Development of Omega 3 Rich Algae from Biodiesel Waste for Use as an Animal Feed Supplement

WSU-American Premix Technologies

Research & Technology Development Program – Phase I

CONFIDENTIAL
PROPOSAL
Project Title
Development of Omega 3 Rich Algae from Biodiesel Waste for Use as a Fish Feed Supplement

One Sentence Description
Optimize a process for converting biodiesel waste to algae biomass that is high in omega 3 fatty acids which in turn can become an additive into a new commercial animal feed

Project Manager
Name: Shulin Chen
Title: Professor
University: Washington State University
Department of Biological Systems Engineering
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Phone: 509-335 37 43
Fax: 509-335 27 22
Email: chens@wsu.edu
Website: http://www.bsyse.wsu.edu/aebe

Company Contact
Name: John Graff
Title: Manager
Company name: American Premix Technologies, Inc.
Mailing address: 20501 Hills Road E., Creston WA 99117
Phone: 509-636-2990
Fax: 509-636-2323
Email: werfeed@odessaoffice.com
Website: NA

Technology Area
Biotechnology/Waste Management

WTC Funds Requested
$40,000

Project Phase
Phase I

Company Cash
$8,000

Non-Disclosure Agreement
Check box if a company non-disclosure agreement was signed
Executive Summary

Background
This project addresses an emerging business opportunity created from the increasing demand for renewable fuel by converting the biodiesel waste into beneficial products. Biodiesel continues to be an alternative fuel of interest as the US struggles to find ways to disentangle themselves from an environmentally and economically unhealthy reliance on foreign oil. Presently, the US biodiesel production capacity hovers around 200 million gallons per year although Department of Energy projections suggest a potential for 1.1 and 2.7 billion gallon capacities by 2007 and 2016, respectively.

During the typical biodiesel production process oils and fats from oilseeds and the oleo-industry are added to methyl alcohol and alkaline catalysts to produce biodiesel, with glycerin and as a primary waste product. The mass conversion of this process is approximately described by: 100 units oil/fat plus 10 units alcohol to produce 100 units biodiesel and 10 units of glycerin. The results are that within a typical biodiesel factory, 7.35 pounds of oil/fat are converted into 1 gallon of biodiesel and 1 pound of crude glycerin (80-70% pure).¹ Current demand for glycerin is around 500 million pounds with uses in medicine, health, cosmetics, foods, and industry. The bulk sale price has been around $0.60-$1.00 with present prices at its lowest because of the availability of European biodiesel.²

Putting all of the above numbers together, a scenario is set up where, by 2007, one billion pounds of biodiesel glycerin could be introduced into a market that as of now can only handle half that amount. Clearly, then, the biodiesel industry will be in need of alternative uses for this waste glycerin.

WSU and our industrial partner, American Premix Technologies (APT), believe that we have a potential and commercially viable answer to this dilemma. We propose to take this crude glycerin waste as a carbon source and feed it to heterotrophic algae that are high in cellular omega-3 fatty acids contents. This algal biomass can then used as a supplement to an organic animal feed that WSU and APT are presently developing.

The heterotrophic algal culture system requires no light, only a cheap source of carbon, to produce a product that can transfer the nutritionally necessary omega-3 fatty acids to the animals and ultimately humans, both of whom can not synthesize these large carbon fatty acids by themselves. Put another way, an industrial and environmental liability is converted to a nutritional and marketable supplement for end-use health benefits.

Project Objectives
The goal is to develop an optimal process that utilizes biodiesel glycerin as a feedstock to produce an algae biomass that is concentrated in omega-3 fatty acids, particularly DHA. The algae will then be used as a feed additive for animals to extract the DHA into the product of the animal. Cultured fish will be the initial focus. Specific objectives include: (1) developing an optimal production process from glycerin; (2) pilot study of the production; and (3) using the algae as a feed additive for fish and testing the nutritional value of the meat. Additional preliminary tests will also be done by APT for palatability and performance on other production animals.

Resources Requested
It is proposed that WTC provide $40,000 to cover personnel, material and travel. APT will match WTC funds with $8,000 in cash and additional in-kind support.

Business and Commercialization Plan

Company Profile
American Premix Technologies, Inc. (APT) was founded in 2002 to develop and supply the Northwestern Livestock Feeding Industry with higher value livestock supplements and to assist in the utilization of locally grown feed products. It was originally founded by John and Teresa Graff who identified this region as lacking in commercial livestock support services and saw as need for better feeding techniques and naturally produced feed supplements.

The current owners of APT is Advanced Protein Technologies, Inc. this business was formed in 2004 to purchase APT and coordinate its livestock supplement business with advanced oilseed crushing interests, protein supplement developments, animal husbandry research, and bio-product development. Advanced Protein Technologies Inc. is made up of APT management and a small agriculturally based investment group. It currently employs 4.5 FTE employees and is poised to grow. It is currently completing construction of a 2 million dollar supplement manufacturing facility at Creston, WA. The staff is known throughout the state for its expertise in designing and producing high performing, natural rations and supplements and is an actively sought consultant with farmers regarding the impact of nutrition and environmental issues on animal health, fertility, and performance.

APT Management Team

John Graff, Manager of Technical Services: John was co-founder of APT and currently acts as the visionary behind the development of APT. He has over 10 years of experience as a college instructor and has both taught and wrote several college courses and curricula in such areas as cow/calf management, beef cattle nutrition, range management, feedlot management, advanced livestock nutrition. He also has worked for several feed companies and as a consulting nutritionist and is still a farmer at heart.

Jerry Emerson, Operations Manager: Jerry has years of experience as a logging manager prior to coming to APT to serve as their operations manager.

Brad Lyons, Sales Manager: Brad has marketing and sales knowledge from his years working as a manager for both a multinational feed ingredient company and later in his privately owned company.

Randy Ames, Process Manager: Randy has parlayed his ventures in water quality, soybean processing, extrusion design, and computer design into capabilities which make him instrumental in managing the construction, manufacturing, maintenance, and process development aspects of the business.

Related Projects with APT
APT is strongly associated with WSU on other biodiesel and aquaculture related projects and in addition are well connected with Columbia Oilseeds LLC, Washington State’s first oilseed crusher, Sustainable Systems, a Montana based biodiesel manufacturer, and Cornerstone Technology; a concentrated animal production sales and marketing venture. This consortium is working together to put together an effective research and commercial team that will facilitate the movement of Washington State oilseed crops to biodiesel, through to feed additives utilizing the seed meal and glycerin waste, and ultimately to the production of an aquaculture industry that is marketed in part by the healthy and safe protein rich meat made possible by the use of the omega-3 organic fish feed; as seen in the schematic below. The schematic below attempts to summarize the interactions between the research and respective business interests.
Market
There is a great market potential for the products resulting from the project. The omega-3 enhanced feed can be used virtually for all types of animal operations. Studies have shown that dairy cattle will gladly tolerate up to a 2% algae supplement to their diet with the desired omega-3 fatty acids capable of being transported across the rumen into the milk and meat supply. With the large dairy and cattle industry in Washington State, there is more than enough of a market to meet the algae-omega-3 production capabilities.

Of particular note, though, is the possibility of inclusion of algae as a supplement to the feeds of other animals, especially farm-raised fish. In 2001, 819 million pounds of aquaculture fish were grown in the US yielding over $935 million in sales. These numbers are growing dramatically at an annual rate above 20%, making it according to the USDA Agriculture Statistics Service, “the fastest growing sector in US agriculture.” The growth is a result of worldwide declines in fish catch, concerns with toxins within the meat of the catch, and a need to supply a rapidly growing world with a ready source of protein and healthy fats.

The omega 3 algae produced in this process could be instrumental in meeting the unique needs of this burgeoning industry. The marketability of their aquaculture product relies on an ability to emphasize the healthy aspects of the meat. Unfortunately, concerns exist about the concentration of heavy metals even within farm raised fish (fish feed is in part fish waste from marine catches). Also, fish, although prized for their high omega-3 content, only obtain these fatty acids through a diet of algae, the primary producers of omega-3. Thus, farm-raised fish fed on an organic diet supplemented with enriched omega-3 algae could heighten the marketability considerably by removing the heavy metal concern while increasing the levels of omega-3 in the meat product.

Impact
The fabrication, installation, and maintenance of a glycerin-based, algae-omega 3-animal feed system will create jobs within Washington State. The jobs fall into several categories with reasonable total numbers over a 5-year period being 12. Numbers are based on the development of a 20 million gallon/year production supply in Washington State by 2007 which is well in line with DOE projections and production projections for potential biodiesel start-ups in the state. This biodiesel supply could result in roughly 300 tons of omega-3 algae annually to then be mixed into the feed supplies for the production animals. The commercial vision is a dedicated glycerin conversion facility built and located next to an existing seed crusher/biodiesel refinery complex that is located in rural Washington. The algae product would then by shipped to local feed lots for mixing and marketing.

- Fabrication/Construction/Management – 4 full time equivalents
- Production/FeedMix/Quality Control – 6 full time equivalents
- Marketing/Sales 2–full time equivalents

The above jobs require a high level of technical skill, especially for fabrication and maintenance personnel. Pay levels would range from $17 to $30 per hour.

The economic impact for Washington State goes well beyond the marketing and job
opportunities already described. Since utilization of the biodiesel waste leads to increased economic viability of the biodiesel industry as well reduction in the potential impact to the environment for the disposal of this waste stream, the project results become one related not to just biotechnology improvement but industry, security, and energy enhancement. Ultimately the development of an algae-omega 3-feed additive, nutraceutical industry in Washington State leads the state closer to realizing its vision for economic and agricultural sustainability; both of which will have long term and profound effects on the economy and jobs.

Intellectual Property
WSU is in the process of generating multiple patents in regards to the organic fish feed and novel recirculation systems.

Project Description
Problem statement
As discussed earlier, if the biodiesel industry takes off as the US DOE suggests, considerable amounts of glycerin waste will be produced that will far exceed the present demand for its uses. Thus, in order to energize the economic viability of the biodiesel industry, not to mention dealing with a potentially harmful industrial waste, new options must be found for utilization of the glycerin. The idea proposed here is to use the glycerin as a carbon feedstock to produce algae that are enriched in the omega 3 fatty acid; DHA (docosahexanoic acid). This essential fatty acid then could be incorporated into production animal feeds, including fish feed which is primarily being proposed here. Whatever the feed use, though, the end result is meat, milk, or eggs that are enriched in omega-3 fatty acids.

Why the need for omega-3 fatty acids? Scientific and clinical research have shown that heart, neurological, and infant diseases and most recently Alzheimers have all been linked to shortages of omega-3 fatty acids in the diet. The latest research shows that the most desired ratio of omega-3s to omega-6s (the other general classification of fatty acids available in a diet) is roughly 4 to 1. However, the typical American diet has this ratio drastically reversed with a ratio of around 20 omega-6s to every omega-3! Although an imbalance in the fatty acid profile often does not lead to immediate health concerns, there is evidence that a prolonged lack of omega-3s will have detrimental effects.

How to deal with the omega-3s deficiency? While reducing the intake of omega-6s can help alleviate this ratio imbalance, getting more omega-3s from food is an even better way to go. The conventional source is marine fish oil, which has some disadvantages such as high recovery and purification cost and stability problems and most notably is affected by seasonal and climatic variations and the dramatic effects of over-fishing. In addition, the quality of fish in terms of nutrient composition is difficult to control. Because fish cannot synthesize omega-3 fatty acids by themselves, they obtain the fatty acids by eating the primary producer in the ocean environment - the algae. Therefore, one way to improve the amount of omega-3 in the diet of Americans is to use the algae-omega-3 concept and feed it to production animals that are liked by Americans. The obvious choice is to supplement it into the diets of cows, pigs, and chickens and get a better fat profile in the milk, meat, and eggs. Some research has been done in this respect and has shown that diets containing up to 2% algae supplementation will be well received by the animals and does result in improved omega-3 concentrations in the animal products. Another possibility is of course, cultured fish and their marketable meat. Although Americans do not in general have a large consumer taste for fish, there is a potential for improved growth in the aquaculture market due to the need for more efficient production of protein and the draw of healthy fats; as evidenced by the 20%
annual growth in the industry.

When algae culturing is mentioned, the most common perception is the growth of algae in a large open-air pond where the algae is exposed to the necessary photosynthetic light source it needs to grow. This culture mode, however, cannot grow very much algae because the growth conditions are difficult to control. Plus, the algae growing in the bottom of the pond cannot get enough light because of light penetration problems. These factors lead to a very low algae concentration in the pond and thus, a very low level of omega-3s produced.

To develop a cost-effective algal omega-3s production process, the algae need to be cultured in heterotrophic conditions because only this growth mode eliminates the requirement of photosynthetic light. With this method very high algal biomass levels and therefore high levels of omega-3s can be obtained. However, during heterotrophic algae cultivation, the cost of the organic substrate (usually in the form of pure sugars) is relatively high. To overcome this high carbon-ingredient cost the process therefore needs to be fed on a cheap, under-valued source of carbon. Glycerin has been reported to be such a substrate for algae, specifically the alga *Schizochytrium limacinum*, which when grown on glycerin-based nutrient solution yields over 4g/L of DHA. This yield is the highest ever reported. If the cultivation process can be optimized the availability of cheaper biodiesel-derived glycerin will provide a great opportunity to reduce the medium/fermentation cost, and thus offer a very competitive price for the produced DHA-containing algal biomass.

The high purification cost of omega-3 fatty acids from algal biomass is another limiting factor in the commercialization of algal omega-3 production. The concept of feeding the algae to production animals like aquaculture fish and using the fish as an “extractor” to get omega-3-containing meat could avoid the high purification cost. In addition, the promotion of farm raised, organic fish as a dietary supplement enriched with omega-3 like DHA could improve the marketing and sale of the fish. Also, the fish may benefit from higher intakes of algal biomass because algae usually contain a high level of protein/amino acids, lipids and vitamins, which are crucial for a fish’s growth. The project can benefit consumers because the regular consumption of omega-3 fatty acids-containing products eliminates the additional intake of EPA/DHA-containing capsules as dietary supplements.


**Project Objectives**

The goal is to develop an optimal process that utilizes biodiesel industrial waste as feedstock to produce omega-3 polyunsaturated fatty acids (DHA) that are contained within the growing algal biomass. The algae will then be used as feed additives for production animals particularly fish to extract the DHA into the product. Specific objectives include: (1) developing an optimal production process from glycerin based biodiesel waste; (2) pilot study of production; and (3) using algal biomass as feed additives for fish and testing the nutritional value of the produced meat. Simultaneous to the fish tests, APT will test the algae supplement’s palatability and performance capabilities on cattle, swine and poultry.

**Outline of Tasks**

The project will be conducted by the Bioproducts Development and Aquaculture Engineering research groups at the Department of Biological Systems Engineering and in collaboration with our industry partner American Premix Technologies. A time line for the research activities is presented at the end of the proposal.
**Objective 1: Developing an optimal DHA production process from glycerin**

In this project, DHA will be selected as the targeted omega-3 product and the alga *Schizochytrium limacinum* will be used as the DHA producer as the alga has been reported to produce a significant amount of DHA when growing on glycerin \(^3,^4\). The algae will be maintained according to the procedures provided from the cell collections and will initially be fed with pure glycerin as their carbon source. The DHA production of this “pure glycerin” culture will be used as control.


The biodiesel-derived glycerin will then replace the pure glycerin and the DHA production from this type of glycerin will be optimized. In addition to glycerin, the medium composition also includes various salts, nitrogen sources such as yeast extract or tryptone, and traces of vitamin B\(_{12}\) and biotin. The cost of all these components, though, is insignificant as their concentration is very low (less than 0.1%).

The optimal glycerin concentration will be investigated at four levels (10, 20, 30, 40 g/l). Once the added amount of glycerin is fixed, a complete statistically-based experimental design will be used to optimize the other medium components as well as temperature and pH.

All of the above experiments will be done in shake flask cultures. Once the optimal conditions are obtained, a fed-batch culture strategy will be developed for the alga. The purpose is to achieve high cell density microalgal cultivation, which can ultimately convert to a high DHA concentration.

Research will be conducted in a 5-L fermentor. The fed-batch culture process will be accomplished through two steps: (1) a suitable feed strategy will be developed by investigating the limiting factors in the medium, with any limiting factor correspondingly being enriched in the feed medium; (2) a “temperature shift” strategy will be developed as the algal cells usually require higher temperatures for their growth while lower temperatures for their DHA formation. The temperature will be maintained at a higher temperature (~30°C) for cell growth during the earlier stage of culture, and then shifted down to a lower level (~20°C) for the alga to accumulate DHA. The algal biomass will be harvested at the end of fed-batch culture and DHA will be analyzed.

The lipids/fatty acids content of the algal biomass will be extracted by standard AOCS methods (The American Oil Chemists’ Society). The fatty acids will then be analyzed by a gas-chromatograph (GC) at WSU Water Quality Laboratory.

**Objective 2: Pilot study of DHA production by the alga Schizochytrium limacinum**

In this objective, a pilot study of the algal culture process will be performed in order to develop a cost-effective process towards commercial scale application of DHA production by the alga. The study will be conducted in batch and fed-batch mode in a 25 L fermentor in the Department of Chemical Engineering at WSU. The growth conditions will be the same as results obtained in the corresponding lab scale process (Objectives 1).

It should be noted that the algae obtained in this task will be collected for fish feed and other animal feed studies. The algal biomass will be freeze dried by a freeze drier and preserved for research in Objective 3. It is expected that pilot scale of algal culture with fed-batch operation mode could provide sufficient algal biomass for the research work of Objective 3.
Objective 3: Using the algal(184,136),(320,148) biomass as additive in fish feed and testing the nutritional value of the fish as well as other production animals

A feeding trial using rainbow trout will be conducted to determine the safety and effectiveness of using algal biomass as a source of omega-3 fatty acids.

Investigations in this objective will be performed at the Aquaculture Engineering Laboratory. A domesticated strain of rainbow trout (average weight, 20-25 g) will be selected from a larger population, counted in groups of 35 fish, weighed, and placed into 150 L fiberglass tanks, each supplied with 4-6 l/min of untreated, constant temperature (14.5⁰ C) water. A fixed photoperiod, controlled by timers and fluorescent lights, will be utilized (14 hrs daylight: 10 hours dark). Fish will be fed 2x per day to apparent satiation, six days per week, for 12 weeks in accordance with normal trout culture practice.

A standard fish meal (control, APT mixture) and fish meal added with three levels of algal biomass will be fed to the fish. Formulations will be held isoenergetic by adding soybean oil as needed. Each diet will be fed to three replicate tanks of trout, and the arrangement of the diets among tanks will be according to a completely randomized design.

Fish will be bulk-weighed at the start and every 21 days for 12 weeks. Feed intake will be recorded and used to calculate feed conversion ratios for each period and for the total study. Three fish sampled from each tank (9 per dietary treatment) at the end of the feeding trial will be used for pathological evaluation. Selected tissues, e.g., liver, heart, will be removed when the fish are killed, and preserved in 10% buffered formalin. These tissue samples will be used for histological preparation and pathological evaluation. Five additional fish will be used for whole-body analysis of proximate fatty acid composition. Fish will be processed into a puree, sub-sampled, and samples will be dried, and analyzed using AOAC (1990) methods for fatty acid analysis by a GC. The protein will be calculated from sample nitrogen content determined using a nitrogen analyzer.

Data will be transformed as necessary and analyzed for statistical significance using analysis of variance (ANOVA).

While the intensive fish studies are being completed, APT will begin initial studies into the palatability and performance of the algae feed on other production animals.

Project Reviews
The project team will present results of the project work to the WTC Advisory Committee at the conclusion of this project.

Team Interaction
Throughout the experimentation and pilot and fish studies, the research team of Dr. Chen, Dr. Wen, and Mr. Frear will work together with the APT team via personal meetings and conference calls. Regular conferences between Dr. Chen and Mr. Graff will be conducted to review the project, its progress, problems encountered, and any changes in the project plan. At the performance evaluation and marketability levels, collaboration will take place between APT and WSU. Monthly telephone conferences will be held among the project team members to evaluate the progress of the project. The project team at WSU will present a progress report to the company in 3-month intervals.

Key Technical Personnel
Dr. Shulin Chen, Professor: Dr. Chen, a registered Professional Engineer, who received his Ph.D. from Cornell University, is the Director of the WSU Water Quality and Agricultural Waste Analysis Laboratory, as well as the Director of the WSU Bioresource Utilization Laboratory and the Aquaculture Engineering Laboratory. As the project leader, he will be
coordinating all aspects of the project at WSU and coordinate the communications with APT. Dr. Zhiyou Wen, Postdoctoral Research Associate: Dr. Wen received his Ph.D. from the University of Hong Kong and will be responsible for the research, pilot and fish studies. Mr. Craig Frear, Ph.D. student will be responsible for the day to day experimentation and analysis.

Related Activities
Dr. Chen has over 10 years of experience in wastewater treatment, waste management, bioprocessing, and aquaculture engineering and has been awarded over $8 million for research related to these fields. Dr. Chen has an active research program that develops various technologies for converting agricultural/industrial biomass into value added products. Dr. Wen is the coordinator of the WSU Bioresource Utilization Laboratory and has more than 8 years of experience in fermentation technology and bioprocessing; specifically in regards to omega-3 synthesis.

Milestone Chart

<table>
<thead>
<tr>
<th>TASKS</th>
<th>WHO</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tbody>
<tr>
<td>1. Optimizing culture conditions</td>
<td>Frear/Wen</td>
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<tr>
<td>1. Fed-batch development</td>
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<tr>
<td>2. Pilot study</td>
<td>Frear/Wen</td>
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<tr>
<td>3. Fish feed experiment plus additional preliminary studies on other production animals by APT</td>
<td>Frear/Wen/APT</td>
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## Budget

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<th>Description</th>
<th>1. WTC Funds</th>
<th>2. Company Cash</th>
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<td></td>
<td>FY2005</td>
<td>FY2006</td>
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<tr>
<td><strong>Salaries</strong></td>
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<tr>
<td>Zhiyou Wen, 3 months @ 100%</td>
<td>$8,926</td>
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<td>Graduate Student 9 month</td>
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<tr>
<td><strong>Benefits</strong></td>
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<td>Research Associate @ 36%</td>
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<td>Graduate Student</td>
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<td><strong>Subtotal Personnel Costs</strong></td>
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<td><strong>Services/Supplies</strong></td>
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<tr>
<td>Media, GC Standards, chemicals</td>
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<tr>
<td>Algae Strains</td>
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<tr>
<td>Glassware and Lab supplies</td>
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<td>Chemical Analysis Costs</td>
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<td>Travel</td>
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<td><strong>Subtotal Other Direct Costs</strong></td>
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<td>Indirect cost rate (%)</td>
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<td>Indirect cost</td>
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<td>Equipment</td>
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<td>Graduate Operating Fee</td>
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<td><strong>TOTAL</strong></td>
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2 For WSU projects, include supplies in this category.
3 Contact the university grant / contract office for information on the applicable indirect cost rate for company cash.
4 Include full cost of graduate student operating fee for your institution.
In-House Personnel / Other In-Kind Commitments

Company Partner: American Premix Technologies, Inc.
WTC Project Title: Development of Omega 3 Rich Algae from Biodiesel Waste for Use as a Fish Feed Supplement
Funding Period: January 1, 2005 to September 30, 2005

A. Company Personnel Costs Associated with Project:

<table>
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<tr>
<th>Name</th>
<th>Title</th>
<th>Hrs/ Month</th>
<th>Total Dollars</th>
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<tbody>
<tr>
<td>John Graff</td>
<td>Technical Services Manager</td>
<td>17</td>
<td>$ 6120.00</td>
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<tr>
<td>Randal Ames</td>
<td>Process Development Manager</td>
<td>20</td>
<td>$ 4500.00</td>
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<td>Jerry Emersen</td>
<td>V.P. Operations</td>
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<td>$ 1800.00</td>
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**Total Direct Labor & Benefits Commitments** $ 12420.00

* Includes employee benefits

B. Other In-Kind Project Commitments

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<tr>
<th>Description</th>
<th>Amount ($)</th>
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<tr>
<td>Overhead</td>
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<tr>
<td>Services performed by Company (describe)</td>
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<tr>
<td>Test palatability of Omega 3 supplement to swine, beef cattle, dairy cattle, equine, and poultry.</td>
<td>$ 1500.00</td>
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<tr>
<td>Test performance of swine and cattle fed Omega 3 supplement</td>
<td>$ 1500.00</td>
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<tr>
<td>Feeding of beef, swine, and dairy animals to test ability to transfer benefits to meat/milk.</td>
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<tr>
<td>Equipment--Donation</td>
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<td>Equipment--Loan or rental</td>
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<tr>
<td>Feed mixing and blending to test mixability and stability (20 tons)</td>
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<tr>
<td>Travel</td>
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<td>6000 miles of travel to/from Pullman and animal feeding trial sites.</td>
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<td>Other expenses (describe, such as materials, etc.)</td>
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**Total Other Project Commitments** $ 9525.00

C. Grand Total

**Personnel and Other In-Kind Commitments** $ 21945.00
October 4, 2004

R. Lee Cheatham
Washington Technology Center
Box 352140
Seattle, WA 98195-2140

Dear Dr. Cheatham,

This letter represents a commitment by American Premix Technologies to provide $29,945.00 in matching support for a sponsored RTD project entitled “Development of Omega 3 Rich Algae from Biodiesel Waste for Use as a Fish Feed Supplement”. This project is the result of a joint discussion and understanding between Washington State University and Dr. Shulin Chen and our company. The support is provided from January 1, 2005 to September 30, 2005. Of this support, $8,000 is committed as direct cash to Washington State University to carry out its work.

In addition, we are providing other in-kind support valued at $21,945.00 which includes salary cost of in-house personnel, use of company equipment, materials, and other services devoted to the project.

For the purpose of determining minimum cash match required, our company was founded in 2002 and has 4.5 full-time equivalent employees. Mr. John Graff will serve as the company’s contact person.

Our company has received and reviewed a copy of the sample Research and Technology Development (RTD) agreement, is aware of its terms and conditions, and understands the agreement must be signed by all parties within three (3) months following WTC’s funding of the project.

Sincerely,

John Graff
Technical Services Manager,
American Premix Technologies, Inc.