 animals starting on treatments one week apart from each other. Animals were randomly assigned to one of 5 treatments of the experiments and every block had two animals receiving each treatment per block.

The experiment consisted of two periods; 1.) adjustment period (21 d) and 2.) metabolism period (7d). The treatments (n = 8 wethers/treatment) for the experiment consisted of: 1.) soybean meal and rice meal as protein supplementation sources (CON); 2.) 5% of algae meal on a DM basis as a protein replacement (5% AM); 3.) 10% of algae meal on a DM basis as a protein replacement (10%AM); 4.) 15% of algae meal on a DM basis as a protein replacement (15%AM); and 5.) 20% of algae meal on a DM basis as a protein replacement (20%AM); All diets were isocaloric and isonitrogenous. A common mineral and vitamin supplement was fed for all treatments. Limestone was added in different quantities into the diets to keep the Ca:P ratio, at a minimum of 2:1, for all diets. All wethers were fed a high concentrate finishing diet once daily. Table 3.1 represents the nutrient analyses for the algal meal and Table 3.2 represents the diets for

the 5 treatments. Both treatment diets were formulated to meet or exceed the NRC (1996) requirements for CP, Ca, K and P. For experimental facilitation purposes, one single mineral and vitamin package was created and included in the formulas for all treatments. One micro-mineral and vitamin supplement was formulated for all animals considering the short feeding period that the animals were going to receive the treatments. Micro-minerals and vitamins minimum requirements were met or exceeded to avoid negative impacts on growth performance.

*Chemical Analysis*

Samples of the algal meal, diet, orts, feces and urine were analyzed for DM, crude protein, fat and minerals at the Michigan State University's Diagnostic Center for Population and Animal Health ( DCPAH, Lansing, MI )and at the Colorado State University laboratory (CSU, Fort Collins, CO). Samples of the *Longissimus dorsi* muscle of the wethers were analyzed at the meat laboratory of the Colorado State University. Crude protein values were determined using a method that uses a pure oxygen environment in a ceramic horizontal furnace and large ceramic boats for the macro sample combustion process, followed by a combustion gas collection and handling system using helium carrier gas and a thermal conductivity cell for the detection of nitrogen. The instrument machine utilized was the LECO TruMac N (LECO Corporation; Saint Joseph, Michigan USA)., After that, CP values were calculated from total N values (N x 6.25). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) (Goering and Van Soest, 1970) were determined using a modified Van Soest process utilizing fiber bags technology Ankom 200 and using an Ankom unit (Ankom, Fairport, NY).

*Blood Metabolites*

On d 0 (starting date), d 21 (prior to nutrient balance period) and d 28 (prior harvest), approximately 0.50 mL of blood was drawn with a syringe via a jugular venipuncture and collected in microtainers treated with 15% EDTA K2. The samples were chilled during collection, and a sample was pulled from the microtainer and analyzed using single use iSTAT cartridges EC8+ and CG8+ which were then run through a portable iSTAT analyzer VetScan i-STAT (Abaxis, Union City, CA). Results for pH, Total $CO_2$ ($TCO_2$), Partial Pressure $O_2$, Saturated $O_2$ ($SO_2$), Partial Pressure $CO_2$, $HCO_3$, Base Excess (BE), Sodium (Na), Chloride (Cl), Potassium (K), Blood Urea Nitrogen (BUN), Hematocrit (Hct), Hemoglobin (Hb), ionized Ca (iCa), Anion Gap and Glucose are reported in the results section.

*Nutrient Balance Trial*

From d 21 to d 28 (7 days) a balance trial was conducted and the feed intake, fecal and urinary outputs were measured for each lamb for every 24 hours from d 23 to d 28 (5 days). The wethers were housed in IACUC approved solitary metal metabolism cages; and the animals were placed in an environment-controlled facility from d 21 until harvest on d 28. Both CON and treated groups had cages designed with the feed bowl inside the cage and they were the same size for all animals. Rubber floor mats with holes in it were placed in the suspended cages for better comfort of the animals. Remaining feed was weighed every 24 h, and recorded. Wethers were allowed ad libitum access to feed and water throughout the balance trial. Urine from each wether was collected every 24 h into a 1 gallon clean containers with 25 ml of 6N HCl to maintain a pH of less than 3.0, and prevent volatilization of urinary N. The feces from each wether were collected in a fecal collector bag attached on the dorsal peripheral side of the

animals. During the balance trial, feed, orts, total feces and total urine output were collected and 10% of the daily amount was sub-sampled over the 5 d balance trial, composited by animal, frozen at $-20^0$ C and retained for subsequent analysis.

*Animal harvest and Organ Evaluation*

The wethers were humanely harvested on a commercial abattoir with the usage of a captive bolt gun. The carcasses were then opened from sternum to pelvis and the organs and complete digestive tract was removed. The lungs, small intestine, large intestine, stomach and cecum were visually inspected and disposed. Samples of the brains, livers, kidneys and hearts were taken. These organs were weighed on all 40 animals and data was recorded for simple comparison between treatments. Carcasses were allowed to chill for 24 hours prior to assessment by trained Colorado State University Meat Science personnel to assess carcass quality characteristics. Following visual assessment, the carcasses were ribbed and the *Longissimus dorsi* was dissected away from the surrounding tissues of the rack (ribs 4-12). The right rib was then homogenized for subsequent assessment of fatty acid composition.

*Fatty Acid Composition*

The fatty acid profile was determined via gas chromatography using a Hewlett Packard (Avondale, PA) Model 6890 series II gas chromatograph fixed with a series 7683 injector and flame ionization detector. The instrument was equipped with a 100-m x 0.25-mm (id) fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA). Methyl ester derivatives of fatty acids were prepared using a combination of $NaOCH_3$ followed by HCl/methanol as described by Kramer et al. (1997). Fatty acid methyl ester preparations were injected using the split mode. The

carrier gas was helium (flow rate of 2.1 ml/min), and the split ratio was 100:1 at 250$^{o}$ C, at a pressure of 59.24 and split flow of 205 ml/min. The oven temperature was programmed from an initial temperature of 150$^{o}$ C (0 min) to a final temperature of 160$^{o}$ C at the rate of 1.0$^{o}$ C/min. The final temperature was held for 110 min and run time was of 120 min total. Chromatograms were recorded with a computing integrator (Agilent Technologies, Palo Alto, CA). Detector heater was at 300$^{o}$ C, $H_2$ flow of 35 ml/min, air flow of 350 ml/min. Standard fatty acid methyl ester mixtures were used to calibrate the gas chromatograph system using reference standards KEL-FIM-FAME-5 (Matreya Inc., PA). Identification of the fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards.

*Calculations*

Calculations for DM digestibility and nutrient retention were made using the following formulas:

DM Digestibility = **(**DM Intake (g) – Fecal DM (g))/DM Intake (g) X 100

Nutrient Retention = (Nutrient balance (g/d)/ Average Metabolic BW)

Individual BW gain was calculated by the difference in BW on d 21 and d 0, and d 28 and d 21, on study and reported as adjustment period ADG and metabolism period ADG. During the adjustment period, initial BW, final BW, DMI, and blood samples were collected. Final BW for the adjustment period was utilized as initial BW for the metabolism period. Final BW and blood were collected immediately following the completion of the balance trial.

*Statistical Analysis*

Data for growth performance, blood metabolite chemistry parameters, DM digestibility, DM intake, nutrient retention, organ's weights and fatty acids composition of the *Longissimus dorsi* muscle were analyzed using PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC). The experimental unit was each individual wether, the fixed effects were treatment and block. Interactions were tested, and removed from the model since no treatments by block interactions were observed. Differences between treatments were considered statistically significant if P-value $\leq 0.05$ and trending towards significance if *P*-value $\leq 0.10$.

## RESULTS

*Live Growth Performance*

The wethers stayed healthy throughout the study according to the Colorado State University veterinarians' reports, and were weighed on d0, d 21 and d 28 of the study. Results for live performance are presented on Table 3.3. During the 28 d feeding period, initial average (45.4 kg) and final average (44.5 kg) BW, average daily gain (ADG) for adjustment period (0.24 kg/d), ADG for metabolism period (-0.84 kg/d), DMI (1,366.2 g/d), and gain-to-feed for the entire period (0.187) were similar ($P \geq 0.36$) across treatments.

*Live Health Status and Blood Metabolite Chemistry*

Blood metabolite results are presented on Table 3.4. All measurements were considered within a normal range from pre-study period until harvest time. No difference was observed across treatments for health status ($P \geq 0.22$), except for iCa which presented lower values for

the 10% Algae Meal treatment when compared with all other treatments ($P \leq 0.03$). According to veterinary inspections, no side effects were observed due to treatment and behavior and vital signs were considered the same across treatments. Additionally, during harvest, there were no apparent gross lesions the internal organs noted upon inspection.

Results showed lower levels of iCa on animals in of the treatment group of 10% AM ($P = 0.03$). All blood metabolites levels observed in this study were considered within normal ranges reported on past literature (Smith, 2009). There were no differences in the blood concentrations of $TCO_2$, $SO_2$, $HCO_3$, BE, Na, Cl, K, Hct, Hb, BUN, Anion Gap and glucose ($P > 0.22$) due to treatment. The results presented above agreed with our initial expectations that protein supplementation utilizing algal meal as a source would not influence live health status and blood metabolite concentrations across treatments.

*Nutrient Intake, DM Digestibility, Mineral Retention and Mineral Excretion*

During the 5 d balance trial the algae meal treatment groups consumed the same amounts of feed as compared to the CON group ($P > 0.49$), and DM digestibility was also maintained the same among all treatments ($P = 0.16$), Table 3.5. High digestibility values were observed, and that may be a consequence of the stressed caused by the cages to the animals and their DMI and fecal outputs. Total fecal and urinary outputs were not different between any of the treatments ($P > 0.38$).

The results for the nutrient intake, balance and retention of nitrogen and phosphorus are also reported in Table 3.5. The average intake of Nitrogen and Phosphorous were not affected by the treatment diets ($P \geq 0.28$). The average fecal and urine output of N (g/d), and urine output for P (g/d) were the same among treatments ($P \geq 0.26$). Average fecal output for P (g/d) showed

a tendency of presenting increasing values starting with CON, 5% AM, 10% AM, 15% AM, and 20% AM *(P* > 0.08) respectively. Nitrogen balance (g/d) and retained N expressed in g/kg of metabolic BW were similar between all treatments. However, P balance (g/d) and retained P expressed in g/kg of metabolic BW presented lower values for 20% AM treatment when compared to all other treatments and CON (*P* < 0.04).

Results for nutrient intake, fecal and urine output, and mineral balance values for Ca, Mg, K and Na are presented in Table 3.6. Ca intake increases stepwise from CON to 20%AM treatments and could cause impacts on blood metabolite balance and iCa. Also if intake of calcium is excessive an imbalance between Ca and P may occur causing metabolic problems to the animals. Other salient mineral components of Table 3.6 are the electrolytes Na and K, which participate in numerous biochemical reactions in mammals. Therefore the increase in intake of Na and decreased in intake of K may have possible effects if animals were to be in the study for a much longer period. The results for the same characteristics for S, Cu, Fe and Zn are presented in Table 3.7. Increased levels of S in the diet can cause problems if animal exposed to excess of this mineral for extended periods of times. Corn by-products from the ethanol industry present high levels of S in its composition and it is reported in the literature as the main factor influencing the occurrence of polioencephalomacia in confined animals. T levels of sulfur ultimately cause alterations in enzyme function and brain damage in the animals, resulting in the sulfur toxicosis form of polioencephalomalacia..

Lastly, the results for Mn, Mo and Co are reported in Table 3.8. Slight differences in manganese and cobalt intake occurred across study groups; however, no biological effects were observed throughout the 28 d period that the animals were fed the algae meal.

Considering the fact that the facilities and metabolism laboratory utilized in this trial were not equipped with specific tools for a micro-mineral balance trial, the results observed are inconclusive at this moment since the design of this experiment did not present identical micro-nutrient balance among diets. Further studies designed specifically for micro-mineral measurements are necessary for further analyses of effects of algae meal in animal production.

*Carcass Characteristics*

Results for carcass characteristics are presented on Table 3.9. Furthermore, hot carcass weight, subcutaneous adipose depth, *Longissimus* muscle area, calculated YG, marbling score, dressing percentage, muscle percentage, body wall thickness, leg circumference, flank streaking, quality grade, carcass conformation and carcass length were also similar ($P \geq 0.27$) across treatments. Significant differences were observed on leg score measurements ($P < 0.01$), with higher values presented on treatment 10%A followed by treatments 20%A, CON, 5%A and 15%A respectively.

*Fatty Acids Profile for Longissimus dorsi muscle samples*

Fatty acids profile for *Longissimus dorsi* muscle samples are reported on Table 3.10 and represent the percentage of each fatty acid in relation of all fatty acids measured and identified. There was no significant effect from the dietetic algae meal treatments on fatty acids profile on the wethers' meat samples. Since animals were on feed for the study for only 28 days, it was expected that little to no changes in dentified fatty acids from *Longissimus dorsi* muscle should be observed. Past literature also suggest that with low lipid content in the protein supplement, fatty acid composition should remain relatively unaffected (Turner et al., 2012).

*Organ Evaluation*

At time of harvest in the end of the study period, the wethers were slaughtered with a captive bolt gun, and each organ was removed from the animal and the weights recorded. These data are shown in Table 3.11. There were no differences observed in the weights of lungs, hearts, kidneys, livers or brains between all group treatments *(P* > 0.24). All organs seemed healthy and had no gross abnormalities or abscesses.

## DISCUSSION

Wethers were chosen due to the fact they are an easy to handle, small-ruminant animal that serve as a model for large ruminants and also because of availability of algae meal, it would have been a challenge to utilize cattle or other large ruminants in this experiment. The algal meal utilized in this ruminant study presented 11.88% ADF and 23.16% NDF, which are greater than the concentrations observed generally in commonly fed cereal crops and by-products such as steam flaked corn and soybean meal (Archibeque et al. 2009). Feeds that contain greater concentrations of fiber paired with high levels of protein in its composition may be a very desirable feedstuff to be fed to ruminants and proved to be an adequate model for this type of study. No differences were observed in the DM digestibility, DMI, live performance or carcass characteristics among all five treatments, which was expected for to be seen for ruminant animals. Increased fiber levels in diets may be more beneficial for ruminants than monogastric animals due to fermentation capabilities and slower transit time through the gastrointestinal tract in ruminants. That will mainly result in greater microbial fermentation and more efficient utilization of the fiber portion of the feedstuff.

Blood metabolites measured in the present study showed that blood chemicals and metabolites were mainly not influenced by dietetic treatments. While there was one statistically significant difference for ionized Ca across treatments, this difference does not appear to be upon treatment since no pattern was observed with increased levels of algal meal fed to the wethers. Different Ca intake and Ca retention by individuals may have influenced this measurement. Other variations on blood chemistry and metabolites could have occurred due to handling of the animals during blood collection. External temperatures, during the two first blood sampling and variation of blood collection time between wethers also could have impacted iSTAT blood analyses results.

The apparent DM digestibility and nutrient intake of the major essential minerals such as Nitrogen and Phosphorus targeted for the same amount of CP % in the diet observed in the present experiment did not show any differences among treatments as expected. These average for N intake and P intake in g/d found in this study agrees with those of Cole (1999) and Archibeque et al. (2008), who reported that feeding continuous or oscillating protein in the diet to lambs did not affect nutrient intake unless CP% in the diet increased. Since animals did not differ in daily DMI and CP in the diet was calculated as the same, no increase in intake was expected. Due to low plane of productivity of the wethers in this experiment, it is possible to speculate that these low requirements and constant level of N in the diet restricted also any changes in fecal N output, urine N output and N retention. In addition, N and P levels of fecal N and fecal P in g/d also agreed with those authors following the same pattern throughout treatments. Likewise, Phosphorus retention was in agreement with Archibeque et al. (2008), which reported different values for different sources of feedstuff (corn vs. DDGS). In this

experiment, it is then possible to speculate that due to different protein composition from Algae meal and soybean meal, P retention was different among treatments.

In addition, the current study presented above did not have the intention to balance different diets for micronutrients, but only supply minimal requirements from the NRC for the animals to perform well. With that, no special supplement was formulated and the majority of the differences observed in Tables 3.6, 3.7 and 3.8 had no impact on performance or carcass characteristics. However, some of these differences may be exacerbated if potentially allowed to persist for an extensive period. Additionally, these perturbations of mineral balance, particularly of the electrolytes my have unforeseen effects upon acid/base balance and maintenance of homeostasis during the course of extended periods of feeding.

The elucidation of digestive effects of algal meal will only be met after repeated long term studies that would mimic the traditional commercial sheep feedlot scenario in the United States. The lack of differences seen in the present study may be due to the short period of time that the animals where under dietetic treatment.

This is one of the first studies in ruminants with the purpose of feeding algae meal as a protein supplement feed.

Feeding novel feedstuffs such as algal meal to ruminants was an interesting opportunity to observe not only experimental measurements but also animal behavior, acceptability of the new feed and apparent palatability. It seems that small ruminants did not notice the substitution of the soybean meal by algal meal up to 20% of the total DM. This lead to the conclusion that the unique physical characteristics of the algae meal did not affect bunk behavior of the animals.

As expected, the balance of P in g/d between the groups was different statistically being less for the 20 % algal treatment. It is known that plants store P as phytic acid, become available to ruminant animals due to fermentation in the rumen (D'Mello et al. 2000).

The fatty acid composition of *Longissimus dorsi* muscle, expressed in % of the total identified fatty acids, observed in the present experiment did not show any differences among treatments as expected, except for the saturated fatty acid 16:0. Results showed higher numerical values for the treatments CON and 20% AM when compared to the 5%, 10% and 15% AM. These differences seem numerically different but not biologically different since proportions of the fatty acid 16:0 varies slightly depending on the number of fatty acids identified. These percentages for fatty acid composition found in this study agrees with those of Jeronimo et al. (2009), Qi et al. (2012) and Turner et al. (2012), who reported similar proportion values for all identified fatty acids. The greater values for fatty acid composition were attributed to the 18:1*cis*-9 fatty acid (more than 30% of total fatty acids identified), followed by 16:0 (approximately 25% of the total fatty acids identified), and proceeded by 18:0 (approximately 16% of the total fatty acid identified). Since animals were only fed algal meal treatments for 28 d, no major differences were expected. In this experiment, it is then possible to speculate that due to no difference in fatty acid profile among treatments, substitution of traditional protein supplements for Algal meal up to 20% of total diet DM may not affect meat flavor and aroma.

In addition, the current study presented above did not have the intention to manipulate fatty acid profile. Furthermore, algal meal is still a USDA non-approved feedstuff; therefore no taste panel was conducted.

*Research Importance and Comparisons*

This study was one of the first studies to feed lipid extracted algal meal as a feedstuff for ruminant animals consuming a high concentrate diet. Previous research in our lab evaluated the use of algal meal in the diets of growing rats (Howe et al., 2010) and rabbits (Howe et al., 2011). There was some limitation upon growth parameters and gain:feed in the rats, but not in the rabbits. This was speculated to be due to the more advanced symbiosis between animal and digestive microflora in the cecotrophic rabbits, which allowed them to more fully utilize the highly fibrous algal meal. Similarly, the wethers used in the current study were able to utilize the product similarly to the diets without algal meal. This further confirms that algal meal may serve as a viable feedstuff for animals that have a symbiotic relationship that allows for use of feeds with larger amounts of fiber, in this case 23.16% NDF. It is important to understand the nutritional values of algal meal as a potential animal supplement, and which sectors of the livestock industry would be most likely to effectively utilize the product. According to Archibeque et al. (2009) that compared the nutrient profiles of algae, algae meal, soybean meal, and steam flaked corn, the results showed that the NDF and ADF fiber composition of *Nannochloropsis* meal was higher than soybean meal, and steam flaked corn, 25.12% vs. 11.45% and 9.59%), and 6.64 vs. 5.89, 2.92% respectively.

## CONCLUSIONS

The present study showed that algae meal is a very interesting potential protein supplement for ruminants if available to producers at a reasonable price. When compared to other protein supplements such as soybean meal and distiller's grains algae meal may be rise as a popular protein supplement used in ruminant production systems.

# IMPLICATIONS

Lipid extracted algal meal might be a feasible alternative for protein supplementation for ruminants in the future. Further research may be necessary to determine the response of different levels of supplementation of algal meal for sheep and other ruminants, effects on animals in a different physiological stage or effects on other ruminants in the finishing diet on performance and carcass merit.

**Table 3.1. Analyzed nutrient analysis for algal meal of *Chlorella sp.*, %DM.**

| Nutrient Content | %, DM |
|---|---|
| Crude Protein, % | 21.12 |
| Acid Detergent Fiber, % | 11.88 |
| Neutral Detergent Fiber, % | 23.16 |
| Fat (EE), % | 1.96 |
| Calcium, % | 6.01 |
| Phosphorous, % | 0.435 |
| Potassium, % | 0.52 |
| Magnesium, % | 0.74 |
| Sodium, % | 5.685 |
| Chloride, % | 4.25 |
| Sulfur, % | 0.85 |
| Copper, ppm | 19.95 |
| Iron, ppm | 5355 |
| Lead, ppm | 3.785 |

**Table 3.2. Finishing diets for yearling wethers using algal meal compared to standard industry soybean meal diet at 0, 5, 10, 15, or 20% of the dietary DM.**

| **Ingredient, %** | Treatment | | | | |
|---|---|---|---|---|---|
| | CON | 5% AM | 10% AM | 15% AM | 20% AM |
| Corn | 49.25 | 48.9 | 49.25 | 49.6 | 49.9 |
| Soybean Meal | 15.25 | 13.75 | 12 | 10.3 | 8.75 |
| Rice meal | 29 | 26.25 | 23 | 19.7 | 16.25 |
| Algal Meal | 0 | 5 | 10 | 15 | 20 |
| Limestone | 1.5 | 1.1 | 0.75 | 0.4 | 0.1 |
| Supplement[1] | 5 | 5 | 5 | 5 | 5 |

[1] Supplement composition: Ca max 10.5%, P min 10%, Salt max 28%, K min 1.0%, Mg min 2.0%, Zn min 2,700 ppm, Chelated Zn min 404 ppm, Mn min 1,930 ppm, Chelated Mn min 289 ppm, Se min 24 ppm, Iodine min 45 ppm, Vitamin A min 120,000 IU/lb, Vitamin D min 36,500 IU/lb, Vitamin E min 500 IU/lb

**Table 3.3. Live growth performance of yearling wethers (n=8/treatment) consuming finishing rations with 0, 5, 10, 15, or 20% of the dietary DM as algal meal (AM).**

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | CON | 5% AM | 10% AM | 15% AM | 20% AM | SEM | P-value |
| Initial BW, kg | 46.4 | 45.2 | 45.3 | 44.9 | 44.6 | 1.22 | 0.66 |
| Final BW, kg | 44.6 | 44.7 | 45.5 | 45.1 | 42.6 | 1.51 | 0.36 |
| ADG adjustment[1], kg | 0.23 | 0.24 | 0.27 | 0.27 | 0.23 | 0.06 | 0.89 |
| ADG metabolism[2], kg | -0.82 | -0.81 | -0.79 | -0.81 | -1.03 | 0.25 | 0.84 |
| G:F[3] | 0.158 | 0.195 | 0.193 | 0.213 | 0.174 | 0.04 | 0.73 |
| DMI, g | 1,409.59 | 1,258.25 | 1,431.94 | 1,354.99 | 1,376.20 | 134.13 | 0.73 |

[1]ADG during the 21 days of the individual feedlot pen period.

[2]ADG during the 7 days of the balance metabolism period.

[3] G:F presented for the whole feeding period.

**Table 3.4. Effect of feeding control and algal meal diets to yearling crossbred wethers on blood metabolites.**

| Response[1] | CON | 5%AM | 10%AM | 15%AM | 20%AM | SE | P-value | Normal Ranges |
|---|---|---|---|---|---|---|---|---|
| pH | 7.4 | 7.39 | 7.4 | 7.41 | 7.38 | 0.02 | 0.43 | 7.32 - 7.54 |
| Total $CO_2$ mMol/L | 26.92 | 26.38 | 28.13 | 27.51 | 27.67 | 0.93 | 0.37 | 21 - 28 |
| Partial Pressure $O_2$ mmHg | 33.21 | 32.47 | 35.42 | 32.34 | 31.13 | 1.86 | 0.22 | - |
| Saturated $O_2$ % | 61.63 | 60.13 | 62.5 | 59.71 | 57.13 | 3.76 | 0.66 | - |
| Partial Pressure $CO_2$ mmHg | 41.5 | 42.33 | 42.62 | 41.25 | 44.3 | 1.78 | 0.46 | 37 - 46 |
| $HCO_3$, mMol/L | 25.32 | 25.3 | 25.93 | 26.35 | 25.97 | 0.64 | 0.41 | - |
| BE, mMol/L | 0.71 | 0.59 | 1.04 | 1.79 | 0.92 | 0.73 | 0.5 | - |
| Na, mMol/L | 143 | 146.83 | 146.25 | 146.92 | 147.33 | 3.1 | 0.64 | 139 - 152 |
| Cl, mMol/L | 108.27 | 107.54 | 107.18 | 107.18 | 107.05 | 0.66 | 0.34 | - |
| K, mMol/L | 4.69 | 4.56 | 4.71 | 4.58 | 4.76 | 0.16 | 0.66 | 3.9 - 5.4 |
| iCa, mMol/L | 1.15[a] | 1.17[a] | 1.09[b] | 1.14[a] | 1.15[a] | 0.02 | 0.03 | 11.5 - 12.8 |
| Anion Gap, mMol/L | 18.95 | 20.55 | 18.73 | 19.14 | 19.05 | 0.99 | 0.38 | - |
| Hematocrit, %PCV | 32.21 | 32.88 | 31.71 | 33.25 | 33.13 | 0.97 | 0.46 | - |
| Hemoglobin, g/dL | 11.01 | 11.23 | 10.82 | 11.33 | 11.27 | 0.33 | 0.49 | 9 - 15 |
| Glucose, mg/dL | 82.8 | 84.13 | 83.21 | 80.04 | 83.29 | 2.6 | 0.58 | 50 - 80 |
| BUN, mg/dL | 19.78 | 19.01 | 19.36 | 21.55 | 16.69 | 2.19 | 0.29 | 8 - 20 |

[1]No treatment by date interaction was observed.

**Table 3.5. Nutrient intake, balance, retention of Nitrogen and Phosphorus and DM digestibility on yearling crossbred wethers fed 0, 5, 10, 15 and 20% of lipid extracted algae meal on the diets during metabolism period (n=8 observations/treatment).**

| Item | CON | 5% AM | 10% AM | 15% AM | 20% AM | SE | *P*-value |
|---|---|---|---|---|---|---|---|
| DMI met.[1], g/d | 1079.11 | 1001.43 | 1063.53 | 1016.98 | 854.49 | 134.44 | 0.49 |
| DM digestibility % | 95.13 | 94.93 | 94.74 | 94.22 | 94.18 | 0.45 | 0.16 |
| *Nitrogen* | | | | | | | |
| N intake, g/d | 22.5 | 19.08 | 22.44 | 19.69 | 17.56 | 2.67 | 0.28 |
| Feces N, g/d | 0.78 | 0.86 | 0.98 | 1.13 | 1.01 | 0.16 | 0.26 |
| Urine N, g/d | 1.54 | 1.3 | 1.24 | 1.42 | 1.13 | 0.32 | 0.75 |
| N balance, g/d | 20.19 | 16.93 | 20.22 | 17.14 | 15.42 | 2.44 | 0.21 |
| N retained, g/kg metabolic BW | 1.15 | 0.98 | 1.16 | 0.98 | 0.92 | 0.14 | 0.3 |
| *Phosphorus* | | | | | | | |
| P intake, g/d | 10.53 | 9.71 | 10.22 | 10.21 | 8.24 | 1.29 | 0.43 |
| Feces P, g/d | 2.27 | 2.46 | 2.94 | 3.39 | 3.28 | 0.46 | 0.08 |
| Urine P, g/d | 0.21 | 0.15 | 0.11 | 0.09 | 0.15 | 0.06 | 0.43 |
| P balance, g/d | 8.06$^{y}$ | 7.11$^{y}$ | 7.16$^{y}$ | 6.74$^{y}$ | 4.81$^{z}$ | 0.96 | 0.03 |
| P retained, g/kg metabolic BW | 0.46$^{y}$ | 0.41$^{y}$ | 0.41$^{y}$ | 0.38$^{y}$ | 0.29$^{z}$ | 0.05 | 0.04 |

$^{y, z}$ Least square means within a row without a common superscript differ ($P < 0.05$)

[1]DMI during balance metabolism period

**Table 3.6. Nutrient intake, fecal and urine output and mineral balance of Calcium, Magnesium, Potassium and Sodium on yearling crossbred wethers fed 0, 5, 10, 15 and 20% of lipid extracted algae meal on the diets during metabolism period (n=8 observations/treatment).**

| Item | CON | 5% AM | 10% AM | 15% AM | 20% AM | SE | *P*-value |
|---|---|---|---|---|---|---|---|
| *Calcium* | | | | | | | |
| Ca intake, g/d | 11.76 $^{z}$ | 12.86 $^{z}$ | 19.56 $^{y}$ | 21.51 $^{y}$ | 18.98 $^{y}$ | 2.74 | <0.01 |
| Feces Ca, g/d | 2.27 $^{z}$ | 2.46 $^{z}$ | 2.94 $^{yz}$ | 3.39 $^{y}$ | 3.40 $^{y}$ | 0.45 | 0.05 |
| Urine Ca, g/d | 0.12 $^{y}$ | 0.06 $^{z}$ | 0.03 $^{z}$ | 0.04 $^{z}$ | 0.04 $^{z}$ | 0.03 | 0.01 |
| Ca balance, g/d | 9.36 $^{z}$ | 10.34 $^{z}$ | 16.58 $^{y}$ | 18.09 $^{y}$ | 15.53 $^{y}$ | 2.37 | <0.01 |
| *Magnesium* | | | | | | | |
| Mg intake, g/d | 3.49 $^{yz}$ | 3.46 $^{yz}$ | 4.46 $^{y}$ | 4.01 $^{y}$ | 2.98 $^{z}$ | 0.5 | 0.05 |
| Feces Mg, g/d | 0.36 | 0.39 | 0.41 | 0.43 | 0.38 | 0.06 | 0.8 |
| Urine Mg, g/d | 0.36 | 0.23 | 0.31 | 0.26 | 0.23 | 0.08 | 0.43 |
| Mg balance, g/d | 2.78 $^{z}$ | 2.84 $^{z}$ | 3.75 $^{y}$ | 3.32 $^{y}$ | 2.37 $^{z}$ | 0.42 | 0.03 |
| *Potassium* | | | | | | | |
| K intake, g/d | 11.34 $^{y}$ | 9.68 $^{y}$ | 9.57 $^{y}$ | 8.13 $^{yz}$ | 6.20 $^{z}$ | 1.14 | <0.01 |
| Feces K, g/d | 0.14 | 0.1 | 0.12 | 0.15 | 0.12 | 0.03 | 0.55 |
| Urine K, g/d | 4.13 | 3.08 | 3.25 | 2.84 | 2.67 | 0.77 | 0.39 |
| K balance, g/d | 7.07 $^{y}$ | 6.49 $^{y}$ | 6.21 $^{y}$ | 5.13 $^{y}$ | 3.40 $^{z}$ | 1.02 | 0.01 |
| *Sodium* | | | | | | | |
| Na intake, g/d | 4.83 $^{z}$ | 8.23 $^{y}$ | 10.12 $^{y}$ | 13.76 $^{w}$ | 12.18 $^{wy}$ | 1.69 | <0.01 |
| Feces Na, g/d | 0.065 | 0.033 | 0.055 | 0.082 | 0.073 | 0.03 | 0.41 |
| Urine Na, g/d | 2.59 $^{z}$ | 3.66 $^{z}$ | 6.07 $^{y}$ | 5.78 $^{y}$ | 5.96 $^{y}$ | 1.22 | 0.02 |
| Na balance, g/d | 2.18 $^{z}$ | 4.53 $^{y}$ | 3.99 $^{y}$ | 7.89 $^{w}$ | 6.15 $^{w}$ | 1.25 | <0.01 |

$^{w, y, z}$ Least square means within a row without a common superscript differ ($P < 0.05$)

**Table 3.7. Nutrient intake, fecal and urine output and mineral balance of Sulfur, Copper, Iron and Zinc on yearling crossbred wethers fed 0, 5, 10, 15 and 20% of lipid extracted algae meal on the diets during metabolism period (n=8 observations/treatment).**

| Item | CON | 5% AM | 10% AM | 15% AM | 20% AM | SE | *P*-value |
|---|---|---|---|---|---|---|---|
| *Sulfur* | | | | | | | |
| S intake, g/d | 2.15 $^{z}$ | 2.58 $^{yz}$ | 3.16 $^{y}$ | 3.04 $^{y}$ | 2.44 $^{yz}$ | 0.39 | 0.08 |
| Feces S, g/d | 0.138 $^{z}$ | 0.173 $^{y}$ | 0.215 $^{y}$ | 0.255 $^{wy}$ | 0.247 $^{wy}$ | 0.035 | 0.01 |
| Urine S, g/d | 0.69 | 0.69 | 0.81 | 0.69 | 0.77 | 0.16 | 0.91 |
| S balance, g/d | 1.32 $^{z}$ | 1.71 $^{yz}$ | 2.13 $^{y}$ | 2.09 $^{y}$ | 1.42 $^{z}$ | 0.32 | 0.05 |
| *Copper* | | | | | | | |
| Cu intake, mg/d | 11 $^{z}$ | 17 $^{y}$ | 32 $^{w}$ | 11 $^{z}$ | 11 $^{z}$ | 2 | <0.01 |
| Feces Cu, mg/d | 0.0018 | 0.0022 | 0.0022 | 0.002 | 0.0017 | 0.0003 | 0.49 |
| Urine Cu, mg/d | 0.00003 | 0.00004 | 0.00003 | 0.00003 | 0.00004 | 0.00001 | 0.61 |
| Cu balance, mg/d | 0.01 $^{z}$ | 0.02 $^{y}$ | 0.03 $^{w}$ | 0.01 $^{z}$ | 0.01 $^{z}$ | 0.002 | <0.01 |
| *Iron* | | | | | | | |
| Fe intake, g/d | 0.40 $^{z}$ | 0.64 $^{z}$ | 1.01 $^{y}$ | 1.12 $^{y}$ | 1.17 $^{y}$ | 0.11 | <0.01 |
| Feces Fe, g/d | 0.073 $^{z}$ | 0.089 $^{z}$ | 0.118 $^{y}$ | 0.144 $^{wy}$ | 0.149 $^{w}$ | 0.019 | <0.01 |
| Urine Fe, g/d | 0.0005 | 0.0005 | 0.0003 | 0.0003 | 0.0004 | 0.0001 | 0.24 |
| Fe balance, g/d | 0.33 $^{z}$ | 0.55 $^{y}$ | 0.89 $^{w}$ | 0.98 $^{w}$ | 1.02 $^{w}$ | 0.09 | <0.01 |
| *Zinc* | | | | | | | |
| Zn intake, g/d | 0.137 $^{z}$ | 0.137 $^{z}$ | 0.419 $^{w}$ | 0.183 $^{y}$ | 0.157 $^{y}$ | 0.04 | <0.01 |
| Feces Zn, g/d | 0.034 | 0.036 | 0.035 | 0.035 | 0.029 | 0.005 | 0.66 |
| Urine Zn, g/d | 0.0004 | 0.0004 | 0.0004 | 0.0003 | 0.0005 | 0.0001 | 0.46 |
| Zn balance, g/d | 0.103 $^{z}$ | 0.097 $^{z}$ | 0.383 $^{x}$ | 0.147 $^{w}$ | 0.126 $^{y}$ | 0.03 | <0.01 |

$^{x, w, y, z}$ Least square means within a row without a common superscript differ ($P < 0.05$)

**Table 3.8. Nutrient intake, fecal and urine output and mineral balance of Manganese, Molybdenum and Cobalt on crossbred wethers fed 0, 5, 10, 15 and 20% of lipid extracted algae meal on the diets during metabolism period (n = 8 observations/treatment).**

| Item | CON | 5% AM | 10% AM | 15% AM | 20% AM | SE | *P*-value |
|---|---|---|---|---|---|---|---|
| *Manganese* | | | | | | | |
| Mn intake, mg/d | 0.148 $^{yz}$ | 0.228 $^{w}$ | 0.197 $^{w}$ | 0.144 $^{yz}$ | 0.112 $^{z}$ | 0.031 | <0.01 |
| Feces Mn, mg/d | 0.04 | 0.041 | 0.04 | 0.039 | 0.037 | 0.005 | 0.95 |
| Urine Mn, mg/d | 0.097 | 0.093 | 0.042 | 0.051 | 0.048 | 0.029 | 0.19 |
| Mn balance, mg/d | 0.01 $^{z}$ | 0.09 $^{wy}$ | 0.12 $^{w}$ | 0.06 $^{y}$ | 0.03 $^{z}$ | 0.03 | 0.01 |
| *Molybdenum* | | | | | | | |
| Mo intake, mg/d | 0.0029 | 0.0025 | 0.0028 | 0.0023 | 0.0023 | 0.0003 | 0.21 |
| Feces Mo, mg/d | 0.0004 | 0.0003 | 0.0004 | 0.0004 | 0.0003 | 0.0001 | 0.72 |
| Urine Mo, mg/d | 0.123 | 0.141 | 0.151 | 0.148 | 0.138 | 0.039 | 0.97 |
| Mo balance, mg/d | -0.12 | -0.14 | -0.15 | -0.15 | -0.14 | 0.04 | 0.97 |
| *Cobalt* | | | | | | | |
| Co intake, mg/d | 0.0006 $^{z}$ | 0.0013 $^{y}$ | 0.0018 $^{wy}$ | 0.0020 $^{w}$ | 0.0015 $^{y}$ | 0.0002 | <0.01 |
| Feces Co, mg/d | 0.00014 | 0.00019 | 0.00021 | 0.00026 | 0.00024 | 0.00005 | 0.12 |
| Urine Co, mg/d | 0.012 | 0.016 | 0.015 | 0.011 | 0.014 | 0.0036 | 0.59 |
| Co balance, mg/d | -0.013 | -0.015 | -0.015 | -0.009 | -0.014 | 0.003 | 0.44 |

$^{w, y, z}$ Least square means within a row without a common superscript differ ($P < 0.05$)

**Table 3.9. Carcass characteristics of yearling crossbred wethers (n=8/treatment) consuming finishing rations with 0, 5, 10, 15, or 20% of the dietary DM as algal meal (AM).**

| Treatments | CON[1] | 5% AM | 10% AM | 15% AM | 20% AM | SEM | P-value |
|---|---|---|---|---|---|---|---|
| HCW, kg | 23.4 | 23.1 | 22.9 | 22.9 | 21.8 | 0.96 | 0.53 |
| Dressing, %[a] | 51.6 | 51.7 | 50.3 | 51.1 | 51.8 | 0.99 | 0.53 |
| 12$^{th}$ rib fat, cm | 0.35 | 0.39 | 0.43 | 0.58 | 0.41 | 0.15 | 0.6 |
| Marbling score | 48.29 | 48.09 | 47.36 | 47.17 | 48.27 | 0.44 | 0.27 |
| LM area, cm$^2$ | 13.54 | 13.51 | 13.1 | 12.54 | 13.27 | 0.88 | 0.79 |
| USDA yield grade[b] | 1.78 | 1.94 | 2.09 | 2.68 | 2 | 0.58 | 0.6 |
| Percentage of muscle,% | 48.29 | 48.09 | 47.67 | 47.17 | 48.28 | 0.58 | 0.27 |
| Body Wall Thickness, cm | 1.32 | 1.44 | 1.62 | 1.64 | 1.4 | 0.17 | 0.28 |
| Leg Score | 11.25$^{y}$ | 10.75$^{yz}$ | 11.38$^{y}$ | 10.12$^{z}$ | 11.38$^{y}$ | 0.32 | <0.01 |
| Leg Circumference, cm | 64.8 | 63.4 | 63.95 | 63.23 | 63.39 | 0.95 | 0.47 |
| Carcass Length, cm | 108.06 | 107.69 | 107.69 | 108.38 | 106.94 | 0.77 | 0.75 |

[a]Dressing percentage = carcass weight / average live weight (4% shrink).

[b]USDA calculated yield grade = (Fat thickness in inches * 10) + 0.4

[y, z] Least square means within a row without a common superscript differ ($P < 0.05$)

**Table 3.10. Fatty acid composition (% of identified fatty acids) of *Longissimus dorsi* of finishing crossbred wethers fed corn-based diets containing 0, 5, 10, 15 or 20% of lipid extracted algae meal (AM).**

| Item | Control | 5% AM | 10% AM | 15% AM | 20% AM | SE | P- value |
|---|---|---|---|---|---|---|---|
| 12:0 | 0.29 | 0.14 | 0.16 | 0.14 | 0.2 | 0.04 | 0.46 |
| 14:0 | 3.27 | 2.63 | 2.9 | 2.71 | 3.36 | 0.37 | 0.18 |
| 16:0 | 27.16$^{y}$ | 25.65$^{z}$ | 25.99$^{z}$ | 25.81$^{z}$ | 26.76$^{zy}$ | 0.55 | 0.04 |
| 16:1*cis-9* | 2.48 | 2.52 | 2.46 | 2.35 | 2.51 | 0.13 | 0.66 |
| 18:0 | 16.07 | 16.15 | 16.45 | 16.91 | 17.29 | 0.74 | 0.4 |
| 18:1*trans*-11 | 3.49 | 3.34 | 3.94 | 4.17 | 3.97 | 0.46 | 0.35 |
| 18:1*cis*-9 | 41.68 | 43.37 | 42.27 | 41.95 | 40.18 | 1.1 | 0.15 |
| 18:2*cis*-9,12 | 4.32 | 4.76 | 4.64 | 4.56 | 4.49 | 0.4 | 0.86 |
| 18:2*cis-9,trans*11 | 0.4 | 0.4 | 0.41 | 0.42 | 0.4 | 0.06 | 0.99 |
| 18:2*trans-10,cis*12 | 0.023 | 0.025 | 0.02 | 0.015 | 0.018 | 0.004 | 0.15 |
| 18:3*cis*-9, 12, 15 | 0.28 | 0.18 | 0.14 | 0.31 | 0.2 | 0.14 | 0.69 |
| 20:0 | 0.1 | 0.11 | 0.12 | 0.11 | 0.13 | 0.015 | 0.34 |
| 20:4*cis*-5, 8, 11, 14 | 0.39 | 0.59 | 0.4 | 0.42 | 0.39 | 0.09 | 0.22 |
| 20:5*cis*-5, 8, 11, 14, 17 | 0.07 | 0.09 | 0.07 | 0.08 | 0.07 | 0.02 | 0.65 |
| 20:6*cis*-4, 7, 10, 13, 16, 19 | 0.05 | 0.05 | 0.04 | 0.05 | 0.04 | 0.01 | 0.83 |
| SFA | 46.79 | 44.68 | 45.62 | 45.69 | 47.73 | 1.16 | 0.14 |
| MUFA | 47.66 | 49.23 | 48.66 | 48.46 | 46.66 | 1.18 | 0.22 |
| PUFA | 5.54 | 6.09 | 5.72 | 5.85 | 5.61 | 0.52 | 0.84 |

**Table 3.11. Average Organ Weights at harvest of crossbred wethers fed 0, 5, 10, 15 and 20% of lipid extracted algae meal (AM).**

| Organ | CON | 5%AM | 10% AM | 15% AM | 20% AM | SE | P Value |
|---|---|---|---|---|---|---|---|
| Brain, g | 110.96 | 113.59 | 114.41 | 111.21 | 114.85 | 4.48 | 0.86 |
| Heart, g | 251.8 | 249.85 | 242.82 | 249.92 | 243.43 | 15.82 | 0.97 |
| Right Kidney, g | 72.39 | 74.4 | 72.23 | 76.75 | 67.88 | 5.05 | 0.51 |
| Left Kidney, g | 72.88 | 69.58 | 73.96 | 74.29 | 70.28 | 5.7 | 0.88 |
| Liver, g | 710.63 | 743.82 | 690.73 | 724.82 | 668.35 | 68.17 | 0.83 |
| Lungs, g | 726.17 | 861.28 | 779.74 | 779.96 | 719.1 | 65.99 | 0.24 |

# REFERENCES


Abaxis – VetScan iSTAT. Union City, CA.

Archibeque, S. L., H. C. Freetly, C. L. Ferrell. 2008. Feeding distiller's grains supplements to improve amino acid nutriture of lambs consuming moderate-quality forages. J. Anim. Sci. 86:691-701.

Archibeque, S. L., A. Ettinger, B. D. Willson. 2009. *Nannochloropsis oculata* As A Source For Animal Feed Acta. Agron. Hung. 57:245-248.

ARS-USDA. 2012. http://www.ars.usda.gov/research/ programs/programs.htm?np_code=102&pf=1. Accesed on Aug 12, 2012.

Bremer, V. R., K. J. Hanford, G. E. Erickson, T. J. Klopfenstein. 2011. Meta-Analysis of Feeding Calf-Feds or Yearlings Wet Distiller's Grains with Different Corn Processing Types. Nebraska Beef Rep. MP: 40-41.

Cole, N. A. 1999. Nitrogen retention by lambs fed oscillating dietary protein concentrations. J. Anim. Sci. 77:215-222.

Dib, M. G., K. Rolfe, J. O. Sarturi, G. E. Erickson, T. J. Klopfenstein, R. Lindquist. 2010. Effects Of Feeding A Combination Of Modified Distiller's Grains Plus Solubles and Wet Corn Gluten Feed to Adapt Cattle to Finishing Diets. Nebraska Beef Rep. MP:57-59.

D'Mello, J. P. F., Officer, D. I.. 2000. Farm Animal Metabolism and Nutrition: Feed Enzymes. CABI Publishing, N.Y., N.Y. Pp. 405-426.

ERS-USDA. 2012. http://www.ers.usda.gov/Briefing/ Corn/2010baseline.htm. Accessed on Aug 12, 2012.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st red. Ed. Fed. Anim. Sci. Soc., Savoy, IL.

Gill, R. K., D. L. VanOverbeke, B. Depenbusch, J. S. Drouillard and A. DiCostanzo. 2008. Impact of beef cattle diets containing corn or sorghum distiller's grains on beef color, fatty acid profiles, and sensory attributes. J. Anim. Sci. 86:923-935.

Howe, B. A, I. N. Roman-Muniz, B. D. Willson, and S. L. Archibeque. 2011. Nannochloropsis oculata meal did not alter nutrient usage and had no adverse health effects when fed to rabbits as a protein source. J. Anim. Sci. 89: (Suppl 2) (Abstr.)

Howe, B. A., I. N. Roman-Muniz, B. D. Willson, and S. L. Archibeque. 2010 Evaluation of algae meal from Nannochloropsis oculata as a protein source for non-ruminant animals. J. Anim. Sci. 88: (Suppl 2) (Abstr.)

Jeronimo, E., S. P. Alves, J. A. M. Prates, J. Santos-Silva, R. J. B. Bessa. 2009. Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat. J. Meat Sci. 83:499-505.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board Invited Review: Use of distiller's by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

Qi, J., C. Li, Y. Chen, F. Gao, X. Xu, G. Zhou. 2012. Changes in meat quality of ovine longissimus dorsi muscle in response to repeated freeze and thaw. J. Meat Sci. 92:619-626

SAS Institute Inc. 2003. SAS 9.1.2. Cary, NC, USA.

Schoonmaker, J. P., A. H. Trenkle and D. C. Beitz**.** 2010. Effect of feeding wet distiller's grains on performance, marbling deposition, and fatty acid content of beef from steers fed low- or high-forage diet.J. Anim. Sci. 88:3657-3665.

Smith, B. P. 2009. Large animal internal medicine. 4th ed. Mosby inc. St. Louis, MO.

Turner, T. D., L. Karlsson, C. Mapiye, D. C. Rolland, K. Martinsson and M. E. R. Dugan. 2012. Dietary influence on the m. longissimus dorsi fatty acid composition of lambs in relation to protein source. J. Meat Sci. 91:472-477.

# CHAPTER IV

## EFFECTS OF ALGAL MEAL SUPPLEMENTATION TO GROWING AND FINISHING PIGS ON PERFORMANCE AND CARCASS CHARACTERISTICS

### INTRODUCTION

In swine production systems, like all modern livestock production, animal nutrition is considered the most costly component over all elements (ARS-USDA, 2012). Similar to other livestock production systems, swine professionals are constantly looking for new alternatives in the nutrition area to increase production efficiency. Practices of more biologically, economically and environmentally sustainable are being seeked to enhance performance and quality of pork protein production for the present and future. Grain commodities and fuel prices have been rising in the past few years (ERS-USDA, 2012), and development of alternative sources of fossil fuels such as biodiesel, ethanol and other biofuels is of extreme importance and demand for more economically and environmentally friendly commodities is increasing.

Many different protein supplements have been utilized in swine diets such as soybean meal and also some by-products from the ethanol industry such as distiller's grains, distiller's solubles, corn gluten feed have been used lately. These corn distiller's products may be used up to 30% of the total DM of the diet without affecting growth performance (Cromwell et al., 1983; Gralapp et al., 2002; Cook et al., 2005). However if DDGS is included in greater levels such as 40%, a decrease in performance is expected (Stender and Honeyman, 2008).

Novel processes to produce biodiesel have been researched for the past few years and the utilization of large-scale production of microalgae is now close to becoming a reality in the United States. Various results from research over the past 20 years, and the interest on the

cultivation of microalgae species for biodiesel and glycerin production has renewed the interest and demand for this potential biofuel industry to be developed. Lipid production varies greatly among different species of microalgae, and some of them can produce approximately 5000 gallons of biodiesel per acre of land. Comparisons are being made to the little18 gallons per acre of land that corn produces of ethanol and approximately 48 gallons per acre of land biodiesel from soybeans according to the results reported by Sheehan et al. (1998). Therefore, microalgae seems to be a very attractive alternative for the future production of biofuel. Remarkably, another advantage of microalgae production is the relatively low cost and pro-environment facilities, where ponds can be placed in less expensive and non-fertile land. In addition to biodiesel and glycerin production, the process creates the microalgae meal, which is a high protein supplement with nutrient specifications comparable to distiller's, and soybean meal. The main hypothesis is that lipid extracted algal meal would behave like soybean meal or distiller's grains not affecting live growth performance, carcass characteristics and health status.

The microalgae biodiesel industry is rising not only as a potential future fuel resource, but also as a possible source a high protein by-product where livestock production systems could take advantage of, utilizing it as a replacement of any protein supplement such as soybean meal, corn gluten meal, and distiller's grains, among others. The use of an algal meal could be a valuable, economic, and environmentally sustainable substitution for these common agricultural crops. Since algal meal has not yet been approved for feeding livestock, there is a paucity of data regarding their use by large producing animals, including swine species.

The main hypothesis of the present is that the algae meal may be included in finishing rations at varying concentrations, without altering the performance of finishing barrows.

Therefore, this experiment was designed to determine the effects of varying inclusions of algal meal supplementation on performance, blood chemistry and lives health status, and carcass characteristics of growing-finishing pigs.

## MATERIALS AND METHODS

This experiment was reviewed and approved by the Colorado State University Institutional Animal Care and Use Committee.

### *Lipid Extracted Algae Meal*

The algal meal that was utilized in this study was derived from *Chlorella sp.* utilizing a production method of photosynthetic growth in open pond system. The harvest method used in this algal meal product was the flocculation with the assistance of a flocculating agent, and then the material was dewatered with the usage of a screen and afterwards spray dried. Lipid extraction was performed using the continuous extraction method, which applies mechanical cell lysis in a beadmill with methyl pentane solvent. The microalgae meal leftover was processed and dried at $100 \pm 5^{o}$ C. The remaining dried algal mass was then packed and shipped to the Colorado State University Facilities. The nutrient results for the algal meal are presented in Table 4.1. These results were utilized to formulate the manufacture the Algal diets for the different treatments.

*Animals and Treatments*

The experiment was conducted to conform to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and was approved by the Colorado State University Institutional Animal Care and Use Committee. A veterinarian, before the study started and also once a week throughout the experiment, inspected all the animals to assure normal health conditions.

Forty crossbred barrows (initial BW = 42.3 kg $\pm$ 3.4) were purchased and transported to the research center from a commercial feeding operation. Upon arrival, the barrows were processed, tagged, weighed for two consecutive days, and inspected by the attending veterinarian to assess initial health status. During the first 3 days the barrows received a commercial TMR for growing pigs at ad libitum. On the third day, the barrows were randomized and allocated to individual pens where they stayed for 1 week to adapt to the pen and diets.

The barrows were evaluated in a generalized randomized complete block design for the data collection of feed efficiency, ADG, blood metabolites chemistry, live health status, live final BW, carcass characteristics and fatty acid composition of the *Longissimus dorsi*. These characteristics of measurement were chosen to address the effects of titrated concentrations of algal meal on grower-finisher commercial pigs. The animals were blocked by time with each block consisting of 20 barrows starting on treatments one week apart from each other. Barrows were randomly assigned to one of 5 treatments of the experiments and every block had four animals receiving each treatment.

The experiment consisted of two periods; 1.) adjustment period (21 d) and 2.) metabolism period (7d). The treatments (n = 8 barrows/treatment) for the experiment consisted of: 1.)

soybean meal as protein supplementation source; 2.) 5% of algae meal on a DM basis as a protein replacement (5% AM); 3.) 10% of algae meal on a DM basis as a protein replacement (10%AM); 4.) 15% of algae meal on a DM basis as a protein replacement (15%AM); and 5.) 20% of algae meal on a DM as a protein replacement (20%AM); All diets were isocaloric and isonitrogenous. A common mineral and vitamin supplement was fed for all treatments. Mono-calcium phosphate was added in different quantities into the diets to keep the Ca:P ratio optimal for growing pigs, for all diets and to avoid metabolic health problems caused by imbalance of macro-minerals. In the present experiment, to facilitate management of treatment and its effects on barrows at the ARDEC facilities and due to availability of algal meal, animals were limited fed to 2 kg of each treatment diet daily. All pigs were fed a high concentrate finishing diet once daily. Table 4.1 represents the nutrient analyses for the algal meal and Table 4.2 represents the diets for the 5 treatments. Both treatment diets were formulated to meet or exceed the NRC (1998) requirements for CP, Ca, K and P. For experimental facilitation purposes, one single mineral and vitamin package was created and included in the formulas for all treatments. One micro-mineral and vitamin supplement was formulated for all animals considering the short feeding period that the animals were going to receive the treatments. Micro-minerals and vitamins minimum requirements were met or exceeded to avoid negative impacts on growth performance.

*Chemical Analysis*

Samples of the algal meal, diet, orts, feces and urine were analyzed for DM, crude protein, fat and minerals at the Michigan State University's Diagnostic Center for Population and Animal Health (DCPAH, Lansing, MI) and at the Colorado State University laboratory (CSU,

Fort Collins, CO). Samples of the *Longissimus dorsi* muscle of the barrows were analyzed at the meat laboratory of the Colorado State University.

Crude protein values were determined using a method that uses a pure oxygen environment in a ceramic horizontal furnace and large ceramic boats for the macro sample combustion process, followed by a combustion gas collection and handling system using helium carrier gas and a thermal conductivity cell for the detection of nitrogen. The machine utilized was the LECO TruMac N (LECO Corporation; Saint Joseph, Michigan USA)., After that, CP values were calculated from total N values (N x 6.25). NDF and ADF (Goering and Van Soest, 1970) were determined using a modified Van Soest process utilizing fiber bags technology Ankom 200 and using an Ankom unit (Ankom, Fairport, NY).

*Blood Metabolites*

On d 0 (starting date), d 21(previously to nutrient balance period) and d 28 (prior harvest), approximately 0.50 mL of blood was drawn with a syringe via jugular venipuncture and collected in microtainers treated with 15% EDTA K2. The samples were chilled during collection, and a sample was pulled from the microtainer and analyzed using single use iSTAT cartridges EC8+ and CG8+, which were then run through a portable iSTAT analyzer. pH, Total $CO_2$ ($TCO_2$), Partial Pressure $O_2$, Saturated $O_2$ ($SO_2$), Partial Pressure $CO_2$, $HCO_3$, Base Excess (BE), Sodium (Na), Chloride (Cl), Potassium (K), Blood Urea Nitrogen (BUN), Hematocrit (Hct), Hemoglobin (Hb), ionized Ca (iCa), Anion Gap and Glucose were reported.

*Animal Harvest and Organ Evaluation*

The pigs were humanely harvested on a commercial abattoir with the usage of a captive bolt gun. The carcasses were then opened from sternum to pelvis and the organs and complete digestive tract was removed. The lungs, small intestine, large intestine, stomach and cecum were visually inspected and disposed. Samples of the brains, livers, kidneys and hearts were taken. These organs were weighed on all 39 barrows at harvest and data was recorded for simple comparison between treatments.

*Fatty Acid Composition*

The fatty acids content of the barrows' *Longissimus dorsi* is shown in Table 4.6. The fatty acid profile was determined via gas chromatography using a Hewlett Packard (Avondale, PA) Model 6890 series II gas chromatograph fixed with a series 7683 injector and flame ionization detector. The instrument was equipped with a 100-m x 0.25-mm (id) fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA). Methyl ester derivatives of fatty acids were prepared using a combination of $NaOCH_3$ followed by HCl/methanol as described by Kramer et al. (1997). Fatty acid methyl ester preparations were injected using the split mode. The carrier gas was helium (flow rate of 2.1 ml/min), and the split ratio was 100:1 at $250^{o}$ C, at a pressure of 59.24 and split flow of 205 ml/min. The oven temperature was programmed from an initial temperature of $150^{o}$ C (0 min) to a final temperature of $160^{o}$ C at the rate of $1.0^{o}$ C/min. The final temperature was held for 110 min and run time was of 120 min total. Chromatograms were recorded with a computing integrator (Agilent Technologies, Palo Alto, CA). Detector heater was at $300^{o}$ C, $H_2$ flow of 35 ml/min, air flow of 350 ml/min. Standard fatty acid methyl ester mixtures were used to calibrate the gas chromatograph system using reference standards

KEL-FIM-FAME-5 (Matreya Inc., PA). Identification of the fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards.

*Calculations*

Individual BW gain was calculated by the difference in BW on d 21 and d 0, and d 28 and d 21, on study and reported as adjustment period ADG and metabolism period ADG. During the adjustment period, initial BW, final BW, and blood samples were collected. Final BW for the adjustment period was utilized as initial BW for the metabolism period. During the balance trial, feed, orts, total feces and total urine output were collected and 10% of the daily amount was sub-sampled over the 5 d balance trial, frozen and retained for subsequent analysis. Final BW and blood were collected immediately following the completion of the balance trial.

*Statistical Analysis*

Data for growth performance, blood metabolite chemistry parameters, organ's weights and fatty acids composition of the *Longissimus dorsi* muscle were analyzed using PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC). The experimental unit was each individual pig. Fixed effects were treatment and block. Interactions were tested, and removed from the model since no treatments by block interactions were observed. Differences between treatments were considered statistically significant if P-value $\leq 0.05$ and trending towards significance if *P*-value $\leq 0.10$.

# RESULTS

*Live Growth Performance*

The barrows stayed healthy throughout the study, and were weighed on d 0, d 21 and every d 28 of the study; except for one barrow receiving the 15% AM treatment, which was euthanized after being diagnosed with a spinal tumor by a Colorado State University certified veterinarian at the Veterinarian Teaching Hospital at CSU. This animal was treated with LA-200 3 mls IM every other day for 3 doses, Flunixin Meglumine (Banamine) 1 mls SQ once a day, Bo-Se 2.5 mls SQ once, and Pepsid (Famotidine) 2.5 tabs orally once a day. After 5 days, no response was observed and the animal was taken to the Veterinarian Teaching Hospital at Colorado State University in Fort Collins, CO. At the hospital the barrow was euthanized and the post-mortem report described bicavitary effusion, moderate with blood clot present within the pericardial sac; fibrinous peritonitis; multiple renal cysts bilateral; lymphadenopathy and replacement of bone marrow with tan, homogenous mass with suspicion of lymphosarcoma, which would could explain the neurologic signs.

Initial and final BW, average daily gain (ADG) for feedlot period, ADG for metabolism period, and gain-to-feed were significantly different ($P < 0.05$) across treatments. There was a step-wise reduction in these performance traits as the inclusion of AM increase.

Results for live performance are presented on Table 4.3. During the feeding period, initial average BW (39.6 kg) were similar across all the treatments ($P \geq 0.73$). Final BW in kg, ADG adjustment, ADG metabolism, and G:F results were inversely related to the increase of algal meal included in the diet causing a stepwise decrease on performance responses. Greater values were observed in the CON treatment and smallest values were observed always in the 20% AM treatment ($P < 0.01$).

*Live Health Status and Blood Metabolites Chemistry*

Blood metabolite results are presented on Table 4.4. All measurements were considered within a normal range (Van Metre and Angelos, 1999) from pre-study period until harvest time. In the present study, differences were observed across treatments for health status ($P \leq 0.02$) for blood pH, Total $CO_2$, Partial Pressure $CO_2$, $HCO_3$, BE, iCa, Hematocrit, Hemoglobin and blood urea nitrogen. No similar issue was seen during previous sheep experiment. According to veterinarian inspections, no side effects were observed due to treatment and behavior and vital signs were considered the same across treatments. Additionally, during harvest, there were no apparent abscesses of the internal organs noted upon inspection. The differences observed in blood metabolites were not previously expected.

*Carcass Characteristics*

Results for carcass characteristics are presented on Table 4.5. Furthermore, hot carcass weight, unribbed carcass weight, ham, loin, belly, butt, shoulder, feet, total parts, carcass length, ham circumference, and lion eye area were statistically different among treatment following the same pattern of the live performance data ($P \leq 0.01$). Last rib fat, last lumbar vertebrae fat, first rib fat, belly thickness, $10^{th}$ rib fat, LE L, LE a*, LE b*, marbling score and color score were similar ($P > 0.17$) across treatments.

*Fatty Acids Profile for Longissimus dorsi muscle samples*

Fatty acids profile for *Longissimus dorsi* muscle samples are reported on Table 4.6 and

represent the percentage of each fatty acid in relation of all fatty acids measured and identified. There was no significant effect from the dietetic algae meal treatments on fatty acids profile on the barrows' meat samples. Since animals were on feed for the study for only 28 days, it was expected that little to no changes in identified fatty acids from *Longissimus dorsi* muscle should be observed. Past literature also suggest that with low lipid content in the protein supplement, fatty acid composition should remain relatively unaffected (Turner et al., 2012).

*Organ Evaluation*

At the time of harvest in the end of the study period, the barrows were slaughtered with the utilization of a captive bolt gun, and each organ was removed from the animal and the weights recorded, these data are shown in Table 4.7. There were no differences observed in the brain weights ($P = 0.88$). However, lungs, hearts, kidneys, and liver presented a stepwise decline in observed values from CON to 20% AM treatments ($P < 0.02$). All organs seemed healthy and had no gross abnormalities or abscesses.

## DISCUSSION

Barrows were selected to suit the necessity of an animal model for monogastrics and also because of availability of algae meal. Greater concentrations ADF and NDF were found in the algal meal utilized in this study (11.88% and 23.16% respectively), when compared to common concentrations observed generally in cereal crops and by-products such as steam flaked corn and soybean meal (Archibeque et al. 2009).

Because of the inferior potential of microbial fermentation of feedstuff on monogastrics when compared to ruminants, feeds that contain greater concentrations of fibers may be undesirable. Lower fiber digestion is generally observed and reported in past literature for non-ruminants. Differences observed in live performance and carcass characteristics among all five treatments, may be explained by the lower utilization of the fiber and lower digestibility of the algal meal diets by the barrows which was expected for to be seen. Increased fiber levels in diets may be unbeneficial monogastric animals due to lack of pre-gastric fermentation capabilities and faster transit time through the gastrointestinal tract (Bach-Knudsen et al 1983). That will mainly result in lower microbial fermentation and less efficient utilization of the fiber portion of the feedstuff.

Blood metabolites measured in the present study showed that blood chemicals and metabolites were arbitrary across the board. While there were statistically significant differences for many different measurements across treatments, this difference does not appear to be upon treatment since no pattern was observed with increased levels of algal meal fed to the barrows. The most intriguing observation presented in Table 4.4 is the elevated levels of Partial Pressure of $CO_2$, which were higher than normal ranges previously published in the literature. The speculation for this elevation in blood Partial Pressure $CO_2$ resides on the fact that barrows had to be constrained manually and handled for more than two minutes each for blood collection. In addition, the use of a mechanical snare to restrain the animals increased stress levels and painting was observed on the majority of the animals after blood collection. Difficulty of body constriction also increased animal respiration ratio and collection time for jugular venipuncture blood sampling was variable. Variations on blood chemistry and metabolites could have occurred due to excessive handling of the animals during blood collection.

The elucidation of digestive effects of algal meal in non-ruminant animals will only be met after repeated long-term studies that would mimic the traditional commercial livestock scenario in the United States. The statistically significant differences seem in the present study may be due to the short period of time that the animals where under dietetic treatment and need to be confirmed with more experiments to replicate results.

This is one of the first large scale studies in non-ruminant commercial animals with the purpose of feeding algae meal as a protein supplement feed.

Feeding novel feedstuffs such as algal meal to non-ruminants was interesting, due to the fact that empirical observations on intake behavior were achievable and acceptability of the new feed and apparent palatability was very different from the ruminant experiment cited above. It seems that growing barrows did not appreciate the substitution of the soybean meal by algal meal up to 20% of the total DM in the diet. Barrows receiving algae treatments required 4 to 6 extra hours to ingest the same amount of feed that animals that received CON diets. This leads to the conclusion that algae meal physical characteristics did affect bunk behavior of the animals and might influence total daily DMI if animals were allowed ad libitum access to the feed. Apparent color and quality appeared not to be affected by algal meal supplementation, however size of muscle was clearly smaller for animals ingesting increasing levels of algae meal in their diets. The fatty acid composition of *Longissimus dorsi* muscle, expressed in % of the total identified fatty acids, observed in the present experiment did not show any differences among treatments as expected, except for a tendency for the saturated fatty acid 16:0. Results showed higher numerical values for the treatments CON and 5% AM when compared to the 10%, 15% and 20% AM. The percentages for fatty acid composition found in this study agrees with those of Cameron and Enser (1991) and Wasilewski et al. (2010), who reported similar proportion values

for all identified fatty acids. The greater values for fatty acid composition were attributed to the 18:1*cis*-9 fatty acid (more than 30% of total fatty acids identified), followed by 16:0 (approximately 25% of the total fatty acids identified), and proceeded by 18:0 (approximately 16% of the total fatty acid identified). Since animals were only fed algal meal treatments for 28 d, no major differences were expected. In this experiment, it is then possible to speculate that due to no difference in fatty acid profile among treatments, substitution of traditional protein supplements for algal meal up to 20% of total diet DM may not affect meat flavor and aroma. In addition, the current study presented above did not have the intention to manipulate fatty acid profile, and it was unlikely that changes in meat fatty acid profile would occur since there was little manipulation of the fatty acid composition of the diet fed to the barrows in this study. Furthermore, algal meal is still a USDA non-approved feedstuff, therefore no taste panel was conducted.

## CONCLUSIONS

The present study showed that algae meal may not be a feasible protein supplement for growing and-finishing barrows, and if available to producers may decrease the interest and competition for soybean meal by ruminant production systems, which may decrease both products prices.

# IMPLICATIONS

Research results suggest that feeding up to 20% of algae by-product meal to finishing pigs have negative impact on performance and carcass characteristics as compared to the standard protein sources that have been used by the industry. Further research may be necessary to determine the response of different levels of supplementation of algal meal for pigs and other monogastric species, effects on animals in a different physiological stage and maximal tolerable levels of algae co-product supplementation.

**Table 4.1. Analyzed nutrient analysis for algal meal of *Chlorella sp.*, %DM.**

| **Nutrient content** | %, DM |
|---|---|
| Crude Protein, % | 21.12 |
| Acid Detergent Fiber, % | 11.88 |
| Neutral Detergent Fiber, % | 23.16 |
| Fat (EE), % | 1.96 |
| Calcium, % | 6.01 |
| Phosphorous, % | 0.435 |
| Potassium, % | 0.52 |
| Magnesium, % | 0.74 |
| Sodium, % | 5.685 |
| Chloride, % | 4.25 |
| Sulfur, % | 0.85 |
| Copper, ppm | 19.95 |
| Iron, ppm | 5355 |
| Lead, ppm | 3.785 |
| Zinc, ppm | 41.6 |

**Table 4.2. Finishing diets for grower-finisher barrows using algal meal compared to standard industry soybean meal diet at 0, 5, 10, 15, or 20% of the dietary DM.**

| Ingredient, % | Treatment | | | | |
|---|---|---|---|---|---|
| | CON | 5% AM | 10% AM | 15% AM | 20% AM |
| Corn | 78.2 | 75.6 | 74.55 | 72.7 | 72.05 |
| SoybeanMeal | 18.5 | 15 | 10 | 5 | 0 |
| Algal Meal | 0 | 5 | 10 | 15 | 20 |
| Fat | 1 | 2.15 | 3.15 | 4.55 | 5.2 |
| Ca carbonate | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 |
| Monocalcium Phosphate | 0.75 | 0.7 | 0.75 | 1.2 | 1.2 |
| Salt | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Suppement[1] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| L-Lysine | 0.0314 | 0 | 0 | 0 | 0 |

[1]Supplement was created by Akey Animal Nutrition to meet or exceed the mineral and vitamin requirements for optimal growth performance of growing pigs.

**Table 4.3. Live growth performance of growing-finishing barrows (n=8/treatment) consuming corn-based finishing rations with 0, 5, 10, 15, or 20% of the dietary DM as algal meal.**

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | CON[1] | 5% AM | 10% AM | 15% AM | 20% AM | SEM | P-value |
| Initial BW, kg | 39 .1 | 39.3 | 39.3 | 40 | 40.1 | 3.62 | 0.73 |
| Final BW, kg | 59.6 | 56.4 | 52.5 | 48.8 | 43.7 | 0.57 | <0.001 |
| ADG adjustment[1], kg | 0.82 | 0.66 | 0.63 | 0.37 | 0.2 | 0.05 | <0.001 |
| ADG metabolism[2], kg | 0.33 | 0.34 | -0.12 | 0.11 | -0.09 | 0.12 | 0.01 |
| G:F[3] | 0.361 | 0.302 | 0.233 | 0.166 | 0.076 | 0.01 | <0.001 |

**[1]**ADG during the 21 days of the individual feedlot pen period.

**[2]**ADG during the 7 days of the balance metabolism period.

[3] G:F presented for the whole feeding period.

**Table 4.4. Effects of feeding control and algal meal diets to growing-finishing barrows on blood chemistry metabolites measurements.**

| Response[1] | CON | 5%AM | 10%AM | 15%AM | 20%AM | SE | P-value | Normal Ranges |
|---|---|---|---|---|---|---|---|---|
| pH | $7.32^{z}$ | $7.38^{y}$ | $7.30^{z}$ | $7.29^{z}$ | $7.33^{z}$ | 0.02 | 0.01 | 7.25 – 7.46 |
| Total $CO_{2y}$ mMol/L | $34.50^{z}$ | $37.50^{y}$ | $33.50^{z}$ | $36.06^{y}$ | $36.92^{y}$ | 0.96 | <0.001 | 23.8 - 33.8 |
| Partial Pressure $O_2$ mmHg | 24.92 | 23.92 | 25.33 | 24.77 | 26.67 | 1.16 | 0.21 | |
| Saturated $O_2$ % | 40 | 36.42 | 37 | 36.01 | 40.75 | 2.83 | 0.33 | |
| Partial Pressure $CO_2$ mmHg | $62.45^{z}$ | $60.63^{z}$ | $65.58^{zy}$ | $70.13^{zy}$ | $65.80^{xy}$ | 3.1 | 0.02 | |
| $HCO_3$ mMol/L | $33.03^{y}$ | $35.60^{y}$ | $31.45^{z}$ | $34.00^{y}$ | $34.36^{z}$ | 0.9 | <0.001 | |
| BE, mMol/L | $6.91^{y}$ | $10.33^{z}$ | $4.91^{x}$ | $7.19^{y}$ | $8.75^{zy}$ | 1.09 | <0.001 | |
| Na, mMol/L | 143.42 | 141.25 | 140.17 | 141.28 | 141.5 | 1.34 | 0.19 | 141 - 152.6 |
| Cl, mMol/L | 97.58 | 98.83 | 100.25 | 98.66 | 100.08 | 1.33 | 0.25 | 94.4 - 114 |
| K, mMol/L | 5.13 | 4.85 | 5.5 | 5.09 | 5.17 | 0.25 | 0.14 | 4 - 5.2 |
| iCa, mMol/L | $1.40^{y}$ | $1.41^{y}$ | $1.46^{z}$ | $1.41^{y}$ | $1.42^{y}$ | 0.02 | 0.01 | |
| Anion Gap, mMol/L | 18 | 11.83 | 13.75 | 14.15 | 13.42 | 2.09 | 0.06 | |
| Hematocrit, %PCV | $35.58^{y}$ | $32.83^{z}$ | $35.17^{y}$ | $32.76^{z}$ | $31.92^{z}$ | 1.14 | 0.01 | 36.4 - 52.8 |
| Hemoglobin, g/dL | $11.95^{xy}$ | $11.15^{yz}$ | $12.04^{x}$ | $11.01^{z}$ | $10.94^{z}$ | 0.39 | 0.01 | 12.5 - 17.3 |
| Glucose, mg/dL | 91.67 | 93.17 | 103 | 92.46 | 101.42 | 4.97 | 0.06 | |
| BUN, mg/dL | $11.42^{yz}$ | $12.58^{yz}$ | $11.33^{yz}$ | $12.00^{yz}$ | $10.75^{z}$ | 0.79 | 0.2 | 9.2 - 29.2 |

[1]No treatment by date interaction was observed.
[x, y, z] Least square means within a row without a common superscript differ ($P < 0.05$)

**Table 4.5. Carcass characteristics of growing-finishing barrows (n=8/treatment) consuming corn-based finishing rations with 0, 5, 10, 15, or 20% of the dietary DM as algal meal.**

| Treatments | CON[1] | 5% AM | 10% AM | 15% AM | 20% AM | SEM | P-VALUE |
|---|---|---|---|---|---|---|---|
| HCW, kg | 39.6 | 36.8 | 35.1 | 32.8 | 29.9 | 0.64 | <0.001 |
| CW, kg | 19.1 | 17.8 | 17.1 | 13.9 | 14.4 | 0.91 | 0.002 |
| Ham | 4.79 | 4.43 | 4.26 | 3.42 | 3.64 | 0.23 | 0.001 |
| Loin | 5.3 | 4.87 | 4.74 | 4.68 | 3.92 | 0.36 | <0.001 |
| Belly w/Spear rib | 3.65 | 3.65 | 3.39 | 2.98 | 2.82 | 0.11 | <0.001 |
| Butt | 2.65 | 2.6 | 2.62 | 2.36 | 2.28 | 0.1 | <0.001 |
| Shoulder | 2.33 | 2.04 | 1.95 | 1.84 | 1.67 | 0.06 | <0.001 |
| Feet | 0.68 | 0.71 | 0.64 | 0.63 | 0.58 | 0.02 | <0.001 |
| Total | 19.3 | 18.2 | 17.5 | 16.3 | 14.8 | 0.99 | <0.001 |
| Carcass Length | 66.7 | 67.1 | 65.5 | 65.4 | 62.8 | 0.72 | 0.001 |
| Ham Circ. | 56.63 | 56.31 | 54.25 | 53.82 | 52.5 | 1.02 | 0.04 |
| Last Rib fat | 0.49 | 0.43 | 0.37 | 0.41 | 0.34 | 0.05 | 0.17 |
| Last Lumbar Vert Fat | 0.29 | 0.34 | 0.27 | 0.23 | 0.25 | 0.03 | 0.19 |
| First Rib Fat | 0.92 | 0.89 | 0.94 | 0.92 | 0.83 | 0.18 | 0.81 |
| Belly Thickness | 1.06 | 1.08 | 1.01 | 1.00 | 0.99 | 0.05 | 0.59 |
| $10^{th}$ Rib Fat | 0.44 | 0.5 | 0.5 | 0.49 | 0.47 | 0.05 | 0.88 |
| Loin Eye Area | 2.85 | 2.65 | 2.56 | 2.42 | 2.04 | 0.32 | 0.002 |
| LE L* | 53.99 | 53.22 | 53,75 | 53.18 | 52.62 | 2.55 | 0.88 |
| LE a* | -0.47 | -.1.30 | -0.3 | -0.56 | -0.43 | 1.05 | 0.54 |
| LE b* | 7.98 | 7.31 | 8.8 | 7.73 | 7.94 | 1.25 | 0.33 |
| Marbling score | 1.48 | 1.87 | 1.51 | 1.72 | 1.43 | 0.35 | 0.23 |
| Color Score | 1.75 | 2.25 | 1.87 | 1.85 | 1.88 | 0.26 | 0.69 |

**Table 4.6. Fatty acid composition (% of identified fatty acids) of *Longissimus dorsi* of finishing barrows fed corn-based diets containing 0, 5, 10, 15 or 20% of lipid extracted algae meal (AM).**

| Item | Control | 5% AM | 10% AM | 15% AM | 20% AM | SE | P- value |
|---|---|---|---|---|---|---|---|
| 12:0 | 0.08 | 0.09 | 0.07 | 0.08 | 0.08 | 0.01 | 0.24 |
| 14:0 | 1.25 | 1.38 | 1.26 | 1.32 | 1.34 | 0.06 | 0.24 |
| 16:0 | 26.92 | 27.53 | 26.41 | 26.39 | 26.4 | 0.43 | 0.09 |
| 16:1cis-9 | 3.26 | 3.03 | 3.06 | 3.23 | 3.1 | 0.15 | 0.47 |
| 18:0 | 14.61 | 15.58 | 14.93 | 14.62 | 14.97 | 0.51 | 0.38 |
| 18:1trans-11 | 11.04 | 21.08 | 16.22 | 9.1 | 11.24 | 8.15 | 0.61 |
| 18:1cis-9 | 33.31 | 23.15 | 28.49 | 35.71 | 33.83 | 7.64 | 0.51 |
| 18:2cis-9,12 | 7.79 | 6.75 | 7.94 | 7.88 | 7.63 | 0.83 | 0.64 |
| 18:2cis-9,trans11 | 0.09 | 0.07 | 0.08 | 0.09 | 0.1 | 0.01 | 0.25 |
| 18:2trans-10,cis12 | 0.35 | 0.013 | 0.016 | 0.021 | 0.025 | 0.01 | 0.19 |
| 18:3cis-9, 12, 15 | 0.47 | 0.47 | 0.54 | 0.56 | 0.33 | 0.12 | 0.33 |
| 20:0 | 0.24 | 0.23 | 0.25 | 0.25 | 0.25 | 0.03 | 0.94 |
| 20:4cis-5, 8, 11, 14 | 0.81 | 0.54 | 0.67 | 0.68 | 0.61 | 0.17 | 0.61 |
| 20:5cis-5, 8, 11, 14, 17 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | 0.01 | 0.16 |
| 20:6cis-4, 7, 10, 13, 16, 19 | 0.06 | 0.05 | 0.04 | 0.05 | 0.08 | 0.02 | 0.55 |
| SFA[1] | 43.1 | 44.81 | 42.92 | 42.65 | 43.04 | 0.82 | 0.12 |
| MUFA[2] | 47.61 | 47.26 | 47.76 | 48.04 | 48.17 | 0.88 | 0.85 |
| PUFA[3] | 9.29 | 7.93 | 9.31 | 9.31 | 8.79 | 0.97 | 0.58 |

[1] SFA: Saturated fatty acids % of identified fatty acids.

[2] MUFA: Mono-unsaturated fatty acids % of identified fatty acids.

[3] PUFA: Poly-unsaturated fatty acids % of identified fatty acids.

**Table 4.7. Average organ weights at harvest of growing-finishing barrows fed 0, 5, 10, 15 and 20% of lipid extracted algae meal (AM)**

| Organ | CON | 5%AM | 10% AM | 15% AM | 20% AM | SE | P Value |
|---|---|---|---|---|---|---|---|
| Brain, g | 88.18 | 84.66 | 87.7 | 87.17 | 85.45 | 4.14 | 0.88 |
| Heart, g | 273.71 | 247.96 | 244 | 237.61 | 207.51 | 16.52 | 0.01 |
| Right Kidney, g | 113.69 | 112.9 | 106.41 | 99.87 | 96.53 | 7.63 | 0.12 |
| Left Kidney, g | 111.91 | 115.23 | 111.6 | 101.9 | 94 | 6.67 | 0.02 |
| Liver, g | 1078.5 | 1043.01 | 997.59 | 901.67 | 843.93 | 46.1 | <0.01 |
| Lungs, g | 766.61 | 773.41 | 645.21 | 573.21 | 553.36 | 65.47 | <0.01 |

# REFERENCES


Abaxis – VetScan iSTAT. Union City, CA.

Archibeque, S. A., A. Ettinger, B. D. Willson. 2009. *Nannochloropsis oculata* As A Source For Animal Feed Acta. Agron. Hung. 57:245-248.

ARS-USDA. 2012. http://www.ars.usda.gov/research/ programs/programs.htm?np_code=102&pf=1. Accessed on Aug 12, 2012.

Bach-Knudsen, K. E., J. Wolstrap, B. O. Eggum.1983 The Influence of Dietary Fiber and Microbial Activity in the Digestive Tract on True Protein Digestibility and Biological Value in Rats. Tierphysiol, 49:173-180.

Bremer, V. R., K. J. Hanford, G. E. Erickson, T. J. Klopfenstein. 2011. Meta-Analysis of Feeding Calf-Feds or Yearlings Wet Distiller's Grains with Different Corn Processing Types. Nebraska Beef Rep. MP: 40-41.

Cameron, N. D., M. B. Enser. 1991. Fatty acid composition of lipid in Longissimus dorsi muscle of Duroc and British Landrace pigs and its relationship with eating quality. J. Meat Sci. 29:295-307.

Dib, M. G., K. Rolfe, J. O. Sarturi, G. E. Erickson, T. J. Klopfenstein, R. Lindquist. 2010. Effects Of Feeding A Combination Of Modified Distiller's Grains Plus Solubles and Wet Corn Gluten Feed to Adapt Cattle to Finishing Diets. Nebraska Beef Rep. MP:57-59.

D'Mello, J.P.F., D. I. Officer. 2000. Farm Animal Metabolism and Nutrition: Feed Enzymes. CABI Publishing, N.Y., N.Y. Pp. 405-426.

ERS-USDA. 2012. http://www.ers.usda.gov/Briefing/ Corn/2010baseline.htm. Accessed on Aug. 12, 2012.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st red. Ed. Fed. Anim. Sci. Soc., Savoy, IL.

Gill, R. K., D. L. VanOverbeke, B. Depenbusch, J. S. Drouillard and A. DiCostanzo. 2008. Impact of beef cattle diets containing corn or sorghum distiller's grains on beef color, fatty acid profiles, and sensory attributes. J. Anim. Sci. 86:923-935.

Howe, B. A, I. N. Roman-Muniz, B. D. Willson, and S. L. Archibeque. 2011. Nannochloropsis oculata meal did not alter nutrient usage and had no adverse health effects when fed to rabbits as a protein source. J. Anim. Sci. 89: (Suppl 2) (Abstr.)

Howe, B. A., I. N. Roman-Muniz, B. D. Willson, and S. L. Archibeque. 2010 Evaluation of algae meal from Nannochloropsis oculata as a protein source for non-ruminant animals. J. Anim. Sci. 88: (Suppl 2) (Abstr.)

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board Invited Review: Use of distiller's by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

SAS Institute Inc. 2003. SAS 9.1.2. Cary, NC, USA.

Schoonmaker, J. P., A. H. Trenkle and D. C. Beitz**.** 2010. Effect of feeding wet distiller's grains on performance, marbling deposition, and fatty acid content of beef from steers fed low- or high-forage diet.J. Anim. Sci. 88:3657-3665.

Turner, T. D., L. Karlsson, C. Mapiye, D. C. Rolland, K. Martinsson and M. E. R. Dugan. 2012. Dietary influence on the m. longissimus dorsi fatty acid composition of lambs in relation to protein source. J. Meat Sci. 91:472-477.

Van Metre, D. C., S. M. Angelos. 1999. Clinical Pathology and Sample Collection: Miniature pigs. 2:519-538.

Wasilewski, P. D., J. Nowachowicz, G. Michalska, T. Bucek, B. Lynch, A. M. Mullen. 2011. Fatty acid profile of Longissimus dorsi muscle of crossbred pigs fed with addition of conjugated linoleic acid or sunflower oil. Archiv Tierzucht. 54:61-68.