Executive Summary:

DOE Award # EE0000393 was awarded to fund research into the development of beneficial uses of surplus algal biomass and the byproducts of biofuel production. At the time of award, Sapphire's intended fuel production pathway was a fairly conventional extraction of lipids from biomass, resulting in a defatted residue which could be processed using anaerobic digestion. Over the lifetime of the award, we conducted extensive development work and arrived at the conclusion that anaerobic digestion presented significant technical challenges for this high-nitrogen, high-ash, and low carbon material. Over the same timeframe, Sapphire's fuel production efforts came to focus on hydrothermal liquefaction. As a result of this technology focus, the residue from fuel production became unsuitable for either anaerobic digestion (or animal feed uses). Finally, we came to appreciate the economic opportunity that the defatted biomass could represent in the animal feed space, as well as understanding the impact of seasonal production on a biofuels extraction plant, and sought to develop uses for surplus biomass produced in excess of the fuel production unit's capacity.

Following rescoping of the project to replace capital intensive anaerobic digestion work with operations-intensive animal study work to be completed at Eastern New Mexico University, the project was focused on several phases of work:

- Analysis of intact as well as defatted biomass samples
- Formulation of biomasses of several representative types into cow, pig, and shrimp diets
- Conducting trials to determine suitability of these biomasses in representative animal diets
- Analysis of data to draw conclusions as to suitability of these algae, as processed, in animal diets

All use of vertebrate animals was conducted under supervision of the acting university's Institutional Animal Care and Use Committee.

Cattle trials

This residual biomass research experiment investigated multiple strains of algae in various forms, to determine the feasibility of utilizing it as a feed for cattle. We added to the knowledge base through the determination of the nutrient content of the residual biomass. How much can be included in the ration of cattle. We determined the proper drying and storage methods of the residual biomass as a feed. We contributed knowledge on the best method of feeding the residual biomass, including the best method of pelleting the residual biomass. Finally, we conducted a large feedlot experiment comparing the residual biomass against soybean meal, a common protein source for feedlot cattle. 2) The residual biomass is a byproduct from the algae biofuels process and while the true economic value of the byproduct for other uses may not be

completely determined. We have shown through our feeding experiments that it is a great alternative to soybean meal in a cattle ration and can be substituted equally. 3) This is extremely beneficial to the public, especially the all livestock industries, as protein is the most expensive feed ingredient in our animals' rations and residual biomass is another valuable protein source.

Comparison of Goals and accomplishments:

Our Goals were:

1) *To understand the nutritional composition of several types of algal biomass*
   We started the experiment using 3 different strains of algae in two different forms, dried or aqueous. Through a series of both chemical and biological experiments we were able to quantify the nutritional composition of each strain in either form. Ultimately, we continued the remainder of the experiment using the best strain (Spirulina).

2) *To examine the effect of the biomass on the growth and health of animals*
   Through a series of biological experiments including the last experiment in a commercial feedlot, we determined the residual biomass was as effective as soybean meal (a common protein source) on the growth of the cattle with no adverse health effects.

3) *To determine appropriate quantities of the biomass in animal diets*
   We conducted a series of live animal experiments that determined the optimal amount of residual biomass for inclusion into the diet. The experiments were conducted using step-wise amounts and growth and feed efficiency were calculated by treatment.

4) *To explore the effect of different storage and processing methods for the biomass*
   We investigated, though a series of experiments, two different drying methods (flash or freeze dried). Further, we conducted a pelleting experiment to determine the proper set-up of a pelleting machine as well as pellet carriers (ground corn of wheat). Finally, we conducted pellet durability tests over time to determine storage capabilities of the pellets.

5) *To determine the risk factors associated with the scale-up of the production process*
   From the beginning of the nutrient analysis experiments through processing and storage until a final commercial feedlot experiment we found no risk factors in utilizing residual biomass as a cattle feed.

6) *To understand the overall value of algal biomass as an animal feed*
   We determined over the entire experiment that an algal biomass is a safe and valuable protein source for a cattle feed. It can be a substitute for soybean meal (a common protein source) in equal parts with no detriment effects on the growth or health of the animal.

Summarized Project Activities:

We started with 6 samples of Algae, 3 dried and 3 aqueous. The three dried samples were analyzed for nutrient content including: normal nutrient content (protein, fat, fiber, amino acid content, etc.) as well as mycotoxins, heavy metals, and minerals. The three aqueous samples

were dried by one of two methods – flash dry (oven 64 degrees Celsius) or freeze dried. Both types of drying methods were analyzed as above. The protein results are presented in table 1.

Table 1. Protein Content Flash versus Freeze Dried

Algae strain	Flash frozen protein, %	Freeze dried protein, %
D	53.4	50.6
E	76.1	74.5
F	60.9	57.5

We used a conventional rooster model at West Virginia University to determine nitrogen corrected true metabolizable energy with good results except for strain A which was toxic to the birds and was removed from the experiment. Results are in table 2.

Table 2. Nitrogen corrected true metabolizable energy (TMEn) for six algae products determined using a conventional rooster model

Sample	**Crude Fat (%)**	**TMEn[1] (kcal/kg) ± SD[2]**
Algae A[*]	6.90	------
Algae B	1.80	3191 ± 29.90[e]
Algae C	4.68	4082 ± 24.85[b]
Algae D	6.32	3992 ± 30.16[c]
Algae E	5.35	4481 ± 39.55[a]
Algae F	0.20	3836 ± 35.69[d]
ANOVA P-value	-----	**< 0.0001**
SEM[3]	-----	12.25
Fisher's LSD[4]	-----	35.405

[1]True Metabolizable Energy corrected for nitrogen. TMEn can be calculated by the following equation:

TMEn = (A – B + C – D) / feed intake

A = total feed energy

B = total excreta energy of fed roosters

C = total excreta energy of fasted roosters

D = 8.22 X nitrogen balance

[2]Standard deviation

[3]Standard Error of the Mean

[4]Fisher's Least Significant Difference

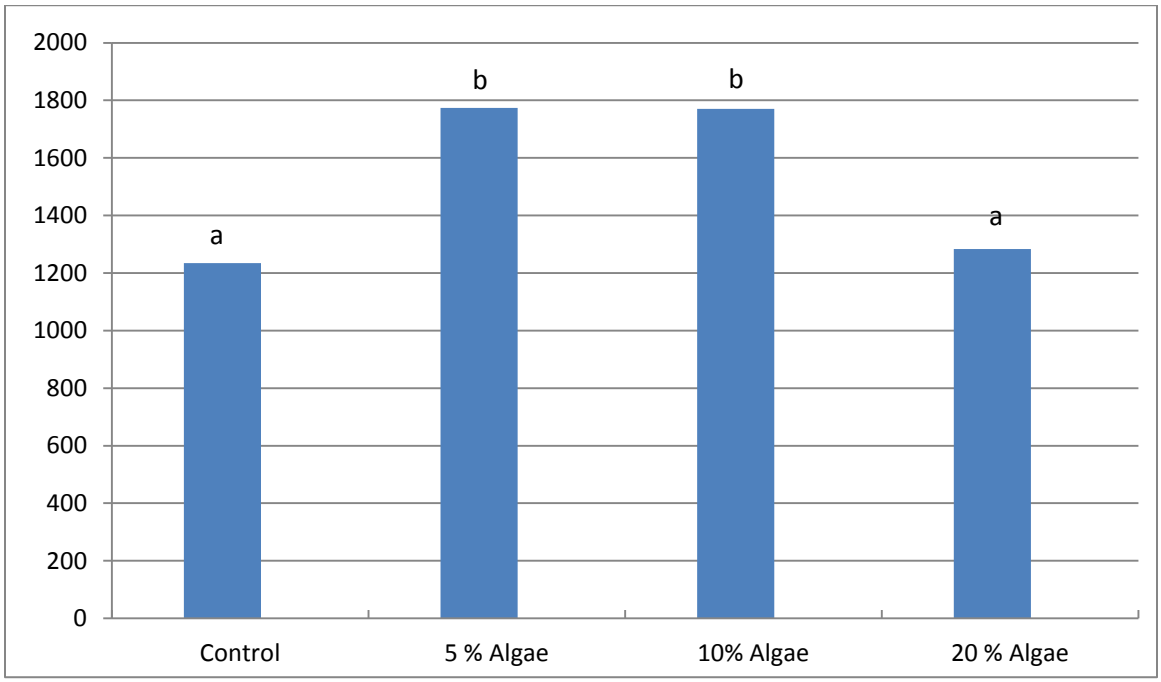
[a-e]Means within a column not sharing a common superscript differ (P < 0.05)

[*]TMEn was not calculated for Algae A due to the fact that roosters fed this product died prior to the completion of the study

Based on the previous results, strain E (Spirulina) was selected and ~125 lbs was dried from aqueous samples. We took the algae to the USDA-ARS center in Lubbock, Texas where we had

feed pellets made using Purina Growena (PG) as the base feed. Our treatments were; control, PG pelleted; 5% algae + PG; 10% algae + PG; 20% algae + PG. The intent was to determine how much algae we could include in a ration. Results were very promising as it appears the 5 and 10 % algae rations were actually an appetite stimulator over both the base feed and 20 % algae ($P<0.05$). Results are in Figure 1.

Figure 1 – Grams of Feed Consumed per Day



Based on the above results we can now formulate balanced rations to conduct growth experiments.

We received a shipment of 17,000 pounds of algae, following a complete nutrient analysis of the new algae, we conducted another 2 precision feeding experiments at West Virginia University (Table 3) and the results were used to formulate 4 beef rations.

Table 3. Nitrogen corrected true metabolizable energy (TMEn) for one algae product, corn, and a 50% mix of corn and algae determined using a conventional rooster model

Sample	TMEn[1] (kcal/kg) ± SD[2]	SEM[3]
Algae[*]	3,289 ± 41.90[c]	18.26
Corn	3,507 ± 40.18[b]	16.67
50% mix of corn and algae	3,785 ± 40.63[a]	16.67
ANOVA P-value	**< 0.0001**	---
Fisher's LSD[4]	2.14479	---

[1]True Metabolizable Energy corrected for nitrogen. TMEn can be calculated by the following equation:

TMEn = (A – B + C – D) / feed intake

A = total feed energy

B = total excreta energy of fed roosters

C = total excreta energy of fasted roosters

D = 8.22 X nitrogen balance

[2]Standard deviation

[3]Standard Error of the Mean

[4]Fisher's Least Significant Difference

[a-c]Means within a column not sharing a common superscript differ ($P < 0.05$)

*One outlier was removed from statistical analysis due to an obvious error in gross energy analysis

The rations were: Control; 2.33% Algae E Flash; 4.66% Algae E Flash; and 7.00% Algae E Flash. The rations were produced in a commercial feed mill in Lubbock, TX. Further, samples of the 4 feed rations were sent to Dr. Harold Harpster, The Pennsylvania State University, for *in situ* degradable and undegradable protein analysis (Table 4).

Table 4. In vitro[1] dry matter degradability (IVDMD)

Item	Wheat straw	Algae	Diet, 0% algae	Sample Diet, 2.74% algae	Diet, 5.49% algae	Diet, 8.24% algae	SEM	P-value[2]	(Contrast[3])
IVDMD, %	74.3[e]	99.2[a]	80.5[d]	87.4[b]	84.3[c]	85.0[c]	0.484	< 0.001	(< 0.001)

[1] In Vitro Digestibility was assayed using the DAISY[II] Incubator (ANKOM Technology Method 3). Samples were analyzed in triplicate.

[2] Main effect of treatment (sample).

[3] Orthogonal contrast: no algae vs. algae-supplemented diets.

[a,b,c,d,e] Means having different superscripts differ at $P < 0.05$.

In preparation for a large scale commercial feedlot feeding experiment we conducted a pelleting experiment at West Virginia University to determine the carrier and percent algae that could be added to a pellet. We determined a 60:40, algae to ground corn pellet was optimal for commercial production. A commercial pellet mill in Roswell, NM was used and 12,000 pounds

of algae pellets were produced. A new precision feeding experiment was conducted using the new pellets.

The large scale feedlot experiment was conducted in Melrose, NM in a commercial feedlot for 70 days. We had 8 pens of 30 steers in each pen. Four pens were control using soybean meal (SBM) as the protein source (the most common in feedlots) and 4 pens of treatment with algae pellets as a substitute for the soybean meal. All other components of the rations were identical. The steers were acclimated to the feed for 10 days and immediately following the 60 day feeding experiment was conducted. This experiment just ended so a complete analysis has not been conducted, but the preliminary data show:

The algae steers gained an average of 2.19 lbs. per day to the SBM steers 2.10 lbs. per day.

The algae steers had an average feed efficiency of 8.71 lbs. of feed per pound of gain to the SBM steers who had an average feed efficiency of 9.11 lbs. of feed per pound of gain.

In both gain and feed efficiency the algae appears to outperform the traditional SBM diet.

Shrimp study and production of biomass for swine study

Over the summer of 2014 our efforts in this area focused on production of biomass in support of swine and shrimp feeding trials, and initiation of the first shrimp feeding trials.

To enable the production of sufficient swine feed, we scaled *Tetraselmis marina* (SE416) to approximately 5000l scale, and conducted frequent centrifuge-based harvests to produce paste at approximately 30% solids. Control samples (~4kg) were lyophilized, and the bulk of the material (currently ~ 25kg) was tray dried by Aveka Inc. (Woodbury, MN) (figure 2).

Figure 2 Thermally dried Tetraselmis SE416

In addition to centrifuge based harvesting, a test batch of SE416 was harvested using flocculant, in a manner similar to Sapphire's dissolved air floatation. This material was also lyophilized.

After production and drying, materials were analyzed for nutrient composition (Tables 1&2). In the tables, sample number SE1117 is centrifuged *Tetraselmis*; SE11120 is flocculated *Tetraselmis*, and SE1119 is centrifuged *Nannochloropsis salina*, which is useful as a control biomass (and was also lyophilized).

Finally, a shrimp feeding trial was initiated and conducted at Texas A&M University's Port Aranses, TX facility under the supervision of Addison Lawrence. The trial had the following characteristics:

- Three types of algae in feed: Centrifuged *Tetraselmis*, DAF'd *Tetraselmis*, centrifuged *Nannochloropsis*
- Shrimp fed 0, 5, 10, 15, or 20% algae in diet
- Fish meal reduced from 13% to maintain total protein

The trial shows no negative impact, and potentially a positive impact, from replacement of fishmeal with the algae. Furthermore, there was no evidence of toxicity from the flocculant polymer present in sample SE1120; as algae concentration increased from 5 to 20% of the diet, performance continued to improve.

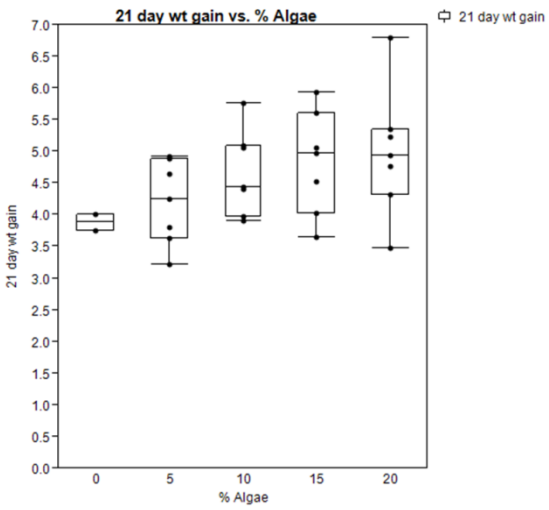


Figure 3 Shrimp trial results SE11120 (Tetraselmis)

Comprehensive swine study

The swine study was designed as three, 14 day grow-out studies with downtime in-between studies for cleaning to make maximal use of the 60 kg of algae available total (130 lbs), while allowing the opportunity to optimize diet parameters between phases.

Feed intake:

Feed intake was estimated to be approximately 6% of body weight. With pigs sourced at approximately 40 lbs, then feed intake during the 14 day experiment could be estimated to be 5 lbs. per pig per day. Thus during a 14 day experimental period one pig would consume approximately 70 lbs. of feed. A 4% algae inclusion would require 3 lbs. of algae per pig during a 14 day experiment.

Studies and algae needed based on theoretical replicatess and pig numbers:

Study 1 –

3 reps of two pigs per pen control – 0 lbs. of algae required
3 reps of two pigs per pen 1% algae – 4.5 lbs. of algae required
3 reps of two pigs per pen 2% algae – 9 lbs. of algae required
3 reps of two pigs per pen 4% algae – 18 lbs. of algae required

Study 2 –

5 reps of two pigs per pen control – 0 lbs. of algae required
5 reps of two pigs per pen 4% algae – 30 lbs. of algae required

Study 3 –

5 reps of two pigs per pen control – 0 lbs. of algae required
5 reps of two pigs per pen 4% algae – 30 lbs. of algae required

Total algae required for feeding would be 92 lbs. with approximately 38 lbs. to be used for feed loss during batching and pelleting.

Diet Formulations (Table 5):

Ingredient (%)	Control [a]	4% Algae Inclusion [a]
Corn	57.15	56.66
Soybean meal	33.79	31.62
Soybean oil	5.97	5.37
Algae	0.00	4.00
Limestone	1.05	0.67

Dicalcium phosphate	1.05	0.98
NB 3000	0.25	0.25
Salt	0.22	0.13
Lysine	0.18	0.19
Bicarbonate	0.17	0.00
Methionine	0.09	0.08
Threonine	0.07	0.08
Total	100	100
Calculated Nutrients		
Metabolizable Energy (kcal/lb)	1481.0	1481.0
Crude Protein %	20.90	20.90
Lysine %	1.15	1.15
Arginine %	1.27	1.27
Isoleucine %	0.86	0.87
Methionine %	0.37	0.37
Methionine + Cysteine %	0.65	0.65
Threonine %	0.74	0.74
Tryptophan %	0.25	0.26
Valine %	0.95	0.97
Calcium %	0.70	0.70
Phosphorus (Available) %	0.32	0.32
Sodium %	0.15	0.15

[a] All diet values were calculated by referencing Merck Veterinarian Manual Dietary Nutrient Requirements of Growing Pigs.

Measurements:

For all studies, pigs were weighed following a 1 wk facility acclimation period and then again at the end of the two week experimental period.

Feed intake was measured per pen of pigs.

Feed efficiency was calculated based on intake and gain.

Health was monitored throughout the experiment.

Study 1

Table 6. Overall comparison of 14 day grower pig performance

Treatment	ADG (kg)	ADFI (kg)	FE (G:F)
Control (No Algae)	0.956	1.782	0.537
1% Algae Inclusion	0.902	1.829	0.493
2% Algae Inclusion	0.864	1.781	0.485
4% Algae Inclusion	1.036	1.999	0.522
Overall P-value	0.21	0.06	0.41

Study 2

Table 7. Overall comparison of 14 day grower pig performance

Treatment	ADG (kg)	ADFI (kg)	FE (G:F)
Control (No Algae)	0.807	1.851	0.442
4% Algae Inclusion	0.862	1.770	0.489
Overall P-value	0.13	0.48	0.13

A total of 21 Duroc x Yorkshire-Landrace pigs (8-week-old) were obtained from Ohio State University. For this study, two diets were manufactured to contain either 0 or 4% algae. Pigs were randomly assigned to pens using a RCBD with 5 replications per treatment. Once again, the control diet (0% algae) was fed from d1-7, and dietary treatments were fed from d7-21.

All performance metrics were statistically similar among treatments. However, ADG and FE (G:F) were numerically increased for the 4% algae treatment. From a management standpoint, pens were more difficult to clean and a “more pungent” odor was noticed for the 4% algae treatments. All performance data would suggest that the algae by-product used in this study, up to 4%, could be utilized in swine diet formulation.

Study 3

Table 8. Overall comparison of 14 day grower pig performance

Treatment	ADG (kg)	ADFI (kg)	FE (G:F)
Control (No Algae)	0.856	1.471	0.583
4% Algae Inclusion	0.794	1.416	0.566
Overall P-value	0.18	0.71	0.73

Study 3 utilized the same experimental design and dietary treatments as Study 2. Once again, a total of 21, 8-week-old Duroc x Yorkshire-Landrace pigs were utilized with 5 replications per treatment.

All parameters were statistically similar, with ADG and FE (G:F) being numerically higher for the control treatment. Based on statistical performance these data also suggest that utilizing this specific algae product up to 4% in swine diet formulation is not detrimental.

Conclusions:

While the literature has a few sparse examples of algae being used in animal feed rations, there is very little quantitative dose-response data around the acceptable levels, and impacts if any of the algae on animal performance. Through the studies funded under this project, we have examined the performance of several fresh and saltwater algae strains in cattle diets and saltwater algae in both shrimp and swine diets, and developed a much greater understanding of limits of inclusion and animal performance. In any algae facility under temperate conditions (including anywhere in the US), productivity will be highly variable across seasons. At the same time, it is very challenging to reduce acreage under cultivation during peak seasons. Sizing the downstream fuel production plants to match peak productivity will necessarily result in underutilization and wasted capital over much of the year. Sapphire's approach through our biofuel program has focused on utilization of excess biomass and/or defatted biomass when available for high value purposes, such as animal feed. The work conducted under this project has significantly advanced our understanding of the economics and technical details of making such use of algal feedstocks.