

REFLECTIVE ESSAY

Before I began my research project on the feasibility of replacing soy and alfalfa protein with Spirulina protein to develop a more nutrient rich horse feed, I studied the advances of using microalgae as a protein source with a low environmental impact in my previous biology and biological engineering courses. My passion for learning about how Spirulina protein could potentially solve the challenges of malnourishment among both humans and animals, as well as how it can decrease our ecological footprint, sparked my interest in conducting further research in Dr. Marcella Reekie's class, Written Communication for Engineers. The basis of the class is to write a feasibility report on an engineering topic that the student is interested in. I decided to develop a better understanding of the positive advancements of Spirulina through the research needed to complete my feasibility report. I had also gained prior knowledge on the care and maintenance of racehorses through peers of mine at Kansas State University. By going through the process of getting my degree in biological systems engineering, I realized that the two topics, racehorse nutrition and easily digestible plant protein, could go hand-in-hand.

To further understand the nutrition requirements of racehorses, I began reading articles such as *Biotechnology Advances*, *USDA*, and *Semantic Scholar*. I was fascinated to learn that the microalgae known as Spirulina has many health and nutritional benefits, including high protein content. I was also able to find that racehorses need a very specific diet that is easy to digest and has a significant amount of protein. If the feed is not easily digestible, it can cause many health issues for the horse. I decided to conduct research on how Spirulina could be used to improve the diet of racehorses and was able to conduct this research in Written Communications for Engineers.

To begin conducting my research, I needed to pick a client that I could present my objectives to and focus on researching information that could benefit them. I eventually found Tribute Superior Equine Nutrition. This horse feed company was the best client for this research because they put an emphasis on using alternative plant protein sources that were easily digestible to their racehorses. After finding the right client, I created my key objectives that the feasibility study would discuss in further detail. Once the objectives were set in stone, I created an outline that would guide my research and organization of the paper. Since the sources cited needed to be in IEEE format, I used Zotero to help keep my cited sources organized and easily accessible.

I needed to make sure that every source I used was reliable. I did this by collecting information from trusted, peer-reviewed articles and websites. I used the Kansas State University online library database to find most of my articles. I knew that these sources were reliable and written by professionals. When I needed to use articles outside of the database, I checked that the articles were written by a professional on the topic being discussed. While this might have added on some extra research time, it was very important that I knew my information could be trusted by the client. I made sure each article was recently written to ensure that the information provided was up-to-date. When discussing food related laws and requirements, I made sure to collect information directly from government sources including the Center for Food Safety and Applied Nutrition, and the U.S. Food and Drug Administration.

One article that really caught my attention was “Special Report: Spirulina Part 7,” written by Algae Industry Magazine. What stuck out to me about this article was the graphic that was used to compare the resources needed for soybeans, corn, beef, and Spirulina. Spirulina uses far less land and water compared to the other protein sources. This image helped provide further proof of the environmental benefits of Spirulina production. By finding this article, I was able to go into further detail and compare the cost analysis and resource use between soy, alfalfa, and Spirulina.

When researching the best articles to use on the Kansas State University library database, I developed key words for each objective that would aid in minimizing the number of articles that would be available. For example, when researching the cost analysis of soybeans, I used keywords such as cost, soybeans, farming, etc. It was also very convenient that the Kansas State database allows you to narrow down the amount of material that shows up with a search. I was able to select that I only wanted peer-reviewed articles written in the last five years to show up on the screen, and it made my research go much smoother. I was able to find exactly what I needed because of the database. I also learned through this research that it is important to pull the specific information that I needed without necessarily reading the whole article. There are a few ways to do this that I found the most beneficial. First, I could read the abstract at the beginning of each article to get a generalized understanding of what it was about. If this article was what I was looking for, I would go directly to the table of contents to find the specific information that I needed. I would finish by reading the conclusion to see if there were any last bits of information that would be helpful in aiding my topic discussion.

When conducting this research for my feasibility report, I learned that creating an outline for the paper is crucial. This allowed me to stay on track with the research that needed to be done over a certain period of time. I was able to create specific deadlines for myself on what topics needed to be researched and when so that I could stay ahead. This was very important for me because the feasibility report was very long and detailed. I needed to stay on track so that I could provide the best information for the client while also giving myself enough time to make proper corrections throughout the report. Using the Kansas State University library database made my research go incredibly smoothly, and I will use the research skills I have gained while writing this report in the future as an engineer.

FEASIBILITY STUDY OF REPLACING SOY AND ALFALFA PROTEIN WITH SPIRULINA PROTEIN TO DEVELOP A MORE SUSTAINABLE AND NUTRIENT RICH HORSE FEED

Submitted To:

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3 May 2022

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Mr. Kalmbach,

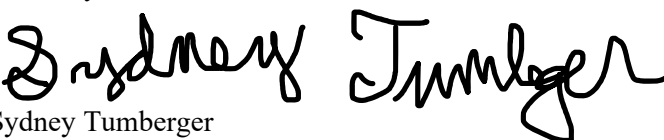
I am pleased to submit my formal report, "Feasibility Study of Replacing Soy and Alfalfa Protein with Spirulina Protein to Develop a More Sustainable and Nutrient Rich Horse Feed," that you approved on March 3, 2022. This report includes the results of my research and explains how and why Spirulina protein should be implemented into all of our horse feeds.

I conducted this research to find a more sustainable and nutrient dense plant protein for our horse feed products. To begin my report, I start by listing my research objectives (p.3). Then, my research is distributed into six main sections: replacing soy and alfalfa protein with Spirulina protein in horse feed (p.3), sustainable growing methods of Spirulina (p.4), impact of unsustainable farming methods of soy and alfalfa (p.8), cost analysis and comparison of soy, alfalfa, and Spirulina production and revenue (p.9), nutrient analysis and comparison (p.15), and FDA rules and regulations (p.19). To end my report, I list my conclusions (p.20), recommendations (p.20), and provide the steps that should be taken to implement Spirulina protein into our horse feed products (p.21).

This report gives all of the information needed for Tribute Superior Equine Nutrition to implement Spirulina protein into our horse feed products to provide our customers with a sustainable and nutrient dense feed. Through my completed research, I proved the feasibility of replacing soy and alfalfa protein with Spirulina protein. Spirulina has the highest protein content, has a wide variety of key vitamins and minerals, uses far less resources for cultivation and production, and can provide a large revenue for our company. At this time, I recommend that we implement Spirulina protein into all of our horse feeds. I also recommend that we install a tubular photobioreactor system for Spirulina cultivation in the fall, winter, and spring, and install an open pond raceway system for cultivation in the summer. Furthermore, we should conduct the proper tests that are necessary for FDA approval of adding Spirulina as a new horse feed ingredient.

Please contact me, by phone or email, if you have any questions about the report.

Sincerely,



Sydney Tumberger

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EXECUTIVE SUMMARY

Tribute Superior Equine Nutrition puts quality and consistency first in every product that we manufacture. Our expertise in proper horse nutrition makes us a top tier horse feed company that offers a variety of feed for each horse's specific needs. We stand above the competition by using whole and natural ingredients that provide key nutrients to horses and support overall health. As new and innovative solutions develop, Tribute must continue to explore better ingredients that will not only benefit horses but will also improve our sustainability and environmental footprint as a company that cares for their clients. Currently, our feed products provide horses with a high protein diet. However, our two most popular plant protein sources, soy and alfalfa, cause the following problems: poor digestibility and other health issues among horses, rapid decrease in farmland, lack of vital nutrients in soy, and degradability rates of alfalfa.

On February 24, 2022, I submitted a proposal to research the feasibility of replacing soy and alfalfa protein with Spirulina protein to develop a more sustainable and nutrient rich horse feed. To conduct my research, I completed six tasks: 1) review FDA rules and regulations regarding the production and distribution of animal feed in the United States; 2) review literature covering the costs of manufacturing soy, alfalfa, and Spirulina protein products that go, or could potentially go, into the horse feed; 3) review literature covering feed related health issues among horses; 4) review literature on current farming methods of soy and alfalfa that are contributing to climate change; 5) review literature on sustainable growing methods for Spirulina; 6) review literature and compare nutrients found in soy, alfalfa, and Spirulina. You approved my proposal on March 3, 2022.

Implementing Spirulina protein into our horse feed in place of soy and alfalfa will allow for easy cultivation and high yield, will provide a feed with higher protein, and will lower negative impacts on the environment. It will keep Tribute Superior Equine Nutrition at the top of all horse feed companies in the race toward a more sustainable future.

CONCLUSIONS (See page 20 for a complete list.)

- 1) The best cultivation methods for Spirulina are to use a tubular photobioreactor system in the winter and an open pond system in the summer.
- 2) Soy and alfalfa contribute to deforestation and soil and land damage and use more resources for production than Spirulina.

RECOMMENDATIONS (See page 20 for a complete list.)

- 1) Tribute Superior Equine Nutrition should implement Spirulina protein into our horse feed.

INTRODUCTION TO THE DISCUSSION

Tribute Superior Equine Nutrition puts quality and consistency first in every product that we manufacture. Our expertise in proper horse nutrition makes us a top tier horse feed company that offers a variety of feed for each horse's specific needs. We stand above the competition by using whole and natural ingredients that provide key nutrients to horses and support overall health.

As new and innovative solutions develop, Tribute must continue to explore better ingredients that will not only benefit horses but will also improve our sustainability and environmental footprint as a company that cares for their clients. If we do not take this leap toward sustainable protein sources now, we risk losing access to soybean and alfalfa sources in the future when croplands become irreversibly damaged. As our population continues to grow, so does our demand for protein. Spirulina protein is the future of food security for all animals and people on Earth. This sustainable and protein-rich plant will be the change that puts Tribute Superior Equine Nutrition and Kalmbach Feeds, Inc. at the head of the race against a sustainable and nutrient dense horse feed.

On March 3, 2022, my proposal to research the feasibility of replacing soy and alfalfa protein with Spirulina protein was approved. The purpose of my research was to find a better plant protein source and develop a more sustainable and nutrient rich horse feed. To conduct my research, I completed six tasks: 1) review FDA rules and regulations regarding the production and distribution of animal feed in the United States; 2) review literature covering the costs of manufacturing soy, alfalfa, and Spirulina protein products that go, or could potentially go, into the horse feed; 3) review literature covering feed related health issues among horses; 4) review literature on current farming methods of soy and alfalfa that are contributing to climate change; 5) review literature on sustainable growing methods for Spirulina; 6) review literature and compare nutrients found in soy, alfalfa, and Spirulina.

The first part of this report discusses the four research objectives. Then, I explain the benefits and drawbacks of replacing soy and alfalfa protein with Spirulina protein. Next, I describe the different cultivation methods of Spirulina. I break this down into phototrophic, heterotrophic, and mixotrophic cultivation. After this, I discuss the impacts of unsustainable farming methods of soy and alfalfa. Then, I compare and analyze the costs of soy, alfalfa, and Spirulina production and revenue. I also do a nutrient analysis and comparison of soy, alfalfa, and Spirulina. Next, I discuss FDA rules and regulations regarding the implementation of ingredients in feed products. Finally, I list my conclusions and recommendations and explain how Spirulina can be implemented into our horse feed products.

Implementing Spirulina protein into our feed in place of soy and alfalfa protein will not only provide a more nutrient dense feed for our clients, but it will also allow Tribute Superior Equine Nutrition to develop a more sustainable feed that will lower negative environmental impacts. I recommend that our company takes the necessary steps to implement Spirulina into our horse feed right away.

DISCUSSION

RESEARCH OBJECTIVES

My proposal for this research contained four objectives that I completed. By accomplishing these objectives, I analyzed all of the topics that are necessary to recommend the best methods for incorporating Spirulina into our horse feed. This report includes all of the information accumulated by finishing these objectives. The four objectives are listed below:

- 1) Complete analysis and discussion of micronutrients and macronutrients found in Spirulina and how they benefit horse health.
- 2) Analysis of efficient growing methods for Spirulina that reduce land and water use.
- 3) Cost analysis of Spirulina protein synthesis versus soy and alfalfa protein synthesis.
- 4) A final recommendation on how Spirulina should be implemented into our company's horse feed.

REPLACING SOY AND ALFALFA PROTEIN WITH SPIRULINA PROTEIN IN HORSE FEED

To continue to produce a high-quality feed, production of alternative protein sources must be a top priority. While soy and alfalfa are the most common protein sources in our feed today [1], replacing them with Spirulina would provide our company with a sustainable and nutrient dense alternative.

Benefits

Using Spirulina in horse feed provides many benefits over the use of soy and alfalfa protein. These benefits include easy cultivation with high yield, higher protein content, successful use in other animal feeds, and low environmental impact.

The cultivation of Spirulina can be done using multiple methods that will be further discussed under the “Sustainable Growing Methods of Spirulina” section. According to Usharani, Saranraj, and Kanchana, key growth factors for producing Spirulina include a carbon source, a sodium source, water, a balanced pH, and sunlight [2]. These sources can be easily obtained and controlled under certain weather conditions. Spirulina produces about 10,000 tons of biomass annually under good conditions, making it the largest microalgal cultivation industry in the world [2].

Spirulina has a high protein content, which falls between 50-70% by dry weight. This superfood also contains all of the essential amino acids [2]. Further comparison of protein content among Spirulina, soy, and alfalfa can be found under the “Nutrient Analysis and Comparison” section.

Approximately 30% of produced Spirulina is used for animal feed [3]. It has been used and demonstrated in feed for pigs, cows, sheep, chickens, domestic and pet animals, and in aquaculture [3]. Adding small amounts of Spirulina biomass to animal feed benefits both the physiology and immune health of the different animals listed [3].

The production of Spirulina can lower the company's carbon footprint because Spirulina can use carbon dioxide that is released into the atmosphere as pollution as its source of nutrients instead [4]. When producing this microalgae on a commercial scale, water, nutrients, and energy can be recycled in the process when it is set up properly [3]. Nutrients found in wastewater that would be a hazard to humans and animals can be used to grow Spirulina [3]. This can lower the costs of purchasing nutrients for cultivation, as well as provide water treatment plants with a sustainable and natural treatment system. Growing Spirulina can benefit multiple companies at once.

Drawbacks

Using Spirulina as a protein source also comes with a few drawbacks including very little research on specific costs of production, a need for a warm area year-round, and no past use of Spirulina in horse feed.

While many methods can be used to grow Spirulina, it is difficult to give a specific cost estimate without knowing the exact process of cultivation and biomass production. However, I will conduct a cost analysis on the best cultivation method of Spirulina for our company. Further information on this analysis can be found in the "Cost Analysis and Comparison of Soy, Alfalfa, and Spirulina Production and Revenue" section.

Temperature is the most important factor in Spirulina biomass production [2]. To produce maximum yield, it needs to grow in conditions at 32 degrees Celsius (89.6 degrees Fahrenheit) [2]. Sandusky, Ohio, the location of Kalmbach Feeds, Inc., has a mean annual temperature of 9.4 degrees Celsius (49 degrees Fahrenheit) [5]. However, this may not be an issue as Spirulina can be grown using multiple methods that will be further discussed in the "Sustainable Growing Methods of Spirulina" section.

From my research, I have not found any evidence of Spirulina being used as a protein source in horse feed. However, research has been conducted on the benefits of adding Spirulina to other farm animals as stated in the "Benefits" section. Adding Spirulina was shown to add mass to pigs and weaned lamb, as well as enhance yellow and red coloring of chicken meat [3].

SUSTAINABLE GROWING METHODS OF SPIRULINA

Spirulina can be cultivated using a number of different methods in an open or closed system. Phototrophic, heterotrophic, and mixotrophic cultivation will be compared in detail below.

Phototrophic Cultivation

Phototrophic cultivation takes place when Spirulina goes through the process of photosynthesis. This cultivation method can be done in either a photobioreactor or an open pond system.

Photobioreactors

Due to the high photosynthetic efficiency and growth rates, *Spirulina* can be grown in open ponds or enclosed photobioreactors. Enclosed photobioreactors use no land for production. Instead, tubes, bags, or plates made of transparent material are used to grow the microalgae [6]. The transparent material allows for light to enter for photosynthesis to take place. Nutrients and water are distributed manually.

Column Photobioreactor

Column photobioreactors are usually arranged vertically with light entering through transparent material [6]. These bioreactors have the best controlled growth conditions, efficient mixing, highest gas transfer rates, and eliminate the need for excess land use [6]. As seen below, Figure 1 demonstrates the transparent columns filled with microalgae.

Diagram of a Column Photobioreactor Labeling its Different Parts

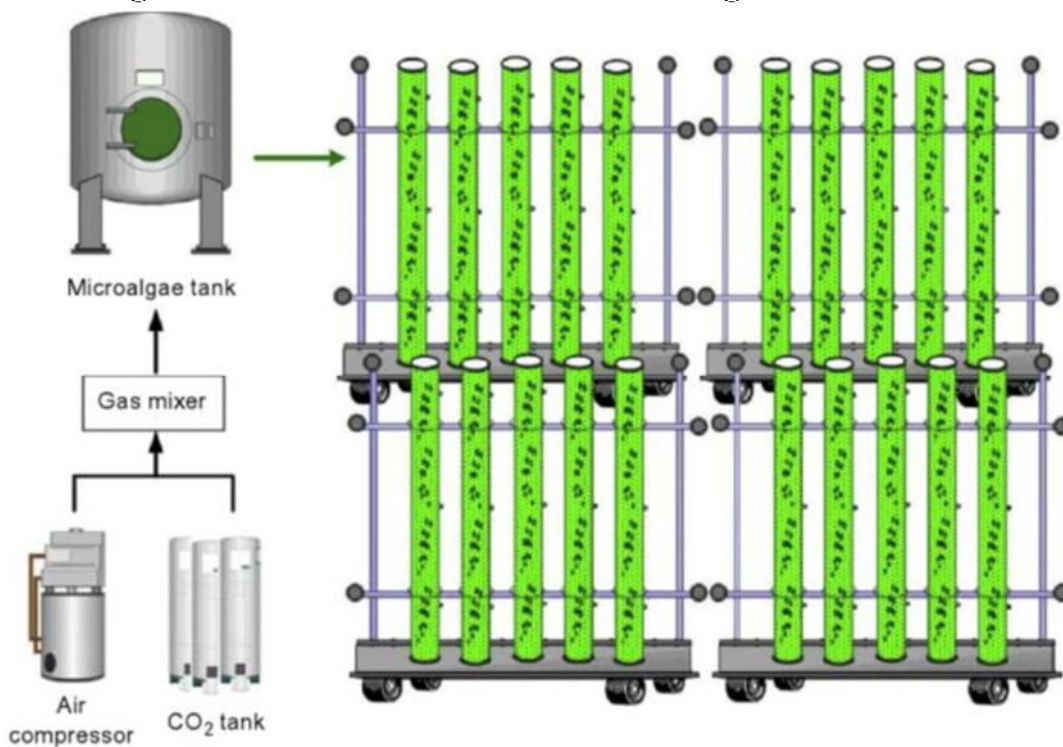


Figure 1: Controlled variables including the carbon source and light source can be controlled in this type of photobioreactor [7].

Tubular Photobioreactor

Tubular photobioreactors are long transparent tubes that pump microalgae through them, as shown in Figure 2. This allows for the exchange of carbon dioxide and oxygen between the liquid medium and the aeration gas [6]. These systems can use natural or artificial light [8].

Diagram of a Tubular Photobioreactor Labeling its Different Parts

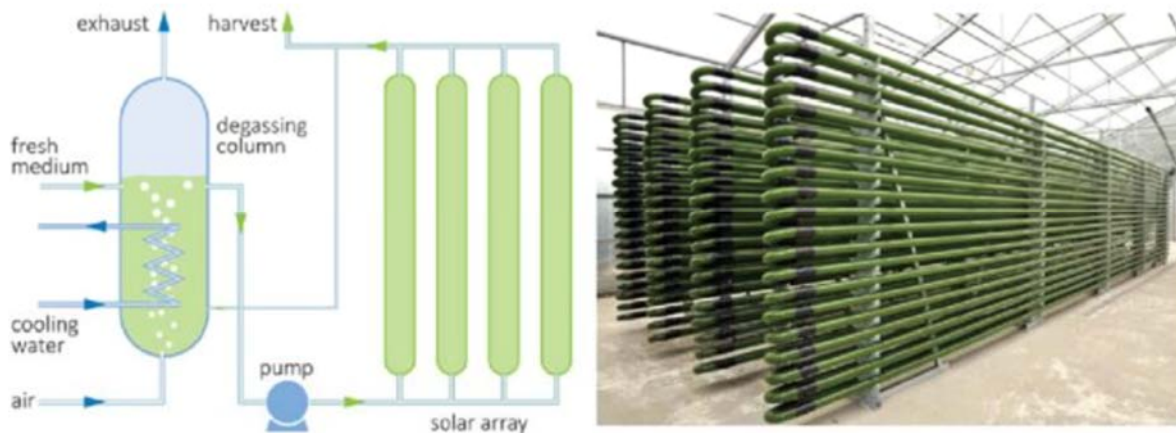


Figure 2: Fresh Spirulina, water, and air enter the tubes and are pumped through the system, and solar energy is able to enter the transparent tubes [8].

Flat-Panel Photobioreactor

Flat-panel photobioreactors promote higher photosynthetic efficiency due to more surface area and support higher growth densities [6]. Figure 3 shows the process of this type of cultivation.

Diagram of a Flat-Panel Photobioreactor Labeling its Different Parts

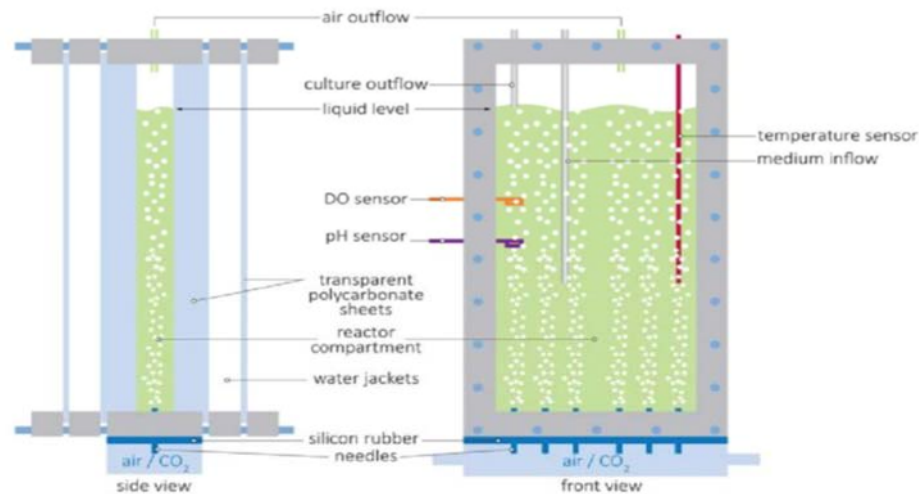


Figure 3: Spirulina cultivation in a flat-panel photobioreactor can include sensors for measuring temperature, pH, and medium flow [8].

Open Pond System

If Spirulina is not grown in a closed tank system, it can be grown in an open pond system. A raceway pond system is found in Figure 4. Approximately 98% of commercial microalgae is produced in open ponds [6]. It is the cheapest system for large-scale cultivation, and it doesn't compete with agricultural land because it can be built in areas with minimal crop production [6]. While open ponds are the cheapest and most commonly used, it is more difficult to control key factors for Spirulina growth such as temperature and pH [6]. This could be a problem for growing Spirulina in Ohio since warm temperatures do not occur year-round. Open ponds also have a higher risk of contamination, but because establishing the system is so cheap, it doesn't make a huge dent in profit if some batches of Spirulina are lost to contamination [9].

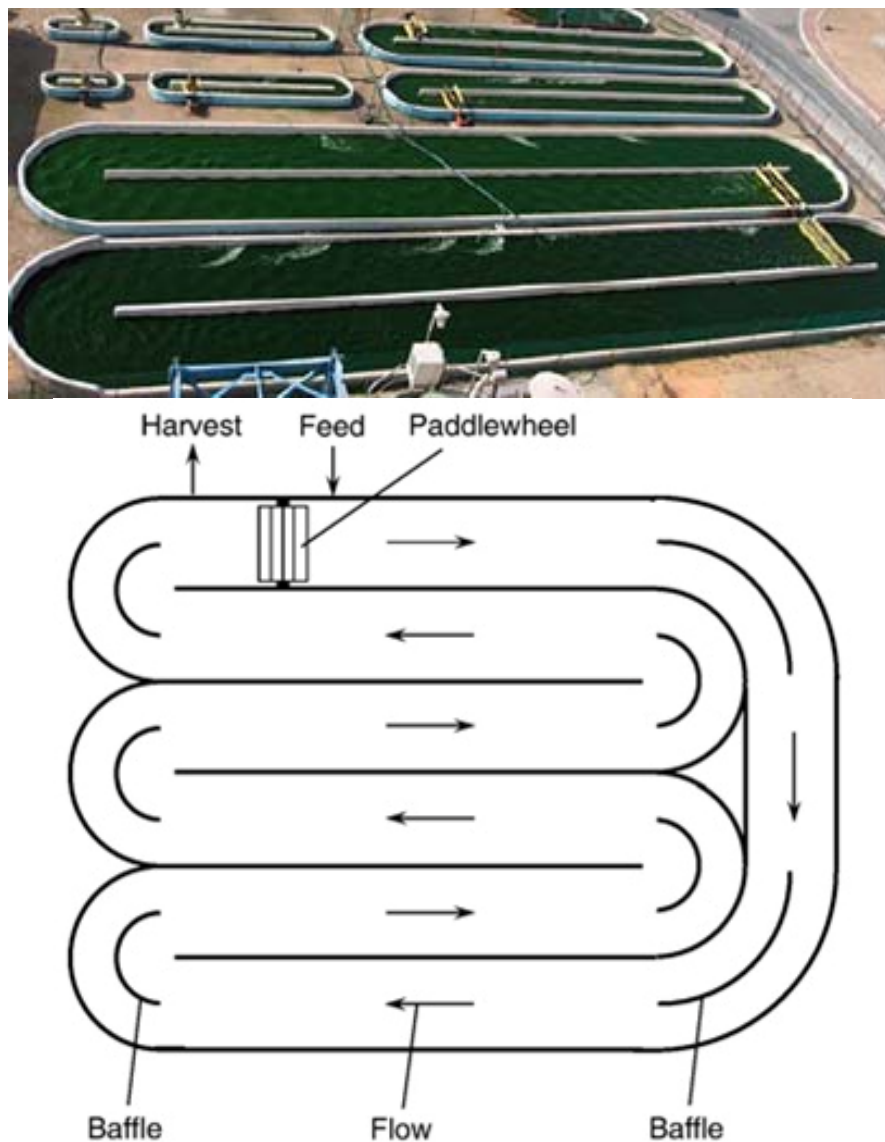


Figure 4: Raceway open pond system and diagram [9].

Heterotrophic Cultivation

Instead of growing Spirulina through photosynthesis, Spirulina can also utilize organic carbon for growth [6]. When growing microalgae at large scales, photosynthesis can be limited if there is not enough surface area for light to spread evenly throughout a batch, or there is simply not enough sunlight available. Because of this, heterotrophic cultivation is often the preferred method [6]. Benefits of this method include cultivation procedure control, elimination of light intensity, and low-cost biomass harvesting [6]. However, heterotrophic cultivation still needs to be further researched due to issues including limited types of microalgae that can grow with this method, little research on technology that uses lignocellulose and glycerol derived glucose, and a lack of sequestering of carbon dioxide from the atmosphere [6]. Heterotrophic cultivation uses the same systems such as open and closed, but they do not need direct sunlight.

Mixotrophic Cultivation

Spirulina exhibits mixotrophic metabolism, which means that it does not depend entirely on photosynthesis because light or organic matter can be used for growth [6]. In this type of cultivation, photosynthesis can take place when light is available, or aerobic respiration can occur when an organic carbon source is available [6]. The media supplemented during the dark phase with no light source can make a big impact on Spirulina growth [6]. No studies have been done to observe if fatty acids or antioxidants are affected by this system, which are two important elements when using Spirulina for food and pharmaceuticals [10].

Summary

After evaluation of the different cultivation methods for Spirulina, the best method to use is a phototrophic tubular photobioreactor in the winter and a raceway pond system in the summer. The tubular photobioreactor allows for a controlled environment where temperature, pH, and nutrient and water addition can be monitored easily. It also takes up less land and allows the Spirulina to have more access to light and nutrients since it is constantly moving throughout the tubes. The raceway pond system is the cheapest when it comes to installation and can make up for profit loss from the expense of the photobioreactor installation because of large biomass production. While it is more difficult to control temperature, pH, and contamination, the profit made from this system is so large that these factors do not make a big impact. It also does not compete with agricultural land use.

IMPACT OF UNSUSTAINABLE FARMING METHODS OF SOY AND ALFALFA

Soybeans and alfalfa use a significant number of resources compared to Spirulina. Figure 5 compares land and water use among soy, alfalfa, and Spirulina. Soy and alfalfa negatively affect the planet compared to Spirulina production.

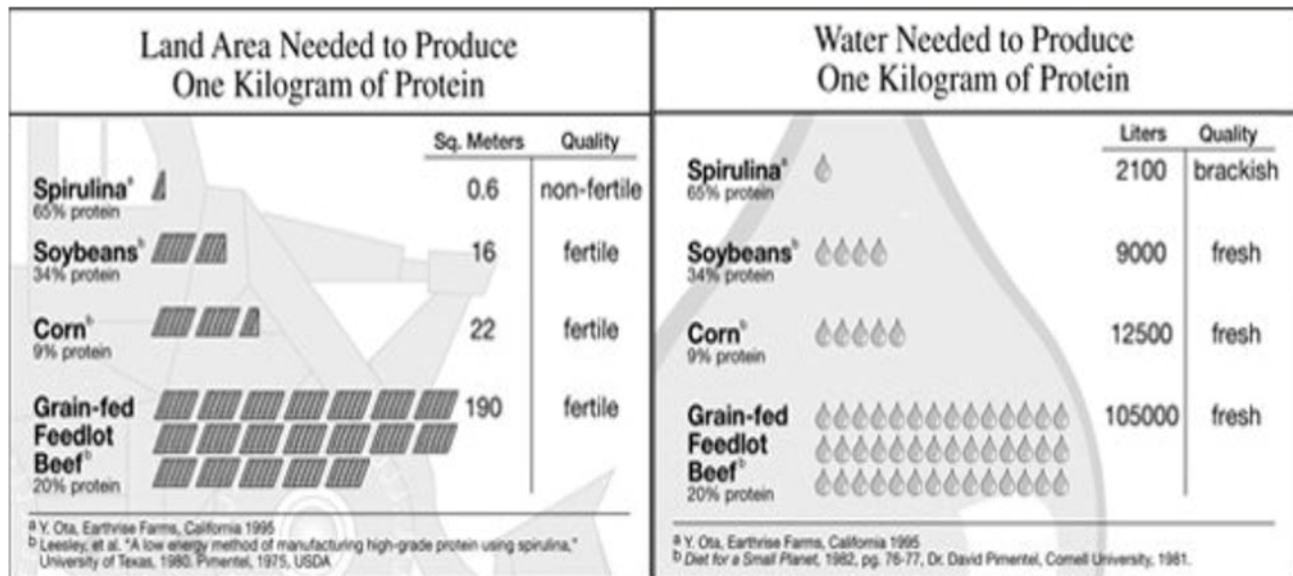


Figure 5: Water and land use comparison among soy, alfalfa, Spirulina, and beef production [11].

Soybeans

Soybean production is booming in the United States. In fact, an estimated 4.4 billion bushels of soybeans were produced in 2021 [12]. This production showed an increase of 200 million bushels compared to 2020 [12]. Unfortunately, the increase in soybean production not only in the U.S. but worldwide is negatively affecting the planet. According to the World Wildlife Fund, “The soybean industry is causing widespread deforestation and displacement of small farmers and indigenous peoples around the globe” [13]. With land being destroyed by these crops, agriculture could be in serious danger without any sustainability efforts taking place.

Alfalfa

Alfalfa has many factors that actually protect cropland. These include improving soil quality due to atmospheric nitrogen fixation and having a perennial crop cycle that ranges three to four years [14]. However, it does use a significant amount of land for production, varying between 672,300 and 717,000 hectares [14]. It also needs a significant amount of water to grow as well. Mechanized field operations, like turning of hay and swathing, make the biggest environmental impact because they affect the soil [14].

Summary

Soy and alfalfa both have a negative effect on the environment. While alfalfa can go through the process of nitrogen fixation, it still uses a large amount of land that is damaged by current farming practices. Furthermore, Soy is a contributor to global deforestation and farmer displacement.

COST ANALYSIS AND COMPARISON OF SOY, ALFALFA, AND SPIRULINA PRODUCTION AND REVENUE

To compare costs between soybean, alfalfa, and Spirulina production, I evaluated the costs of average sized farms and most common farming methods for soy and alfalfa production in the United States. I also evaluated the cost of both the tubular photobioreactor system and the open pond system for Spirulina.

Spirulina

Tubular Photobioreactor Costs

To estimate the cost of a tubular photobioreactor system, I researched the cost of a system located in Almeria, Spain. It includes ten tubular photobioreactors, each being 400 meters long and with a diameter of 0.09 meters [15]. A bubble column, with a 0.4-meter diameter and 3.5-meter height, is also included for degassing and heat exchange [15]. For this system, the expected biomass productivity is as follows [15]:

- 1) 0.42 g/L day at 0.34 1/day dilution rate
- 2) 30 cubic m culture volume
- 3) 3.83 tons/year biomass production

Certain equipment is needed for the tubular system. The necessary equipment includes a Spirulina medium, a process for sterilization, two air blowers, ten photobioreactors, a harvest storage tank, a decanter, a harvest pump, a carbon dioxide supply unit, and a dryer and storage unit [15]. Table 1 lists each mechanism and the average cost in U.S. dollars, which was converted from Euros using an online converter [16]. The total equipment cost sums up to about \$359,936.37.

Item	Cost (\$)	Number of Units	Total Cost (\$)
Spirulina Medium	6,485.34	1	6,485.34
Sterilization Process	16,213.35	1	16,213.35
Air Blower	2,702.23	2	5,404.46
Photobioreactor	16,213.35	10	162,133.50
Harvest Storage Tank	540.44	1	540.44
Decanter	48,640.05	1	48,640.05
Harvest Pump	1,080.89	1	1,080.89
C02 Unit	540.44	1	540.44
Dryer and Storage	118,897.90	1	118,897.90
Total			359,936.37

Table 1: Cost of equipment needed for a tubular photobioreactor [15].

Along with equipment costs, installation and labor also needs to be taken into consideration. Table 2 gives the cost for each factor of installation, including instrumentation, piping, land, engineering and supervision, etc. The total installation cost is approximately \$696,389.44.

Item	Factor	Cost
Installation	0.20	75,250.82
Instrumentation and Control	0.15	56,438.12
Piping	0.20	75,250.82
Electrical	0.10	37,625.41
Yard Improvements	0.12	45,150.49
Land	0.06	22,575.25
Engineering and Supervision	0.30	112,876.24
Construction Expenses	0.05	42,422.65
Contractor's Fee	0.03	21,211.33
Contingency	0.08	81,997.06
Depreciation	1.00	106,180.98
Property Tax	0.01	1,061.82
Insurance	0.006	637.09
Purchase Tax	0.16	17,711.36
Total		696,389.44

Table 2: Installation costs of a tubular photobioreactor [15].

For actual production cost, factors to take into consideration include fertilizers, carbon dioxide, water, power consumption, labor, and maintenance. Table 3 shows costs for each factor in U.S. dollars. The total is approximately \$108,116.14.

Item	Cost (\$)	Number of Units	Total Cost (\$)
Fertilizers (kg)	2,688.00	0.43	1,155.84
Carbon Dioxide (kg)	8,835.00	0.3	2,650.50
Water (m3)	3,070.00	0.1	307.00
Power Consumption (kWh)	134,460.00	0.1	13,446.00
Maintenance (MEC)	13,920.00	0.04	556.80
Labor	30,000.00	3	90,000.00
Total			108,116.14

Table 3: Production costs of a tubular photobioreactor [15].

The sum of total costs is approximately \$1,164,441.95.

Open Pond Raceway System Costs

To observe the average cost of production of an open pond system, I will discuss costs of a farm with 20 ponds measuring 10 x 20 feet [17]. Table 4 shows all production costs of an open pond raceway system. It was difficult to find specific costs in the United States, but information for open pond production was found from India. Costs were converted from Indian Rupees [17] to U.S. dollars using an online converter [18]. The approximate cost is \$16,655.02. Taxes and insurance costs could not be found but should be taken into consideration. Overall cost will be higher with taxes and insurance included than the given total below.

Items	Cost (\$)	Number of Units	Total Cost (\$)
Pond	654.42	20	13,088.40
Plant Machinery	196.33	1	196.33
Lab Equipment	65.44	1	65.44
Water Treatment Plant	1,963.28	1	1,963.28
Piping	327.21	1	327.21
Electrical Work	196.33	1	196.33
Drying Screens	130.89	1	130.89
Harvesting Screens	65.44	1	65.44
Packing Materials	32.72	1	32.72
Chemicals Per Month	26.18	1	26.18
Monthly Operational Cost	327.21	1	327.21
Monthly Labor	235.59	1	235.59
		Total	16,655.02

Table 4: Production costs of an open water system [17].

Revenue

While equipment and production costs are high, extra biomass can be sold to other companies for human protein consumption and pharmaceuticals, and other waste products can be turned into biofuel [3], which will bring in even more profit to Kalmbach Feeds, Inc. A company can make a significant amount of profit off of Spirulina production. Cyanotech, a world leader in Spirulina production that is located in Kailua Kona, Hawaii, released their financial results for the 2021 fiscal year. They made \$2.4 million in cash from operations [16]. They also reported net sales of \$32,345,000 for 2021 compared to \$31,899,000 in 2020 [16]. With Spirulina costs per kilogram increasing, Cyanotech had a Gross profit of \$11,117,000 with a gross profit margin of 34.4% compared to \$12,700,000 with a 39.8% gross profit margin in the previous year [16]. Net income was \$920,000 or \$0.15 per diluted share [16].

Soy

Birgit Meade, Estefania Puricelli, William McBride, Constanza Valdes, Linwood Hoffman, Linda Foreman, and Erik Dohlman conducted research on corn and soybean production cost and exports in Argentina, Brazil, and the United States in 2010. National average yield in the United States was 44.6 bushels per planted acre, national average enterprise size was 303 planted acres per farm, and national average price was \$11.55 per bushel [19]. The Gross value of production was \$515.22 per planted acre [19]. After I found the following costs of soybeans in the United States: operating costs including factors such as seed, fertilizer, chemicals, and more had a national average of \$131.90 per planted acre [19]. Table 5 lists specific operation factors and their prices for a 303 planted acre farm.

Item	Cost (Dollars per Planted Acre)	Cost for 303 Planted Acres (\$)
Seed	59.20	17,937.60
Fertilizer	17.87	5,414.61
Chemicals	17.04	5,163.12
Custom Operations	7.23	2,190.69
Fuel, Lube, Electricity	16.81	5,093.43
Repairs	13.46	4,078.38
Purchased Irrigation Water	0.16	48.48
Interest on Operating Capital	0.13	39.39
Total	131.90	39,965.70

Table 5: Operation costs for 303 planted acres of soybeans [19].

Allocated overhead, which includes labor costs, insurance and taxes, and equipment and machinery had a national average of \$232.19 per planted acre [19]. Table 6 shows each item for allocated overhead and the cost.

Item	Cost (Dollars per Planted Acre)	Cost for 303 Planted Acres (\$)
Hired Labor	2.11	639.33
Unpaid Labor Opportunity Costs	17.33	5250.99
Capital Recovery of Machinery/Equipment	78.18	23688.54
Opportunity Cost of Land	110.30	33420.90
Taxes and Insurance	9.41	2851.23
General Farm Overhead	14.86	4502.58
Total	232.19	70353.57

Table 6: Allocated overhead costs for 303 planted acres of soybeans [19].

The total national average cost is \$364.09 per planted acre [19], or \$110,319.27 for 303 planted acres of soybeans in the United States.

Revenue

With the Gross value of production being \$515.22 per planted acre, a 303-acre farm would have a gross revenue of approximately \$156,111.66.

Alfalfa

Many steps must be taken in the planting and harvesting of alfalfa. Laser leveling costs \$175 per acre. twenty-five pounds of seed per acre is generally used, with seed costing about \$5.84 per pound [20]. Prices for water vary each year depending on conditions in different areas. The estimated average cost is \$130 per acre-foot [20]. Harvesting services including swathing, raking, and baling costs approximately \$40.10 per ton [20]. Labor costs are as follows: \$20.44 per hour for machine operators and \$17.52 for general labor [20]. Cropland with water suitable for alfalfa ranges anywhere between \$15,000-\$25,000 per acre [20]. Other costs to take into consideration are offices, insurance, taxes, and equipment operation. The average total cost for an acre of alfalfa is \$48,133 per acre [20]. Table 7 represents all factors and costs of alfalfa farming. For a 160-acre alfalfa farm, the total cost would be \$7,694,864.

Item	Cost (\$)	Number of Units per Acre	Total Cost (\$)	Cost for 160 Acres (\$)
Laser Leveling	175 per acre	1	175.00	28,000.00
Seed	5.84 per pound	25 pounds	146.00	23,360.00
Water	130 per acre-foot	1	130.00	20,800.00
Labor	37.96	1	37.96	6,073.60
Cropland	25000	1	25,000.00	4,000,000.00
Offices, Insurance, taxes, Equipment Operation	22603.94	1	22,603.94	3,616,630.40
Harvesting	40.10 per ton	1	40.10	6,416.00
Total			48,133.00	7,694,864.00

Table 7: Total costs for 160 acres of alfalfa crops [20].

Revenue

According to Keith Johnson and David Petritz, researchers for the Department of Agricultural Communication at Purdue University, “A selling price of over \$60 per ton and a yield of six tons per acre are required for a positive return to land” [21]. They also state that the alfalfa must be at its highest quality and marketed at top prices for it to be a feasible alternative crop [21]. Because this crop is extremely unpredictable from year to year, the researchers discuss that “It is therefore nearly impossible to predict accurately the revenue side of the alfalfa budget” [21].

Summary

Soybean production is cheap, but revenue is not nearly as high compared to that of Spirulina. Equipment and production costs are high for Spirulina production in a tubular photobioreactor. However, revenue can also make up for money lost to production system installation. Open pond systems can be installed at low cost and can bring a great profit to Kalmbach Feeds, Inc. in the summer. Alfalfa is very expensive, and with revenue being so uncertain from year to year, it is a risky crop to depend on as a protein source for horse feed. Spirulina is the most cost effective for production because while it is expensive to install the systems, the overall biomass produced, and estimated revenue will go far beyond breaking even on production costs.

NUTRIENT ANALYSIS AND COMPARISON

It is essential that horses get quality nutrients as well as a significant amount of protein in their diet. They also need to be able to digest every ingredient easily to avoid complications. To find which plant protein source had the highest protein and nutrient content, I found the nutritional information on soy, alfalfa, and Spirulina and compared them.

Spirulina

Spirulina is very nutrient dense, containing 50-70% protein with all essential amino acids [22]. The least represented amino acids are methionine and cysteine, but they are still present by more than 80% of the ideal level stated by the Food and Agricultural Organization [22]. Spirulina cells also do not contain cell walls. Instead, they have a fragile layer of murein, which makes it extremely easy to digest [22]. No cooking or treatment is needed to increase protein availability because of the easily breakable murein layer [22]. There are also many fatty acids found in Spirulina. Table 8 lists the fatty acids present and their percent concentration.

Distribution of fatty acids in two strains of <i>spirulina</i>		
Fatty acids	<i>Spirulina maxima</i> (% of total fatty acids)	<i>Spirulina platensis</i> (% of total fatty acids)
Palmitic (16:0)	63	25.8
Palmitoleic (16:1 omega-6)	2	3.8
Stearic (18:0)	1	1.7
Oleic (18:1 omega-6)	4	16.6
Linoleic (18:2 omega-6)	9	12
Gamma-linolenic (18:3 omega-6)	13	40.1
Alpha-linolenic (18:3 omega-3)	traces	traces

Table 8: Fatty acid content in two different strains of Spirulina [22].

Spirulina contains 15-25% carbohydrates [22]. It also has Vitamin A, Vitamin B with a full list found in Table 9 below, Vitamin C, Vitamin E, Vitamin K, Folate, Biotin, Panthothenic acid, Inositol [23], and Vitamin B12, which can be very hard to obtain in a meatless diet [22].

Vitamin	Content (mg/kg)	Daily Requirement (mg)
B1	34-50	1.50
B2	30-46	1.80
B6	5.0-8.0	2.00
B12	1.5-2.0	0.00
Niacin	130.00	20.00
Folate	0.50	0.40
Pantothenate	4.6-25	6.0-10.0
Biotin	0.05	0.1-0.3
C	traces	15-30

Table 9: Vitamin content of Spirulina and adult daily Vitamin requirements [22].

Along with vitamins, Spirulina is packed with minerals and trace elements. Table 10 lists all of the minerals found.

Minerals	Content in <i>spirulina</i> (mg/kg)	Required adult daily dose (47) (mg/day)
Calcium	1300-14000	1200
Phosphorus	6700-9000	1000
Magnesium	2000-2900	250-350
Iron	580-1800	18
Zinc	21-40	15
Copper	8-10	1.5-3
Chrome	2.8	0.5-2
Manganese	25-37	5
Sodium	4500	500
Potassium	6400-15400	3500

Table 10: Mineral content of Spirulina and adult daily mineral requirements [22].

Soy

Soybeans contain 37-42% protein [24]. However, it is relatively low in cysteine and methionine. The protein in soy is responsible for initiating allergic responses in susceptible people [24]. Soy also contains lectins. When consumed raw, lectins cause alterations in the small bowel and

negatively affect growth in animals [24]. Soy has protease inhibitors, which can reduce protein hydrolysis and small bowel digestion [24]. Soybeans contain 31% fat. A complete list of fatty acids can be found in Table 11 below.

Fatty Acid	Content (mg/95g)
Hexadecanoic acid	2561
Octadecanoic acid	862
Tetradecanoic acid	67
Hexadecenoic acid	67
Octadecenoic acid	5262
Octadecadienoic acid	12012
Octadecatrienoic acid	1609

Table 11: Fatty acid content of soy [24].

Soy has multiple vitamins including 1% Vitamin A, 8% Vitamin B1, 11% Vitamin B2, 8% Vitamin B3, 15% Vitamin B6, 50% Vitamin B9, 2% Vitamin C, 4% Vitamin E, and 40% Vitamin K [24]. It does not contain Vitamin B12 or Vitamin D. A full list of each vitamin is found in Table 12.

Vitamin	Content (mg/95g)
Beta Carotene	0.114
Thiamin	0.095
Riboflavin	0.138
Niacin	1.339
B6	0.198
Folate	0.20045
C	2.1
E	0.86
Alpha Tocopherol	0.86
K	0.0479
K1	0.0479
Choline	118.1

Table 12: Vitamin content of soy [24].

Soy is also full of minerals, which are listed below in Table 13.

Mineral	Content (mg/95g)
Calcium	131.10
Copper	0.79
Iron	3.71
Magnesium	137.75
Phosphorus	344.85
Potassium	1396.50
Selenium	18.15
Sodium	154.85
Zinc	2.98

Table 13: Mineral content of Soy [24].

Alfalfa

Good quality alfalfa hay has high protein, vitamins, minerals, and is easily digestible for horses [25]. The crude protein content is 18-24.8% and contains all essential amino acids [26]. Table 14 lists amino acids and their percent content found in alfalfa hay. Rare amino acids including citrulline and canavalia can be found in alfalfa hay as well [26], but no percent content for these amino acids could be found.

Amino Acid	Content (%)
Methionine	0.32
Lysine	1.06
Valine	0.94
Threonine	0.86
Phenylalanine	1.27

Table 14: Amino acid content found in alfalfa hay [26].

Alfalfa hay also contains 2.4% crude fat, 35.7% crude fiber, and 8.9% crude ash that contains 1.09% calcium and 0.3% phosphorus [26].

Summary

Spirulina is the best choice for highest protein consumption. It also has the best vitamin and mineral content. It is easily digestible compared to soy, and it does not cause any known digestibility issues.

FDA RULES AND REGULATIONS

The FDA does contain rules and information on procedures for adding new ingredients to food and feed. It also discusses and proves that Spirulina is a generally recognized as safe (GRAS) food that can be used for human and animal consumption.

FDA Food and Safety Modernization Act (FSMA)

In 2011, Congress passed this act to strengthen the food safety system and shift from responding to food contamination to preventing food contamination [27]. The FSMA, title I, section 113 discusses procedures for new dietary ingredients. Also found in this title are section 105, standards for produce safety, section 103, hazard analysis and risk-based preventive controls, and section 104, performance standards. Title II explains procedures for detecting and responding to food safety problems.

Food, Drug, and Cosmetic Act (FD&C Act)

The FD&C Act [28] is found in the *Code of Federal Regulations* (CFR) and requires that any substance added to or that is expected to become a component of animal food must be used in accordance with a food additive regulation unless it is generally recognized as safe (GRAS) for that intended use [29]. GRAS, found under section 201(s) and 409 of the FD&C Act, is a term used to clarify foods that are safe for human and animal consumption [29]. For a food to be considered GRAS, it must go through scientific procedures or through experience based on common use in food for foods consumed in the years before 1958 [29].

Generally Recognized as Safe (GRAS) Notices 000417 and 000424

These documents prove the safety of consumption among humans and animals of Spirulina or *Arthrospira platensis* (Please see Appendix for these specific documents).

CONCLUSIONS

This report is a complete product of my research. My research was on the feasibility of replacing soy and alfalfa protein with Spirulina protein in horse feed. This report contains information on sustainable Spirulina growing methods, impacts of unsustainable farming methods of soy and alfalfa, a cost analysis and comparison of production and revenue of Spirulina, soy, and alfalfa, a nutrient analysis comparison, and FDA rules and regulations for food and feed ingredients. By completing my research, I have made the following conclusions:

- 1) The best cultivation methods for Spirulina are to use a tubular photobioreactor system in the winter and an open pond system in the summer.
- 2) Soy and alfalfa contribute to deforestation and soil and land damage and use more resources for production than Spirulina.

- 3) Spirulina is the most cost effective because of its high estimated revenue and biomass production.
- 4) Spirulina is the best choice for highest protein consumption. It also contains large amounts of vitamins and minerals and is easily digestible.
- 5) FDA rules and regulations will need to be followed for new added ingredients. There is proof that Spirulina is considered a GRAS.

RECOMMENDATIONS

By completing my research, I have developed recommendations for Tribute Superior Equine Nutrition. These recommendations will lead our company in the direction toward a sustainable and nutrient dense horse feed. Moreover, it will put Tribute Superior Equine Nutrition ahead of the race toward a better environment while providing the best quality horse feed for our customers. The recommendations are listed below:

- 1) Tribute Superior Equine Nutrition should implement Spirulina protein into our horse feed.
- 2) Tribute Superior Equine Nutrition should install both a tubular photobioreactor and an open pond system into our facilities to produce our own biomass and create an opportunity for profit.

STEPS TO IMPLEMENT SPIRULINA PROTEIN INTO HORSE FEED

To implement Spirulina protein into our horse feed, the following steps need to be taken:

- 1) An engineer needs to be contacted for a consultation on overall costs of installation of the tubular photobioreactor and open pond system.
- 2) After installing the systems, training needs to take place on how to properly cultivate Spirulina.
- 3) Small amounts of Spirulina should be added to current feeds to test overall health benefits and taste.
- 4) A final Spirulina feed can be made, or Spirulina can be added to all other feeds in place of soy and alfalfa.

APPENDIX: GENERALLY RECOGNIZED AS SAFE (GRAS) DOCUMENTATION FOR SPIRULINA

Material Selected from the U.S. Food and Drug Administration GRAS Notice Inventory [27].

<u>Subject</u>	<u>Page</u>
Generally Recognized as Safe Notice 000417.....	22
Generally Recognized as Safe Notice 000424.....	23

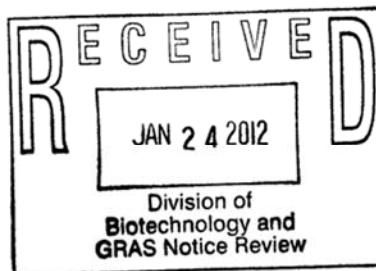


Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835

January 23, 2012

ATTN: Dr. Antonia Mattia, PhD

Our Reference: GRAS Notification and Exemption Claim for Certified Organic Spirulina



Dear Dr. Mattia,

AIBMR Life Sciences, Inc. has been retained as an agent by E.I.D. Parry (India) Limited, Parry Nutraceuticals Division ('the Notifier') to submit a GRAS notification to the FDA for Certified Organic Spirulina, a powdered preparation of organically grown *Arthrospira platensis* to be used as an ingredient in the enclosed specified categories of food.

Please find enclosed three copies of the notification *Notice to US Food and Drug Administration that the use of Certified Organic Spirulina (Arthrospira platensis) is Generally Recognized as Safe*. As stated in the exemption claim, the data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Parry Nutraceuticals Division, E.I.D Parry (India) Ltd., "Dare House" No.234, N.S.C Bose Road, Chennai – 600 001, India; or will be sent to FDA upon request.

Parry Nutraceuticals (the notifier), has determined that Certified Organic Spirulina is Generally Regarded as Safe (GRAS), consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act. This determination has been made based on scientific procedures. Spirulina has a long history of human consumption and has been thoroughly researched in toxicological models, with no results prompting concern for safety. It has been recommended as a food for human consumption by governmental agencies. In summary, the use of Certified Organic Spirulina in the enclosed specified categories of food is exempt from the requirement of pre-market approval.

Yours sincerely,

John R. Endres
Chief Scientific Officer
AIBMR Life Sciences, Inc.
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Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835

March 12, 2012

ATTN: Dr. Antonia Mattia, PhD

Our Reference: GRAS Notification for CyaninPlus™

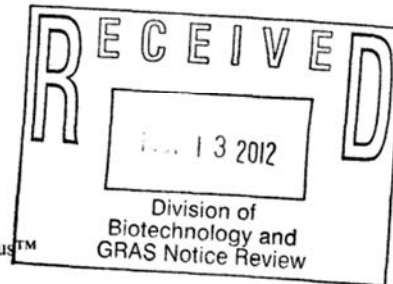
Dear Dr. Mattia,

Desert Lake Technologies, LLC is submitting the attached GRAS notification to the FDA for CyaninPlus™, which is intended for use as an ingredient in food. CyaninPlus™ is a water extract of Spirulina, containing greater than 30% c-phycoerythrin. The GRAS determination has been made based on scientific procedures, using safety assessments of Spirulina and c-phycoerythrin, and is corroborated by a long history of safe consumption of Spirulina. The water extraction method of manufacturing does not chemically alter the ingredient and batch analyses show that the product is made consistently and meets all specifications. Therefore the intended use of CyaninPlus™ is exempt from the requirement of pre-market approval, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act.

Please find enclosed three copies of the notification: *Notice to US Food and Drug Administration that the use of CyaninPlus™ is Generally Recognized as Safe*. Also enclosed are copies of all references cited in the notification. As stated in the exemption claim, the data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at our offices: Desert Lake Technologies, LLC, 3735 Washburn Way, Klamath Falls, Oregon 97603, USA, or will be sent to FDA upon request. Please do not hesitate to contact us with any questions.

Yours sincerely,

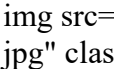
Howard Newman
Desert Lake Technologies, LLC
3735 Washburn Way
Klamath Falls, Oregon 97603
USA



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