Final Report: Production of low-oil/fat distillers' dried grain with solubles (DDGS) to replace fish meal in aquaculture diets (1060-13EU)

Project Title

Production of low-oil/fat distillers' dried grain with solubles (DDGS) to replace fish meal in aquaculture diets

Principal Investigator and Co-Investigator(s)

Timothy Bruce (Principal Investigator: Fish Physiology)

Dr. Michael Brown (Co-Investigator: Fish Nutrition)

Dr. Bill Gibbons (Co-Investigator: Microbiology)

Dr. Jason Bootsma (Co-Investigator: Production Engineer)

Dustin Schulz (Co-Investigator: Fish Nutrition)

Scott Sindelar (Co-Investigator: Fish Nutrition)

Michael Grey (Co-Investigator: Fish Nutrition)

Abstract

Aquaculture is the fastest growing segment of U.S. agriculture and is set to overtake wild capture fisheries. Farmed food fish accounted for 42.2% of the 158 million tons of global fish production in 2012 (FAO 2014). Fish meal, a major protein source in both aquaculture and livestock feeds, has been subject to elevated pricing due to demand and fixed supply. Plant-based protein sources, such as distiller's dried grains with solubles (DDGS) have shown promise as fish meal replacements at an economically feasible pricing structure. Use of traditional DDGS have been limited in aquaculture diets to approximately 20% inclusion, due to relatively low protein content, less than desirable amino acid profile, and high fiber level. Prairie AquaTech, an animal nutrition and health company has developed a novel microbial conversion process that creates a high protein DDGS (HP-DDGS), a protein-rich and more digestible product that may fully replace traditional fish meal-based diets. The initial objectives of the MCRPC project included: 1) the development of a 1st generation HP-DDGS, 2) a performance evaluation in a feeding trial with yellow perch (*Perca flavescens*), and 3) an assessment of mass/energy balance and costs. The resultant HP-DDGS product was 43.43% crude protein with 84.4% protein digestibility, a marked increased from the starting values of 31.93% crude protein and 81.84% protein digestibility. Feeding trial results demonstrated that two initial HP-DDGS products achieved similar relative growth performance to a fish meal reference diet; these products also produced a similar feed conversion ratio (FCR). The continued innovation of this novel microbial conversion process allows for a high quality, cost-effective fish meal alternative for feed manufacturers and fish and livestock producers while creating a novel market for corn producers. Phase II research will focus on process optimization and continued performance evaluation trials with commercially important fish species.

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Introduction

Aquaculture is the fastest growing segment in U.S. food production and globally it is set to overtake capture fisheries as a source of food fish (FAO 2012). The FAO recently reported that 61% of the world's wild fish stocks are now fully exploited and 29% are overexploited, depleted or recovering from depletion (FAO 2014). Furthermore, it was estimated by the NOAA that the U.S. aquaculture industry was worth $1.2 billion in 2011, but growth is hampered by feed cost. The growth of the aquaculture industry in the United States has sprouted both large-scale and small production operations that aim to fuel the economy and offer alternative seafood protein sources in a time where our wild-caught resources are diminishing. Combined wild capture fisheries and aquaculture supplied 158 million tons of finfish and shellfish in 2012 (FAO 2014). Farmed fish production accounted for about 42.2% of production in 2012 (FAO 2014). Wild harvest peaked at 86 million tons in 1996 and has since stabilized at 80 million tons. Per capita consumption is currently about 19 kg and fish constitutes 17% of all animal protein consumed by humans (FAO 2014). The rapid domestic development of this agriculture sector requires both government support and aquaculture research and education.

Similar trends of greater demand for and lower wild harvest of fish meal protein have led to a rapid escalation in price of this meal commodity. In the early 1980s about 10% of annual fish meal production was used in aquafeeds, by 2010 over 70% of annual production was used in the aquaculture industry with the remainder used in other livestock and companion animal feeds (IFFO 2013). In 2005, 2.7 mmt of fish meal was used in aquaculture feeds, while an estimated 6.7 mmt was used in 2012. Meanwhile, the harvest of species used to produce fish meal has dropped by >40% since 2000 (FAO 2012). These trends are unsustainable, as aquaculture will soon consume the entire fish meal resource. This is reflected in current fish meal prices (e.g., Peruvian, 65% protein; 1,560-2,190 $US/mt, 2013; www.indexmundi.com), which are already hampering the economic production of finfish and shellfish products for human consumption. Consequently, there is a significant market opportunity for a sustainable, economical, plant protein concentrate to replace fish meal in feeds manufactured for aquaculture and other feed markets.

Corn distillers' dried grains with solubles (DDGS) has been tested as a partial replacement for fish meal, however the relatively low protein content, less than desirable amino acid profile, and high fiber level limit inclusion rates to ~20% for most species. Some ethanol plants are partially removing fiber or oil from DDGS, resulting in somewhat higher protein contents. However, inclusion rates of those products are still limited to 20-40% of dry diet for most species. The solution to this problem lies in developing an economically feasible process to convert DDGS into a more digestible, enhanced protein product. The development and provision of enhanced DDGS to the feed industry could reduce the strain on wild fish stocks exploited for fish meal (and marine food webs dependent on these prey) and support continued expansion of the domestic and global aquaculture industry but only if there is an economical and sustainable method to increase its nutritional qualities.

DDGS incorporation into aquaculture feeds has challenges, primarily due to its lower protein (28-32%), high fiber and phytic acid contents. Some ethanol facilities have incorporated technologies to remove part of the fiber and/or oil from DDGS, thereby increasing protein content. For example, Still Pro 50 is manufactured by removing some non-protein components

from DDGS. However the amino acid balance and digestibility of this product is still lacking in comparison to the fish meal nutrient. Therefore even these modified DDGS products have been limited to inclusion rates <40% of fish meal, primarily in omnivorous fishes. Thus there is considerable market opportunity for an even higher protein DDGS that could be used for higher value carnivorous species (e.g., salmon, cobia, yellowtail, snappers). Such a product would be especially attractive if the protein component had higher levels of sulfur amino acids such as lysine, methionine, and cysteine that are critical to animal growth. Our microbially based system converts carbohydrates in the DDGS into cell mass resulting in a highly digestible protein concentrate at a lower cost, while also producing an exopolysaccharide with potential immuno-stimulative properties.

Objectives

Objective 1: Produce a first-generation HP-DDGS using microbial conversion.

Objective 2: Evaluate the replacement performance of HP-DDGS in yellow perch feed.

Objective 3: Determine preliminary mass/energy balance and costs.

Materials and Methods

Objective 1: Production of 1st generation HP-DDGS:

In a pretreatment evaluation, we extruded DDG in a single screw extruder to provide a shearing effect against the ridged channels on both sides of the barrel. This was referred to as extrusion method 1. The selected levels of temperature, screw speed and moisture were based on optimized conditions defined previously for defatted soybean meal. Extruded DDG was mixed with water to achieve an acceptable solid loading rate. The slurry was saccharified using a cocktail of cellulase enzymes. Then cooled and pH adjusted for incubation. Samples were removed at 12-24 h intervals for pH, HPLC (sugars), and culture purity analysis. Following incubation the converted slurry was harvested and dried. The recovered solids had a protein concentration of 43.43% on a dry basis. This HP-DDGS (referred to as Submerged WT28) was used in fish feeding trials described in Objective 2.

Solid state trials

Separate trials were conducted with non-extruded DDGS (trial PAT 2.3) vs non-extruded DDG (trial PAT 2.4). Both feedstocks were mixed with water to achieve an acceptable moisture content and pH. These materials were then placed into separate incubation tubes. Following incubation the solids were removed, dried, and analyzed for protein content. The DDGS sample (PAT 2.3) was 39.75% protein and the DDG (PAT 2.4) was 41.28% protein. Thus the protein levels were slightly lower in the solid state trials with non-extruded feedstocks compared to the 1st generation product. We theorize that this was primarily due to the "washing" effect in the prior submerged conversion process. HP-DDGS products were also tested in the fish feeding trials described in Objective 2.

Comparison of DDG pretreatment in a submerged process:

Using non-extruded DDGS and non-extruded DDG as controls, we next evaluated several additional pretreatments on DDG using the submerged process. We also tested a refined commercial DDGS (StillPro) that contains reduced fiber levels.

For conversion, pretreated feedstocks were incubated using a standard protocol. During incubation, samples were removed at 6-12 h intervals. Samples were subjected to HPLC analysis for carbohydrates and hemocytometer counts to assess microbial populations. Samples were also subjected to ethanol precipitation and centrifugation to separate the protein and microbial biomass (HP-DDGS).

Objective 2: Evaluate the performance of HP-DDGS as a fish meal replacement in perch feeds:

Products derived from Objective 1 processes were analyzed for nutritional competencies in view of requirements of targeted species, especially focusing on yellow perch in this project. Samples were subjected to chemical analyses (proximate analysis, fiber, insoluble carbohydrates, amino acids, fatty acids, and minerals) prior to feed formulation.

Experiment Design Summary

Figure 1. Experimental RAS used

The feeding trial was conducted in a recirculating aquaculture system (RAS). The experimental RAS was a 3,370 L system that consists of 30, 110 L tanks equipped with dual drains (for initial solids separation), primary clarifier sump, recirculating moving bed bioreactor, secondary sump with water level control and electronic water quality monitoring sonde, bioreactor filter, UV filter, and a heating/chilling unit. Water flow and pressure were maintained with a centrifugal pump. All tanks were equipped with half-solid/half-screens lids to minimize disturbance when feeding and to prevent escapes. Flow control is monitored (individual tank manometers) daily to ensure optimal even flow throughout the system. Tanks and bioreactors are each supplied with forced air diffusers. Water was sourced from a municipal supply, de-chlorinated and stored in a 15,200 L head tank

Replication of four experimental units (20 fish/110 L tank) per treatment was used in the feeding trial which lasted 112 days. The trial was performed in the RAS described above. A heat pump was used to maintain the optimal temperature (23.6^{o} C) for yellow perch growth. Water quality (e.g., dissolved oxygen, pH, temperature, ammonia and nitrite) was monitored daily.

Experimental diets were delivered according to fish size (~5 g starting weight), split into two daily feedings of 60% daily ration in the morning and 40% daily ration in the evening. Growth performance was determined by total mass measurements taken every four weeks. Rations were

adjusted in accordance with gains, which allowed for satiation with respect to feeding and to reduce waste streams. Consumption was assessed by counting uneaten pellets remaining in the tank 30 minutes after feeding and adjusting to 90% consumption of fed pellets. Survival, weight gain, growth rate, health indices, feed conversion, protein and energy digestibilities, and protein efficiency were compared among treatment groups.

Statistical analyses of diets and feeding trial responses was completed with an a priori $\alpha = 0.05$. An analysis of performance parameters among treatments was done with appropriate analysis of variance or covariance (Proc Mixed) and post hoc multiple comparisons, as needed.

Feed Preparation

Complete practical diets were formulated using DDGS or converted DDGS in accordance with known nutrient requirements for yellow perch (e.g., 45% protein, 9% lipid) in a factorial design. Basal mineral and vitamin premixes for plant-based diets were used to meet micro-nutrient requirements. All feeding trials included a fish meal-based control diet and diets containing a range of DDGS products, both commercial and experimental.

Seven test protein ingredients including experimental DDGS products, commercial DDGS, and a menhaden fish meal control were used in diet formulations (Table 1). Diets were formulated to be isonitrogenous, and isolipidic by adjusting wheat gluten, wheat flour, cellufil, menhaden and corn oils. Targeted diet proximate compositions (dmb) were 45% protein, 9% lipid, and protein to energy ratios (PE) of approximately 27g protein/ MJ GE (Table 2). All diets were formulated as compound practical diets, which included vitamin and mineral supplements as well as palatability and pellet quality augmentations. A completely randomized nested design was implemented wherein each of the DDGS diets were duplicated and supplemented with taurine, methionine, histidine, and arginine to meet or exceed known yellow perch requirements.

Table 1. Base ingredients incorporated in the feeding trials

Ingredient	**Description**
Fish Meal	Control diet
Raw DDG	DDG from Dakota Ethanol (Wentworth, SD)
Still Pro 50	Mechanically fractionated (post-fermentation) DDGS
Novita NovaMeal	Solvent extracted (hexane) DDGS (experimental product)
Converted Wet Cake (**WT28**)	Extruded, saccharified DDG microbially converted in **submerged** reactor.
Converted Wet Cake (**PAT 2.4**)	Non-extruded, non-saccharified DDG microbially converted in a **solid state** reactor.
Converted DDGS (**PAT 2.3**)	Non-extruded, non- saccharified DDGS microbially converted in a **solid state** reactor.

Large particle ingredients were ground with a Fitzpatrick comminutor (Fitzpatrick Company, Elmhurst, IL) with 0.51mm screen prior to dry blending. Dry ingredients were blended for 15 minutes using a Hobart HL200 mixer before water and oils were added and then blended for an additional 5 min. Feeds were then screw-pressed using a Hobart 4146 grinder with a 2.0 mm die and dried with a Despatch conveyor dryer at 210 ^{o} F. Following drying, feeds were placed in frozen storage at -20^{o} C, pending feeding. Approximately 7 kg of each diet were prepared, including 3.5 kg containing 1% (dry diet) chromic oxide for apparent digestibility determinations.

Chemical analyses of primary protein sources (Table 2) and feeds (Table 3) were completed by private labs. Analyses were completed only on the four basal diets because lysine and methionine were supplemented in known concentrations. Analyses were completed for crude protein (AOAC 2006, method 990.03), crude fat (AOAC 2006, method 990.03), crude fiber (AOAC 2006, method 978.10), moisture (AOAC 2006, method 934.01), and ash (AOAC, method 942.05) and amino acids (AOAC 2006, method 982.30 E (a,b,c)).

Table 2. Base ingredient compositional profile (dry weight basis)

Composition (dry basis)	**Raw DDG**	**Still Pro 50**	**Novita**	**Converted Wet Cake (Submerged) WT28**	**Converted Wet Cake (Solid state) PAT 2.4**	**Converted DDGS (Solid state) PAT 2.3**
Protein %	31.93	49.41	34.36	43.43	39.75	41.38
Fat %	8.09	3.24	0.99	1.89	9.93	7.29
Carbohydrate %	48.38	39.50	53.19	41.09	38.68	39.38
Fiber %	5.85	3.58	6.73	8.33	6.67	9.31
Ash %	1.77	4.27	4.73	5.26	4.97	2.64
Dry Matter	0.66	0.963	0.99	0.99	0.96	0.96

Table 3. Predicted dietary proximates (g/100g dmb, unless noted).

	Diet Treatment						
	FM	SP50	WT28	DDG	PAT 2.4	PAT 2.3	Novita
Protein (%)	40.43	37.46	41.76	42.96	41.18	43.03	42.25
Lipid (%)	10.1	8.46	9.42	10	8.97	9.41	9.07
Ash (%)	32.5	42.4	36.2	36.5	37.2	33.1	38.2
Gross energy (MJ/	7.93	5.31	5.09	3.39	5.55	6.44	2.69
PE (g / MJ)	8.99	6.37	7.51	7.17	7.14	7.99	7.85

Feeding Trial Design

560 juvenile yellow perch (μ±SE, 4.13±0.64 g) were randomly stocked into 28 circular plastic tanks (110 L) within the RAS tanks. The initial tank mass (21 fish per tank, 86.78 ±2.94g) was not significantly different (p=0.76) among tanks. After three days of system acclimation on the commercial diet, fish were introduced a graded mixture of the commercial diet and the specific treatment diet for four days and then fed 100% treatment diet for one week. On the start of the trial, the fish biomass per tank was weighed and visible health was monitored.

Fish were fed to satiation by hand twice daily, and feeding rates were modified according to fish weight by tank, observed growth rates, and feed consumption assessments. Consumption rates (%) were estimated from dividing the weight of uneaten from the total feed offered. The weight of uneaten feed was calculated from counting the number of uneaten pellets 30 min after feeding which corresponded with the time when pellets started to disintegrate and individual pellets would no longer be eaten or distinguished. This was chosen as the consumption method because of ease of implementation, and estimated consumption twice per week to correlate with the specific feeding period ration. Tank consumption estimates were performed twice a week and multiplied by rations fed to obtain feed consumption (g). Fish biomass by tank (+ 0.01 g) was measured every four weeks to monitor fish health and calculate growth performance. Individual lengths (mm) and weights (+ 0.01 g) were also measured every four weeks on a randomly sampled fish from each treatment. Other performance variables measured were:

$$\text{Feed conversion ratio (FCR; calculated as: FCR} = \frac{\text{mass of feed consumed (dry,g)}}{\text{growth (wet,g)}}$$

$$\text{Protein conversion ratio (PER); calculated as: PER} = \frac{\text{growth (wet,g)}}{\text{mass of protein consumed (dry,g)}}$$

$$\text{Fulton-type condition factor (K); calculated as: K} = \frac{\text{weight (g)}}{\text{length (mm)}^{3}} \times 100{,}000.$$

$$\text{Specific growth rate (SGR); calculated as: SGR} = \frac{[\ln(\text{final wt (g)}) - \ln(\text{start wt (g)})] \times 100}{\text{n (days)}}$$

Protein and energy digestion of trial ingredients were estimated using a chromic oxide (CrO_3) marker within the feed (Austreng 1978). Fecal material was collected via stripping and necropsy from the distal 1/3 of intestinal tract at the conclusion of the feeding trial.

Objective 3: Determine preliminary mass/energy balance and costs:

Inputs and outputs of the HP-DDGS conversion process were monitored and used to establish a process mass balance. Similarly, energy requirements for the process were measured and/or estimated to calculate total energy use. Together, these inputs were used to assess preliminary costs, which will be compared to the value of HP-DDGS derived from low-oil vs. traditional DDGS.

Results and Discussion

Objective 1: Production of 1st generation HP-DDGS:

The composition of the HP-DDGS was determined and is shown in Table 4.

Table 4. Comparison of DDG microbial pretreatments with in a submerged process

Feedstock	Pretreatment	Incubation pH	Final Protein (%, dmb)
DDGS	Non extruded	5	45.75
DDG	Non extruded	5	38-42
DDG	Dilute acid	5	38.5
DDG	Hot H20 cook	5	48
DDG	Hot H20 cook	3	43
DDG	Extrusion 1	5	38-41
DDG	Extrusion 1	3	46.50
DDG	Extrusion 2	3	49.90
StillPro DDGS	Non extruded	3	64.44

Non-extruded DDGS resulted in a 45.75% protein product in the submerged trial, compared to ~40% protein in the solid state trials (section 4.1.2.1), primarily due to the added "washing" effect in the submerged trial. However in the non-extruded DDG trial the final protein levels were similar: 38-42% in the submerged trial (Table 4) vs ~41% in the prior solid state trial. These protein levels were also comparable to the 41-43% protein of the extruded DDG in the 1st generation product (section 4.1.1), suggesting that extrusion method 1 provided no significant benefit. Of the other pretreatments tested, dilute acid did not improve protein concentrations. However the hot water cook pretreatment showed a significant improvement. While the replications for these treatments are not complete, they do show that pretreatment can substantially improve protein levels. Furthermore, we have since optimized the extrusion conditions for DDG (extrusion method 2, discussed below) and anticipate that will also show improved protein levels.

Comparison of cellulolytic fungi on extruded DDG (method 1) in a submerged process:

To establish whether expensive cellulase enzymes could be replaced by using cellulolytic fungi we tested DDG processed via extrusion method 1 using the same protocol as above, except that the cellulase enzymes and saccharification step were omitted. Trials and replications are not complete, however we have observed 36-45.6% protein levels when cellulase enzymes were replaced by specific cellulolytic fungi compared to the 38-42% protein levels observed when cellulase enzymes were used with a non-cellulolytic strain. Trials will continue and we will explore options such as nitrogen addition to determine if performance of cellulolytic fungi can be enhanced.

Objective 2: Evaluate the performance of HP-DDGS as a fish meal replacement in perch feeds:

Growth Trial Results

Feed Nutrition:
The predicted diet composition of 45% protein is shown in Table 5. All diets were supplemented with arginine, lysine, histidine, methionine, and taurine to meet, or exceed, minimum yellow perch requirements.

Table 5. Calculated diet compositions used in the feeding trial.

Diet	Fish Meal	Raw Wet Cake	Still Pro 50	Novita	Submerged WT28	SSFPAT 2.4	SSF PAT 2.3
Protein %	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Digestible	40.57	41.08	38.39	40.20	39.94	40.27	39.89
Lipid %	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Fiber %	0.97	2.82	1.68	2.95	2.90	2.66	3.24
Ash %	14.09	18.32	15.45	17.74	16.17	16.72	16.47
Carbohydrate %	13.46	40.10	21.25	28.91	22.75	23.03	22.81
GE (MJ/kg)	16.64	21.54	18.10	19.64	18.57	18.58	18.64
Digestible GE	15.02	19.03	15.56	17.12	16.29	16.44	16.26
PE (g/MJ)	27.03	20.88	24.84	22.90	24.21	24.21	24.13
Digestible PE	26.99	21.57	24.66	23.46	24.50	24.48	24.52

Growth Performance:
The growth trial metrics were analyzed following the Day 112 final sampling. Final relative growth is displayed in Figure 2. The fish meal control showed the highest relative growth (443.53±37.63 g) while SSF PAT 2.3 (333.08±52.05 g; p=0.2059) and PAT 2.4 (313.86±40.44 g; p=0.3682) demonstrated similar performance to this reference diet. The submerged treatment (111.61±15.91 g) displayed the lowest relative growth performance and was significantly different from the fish meal control diet ($p<0.0001$).

Fish meal also produced a significantly higher tank biomass (678.90g) than all other treatments. SSF PAT 2.4 (557.33g) and SSF PAT 2.3 (542.65g) produced the next highest tank biomass. Submerged WT28 (248.75g), produced significantly lower biomass than all other treatments. The commercial corn-based diets, Still Pro 50 (485.53g) and Novita (512.68g), produced similar tank biomass.

SGR followed a similar performance trend with fish meal (2.01) outperforming all corn-based diets but was only significantly different from Submerged WT28 (0.88). Survival was significantly different between groups (p=0.3424). SSF PAT 2.4 and Still Pro 50 had the highest survival rates (90%) but were not significantly different from the other dietary treatments. Fulton's condition factor (K) was not significantly different between treatments (p=0.1324), but was highest for fish fed raw wet cake (1.39) and lowest for submerged WT28 (1.24). Feed

conversion ratio (FCR) were not significantly different between diets (p=0.22). The results indicate that raw wet cake displayed the best FCR (1.43) (Figure 2). SSF PAT 2.4 also produced the best FCR (1.37) for the experimental HP-DDG blends. Protein efficiency ratio (PER) was significantly different between treatments (p=0.028). PER was highest in fish meal (1.25) followed by raw wet cake (1.21), and was only significantly different from Submerged WT28 (0.79).

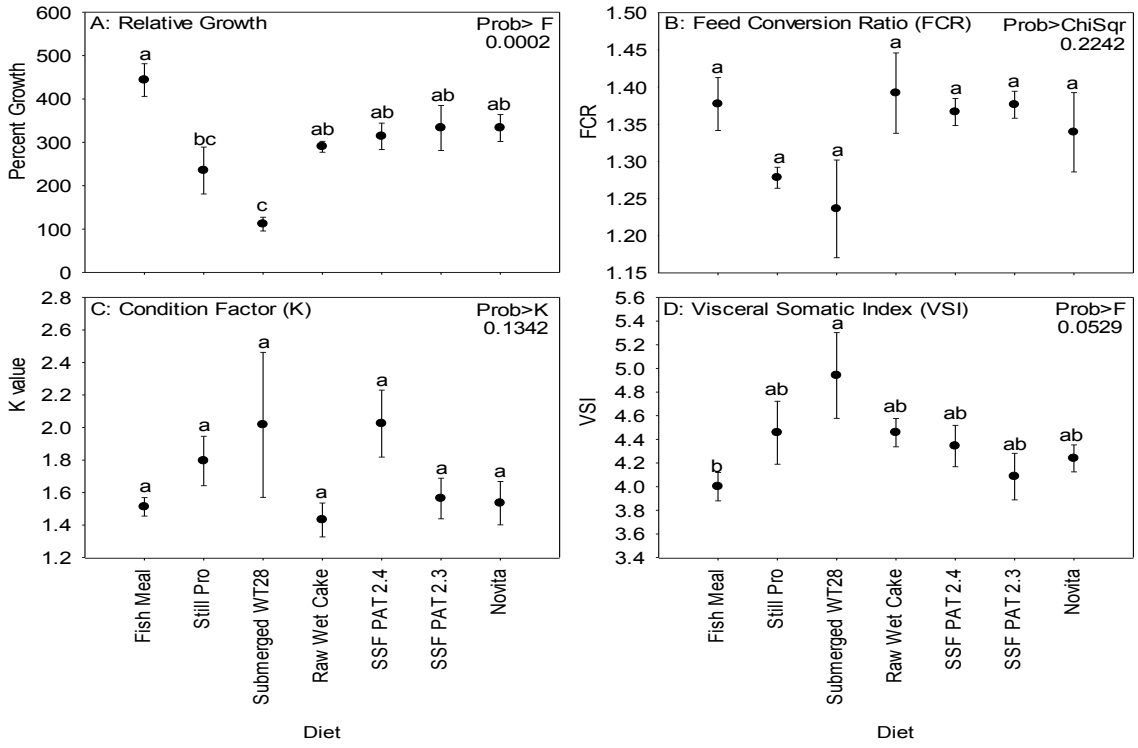

Figure 2. Relative growth, Feed Conversion Ratio, Fulton's Condition Factor (K), and Visceral Somatic Index (VSI) means at Day 112. Letters denote a significant difference between dietary treatments and error bars represent the standard error of the mean (SEM).

Necropsy Variables

Upon completion of the trial, five fish per tank were euthanized and dissected to characterize fish health due to diet responses. There were significant differences in fish morphology and anatomy as a result of the experimental diets (Table 6).

No differences were observed for the visceral fat index (VFI) among treatments (p=0.051). The Submerged WT28 exhibited the lowest VFI (3.41). All of the solid-state fermentation diets (SSF PAT 2.4 and SSF PAT 2.3) produced fish which on average had a higher VFI than the commercial Still Pro 50 diet. Fat in the visceral cavity is considered an indication of poor health

(Craig et al., 1999; Mathis et al., 2003). In addition, excess lipids can affect the visual sense, odor of the final product (Grigorakis 2007) and decrease the carcass yield (Mathis et al., 2003).

Table 6. Summary (means ± standard error) of health indices (HSI, hepatosomatic; VSI, visceral somatic; VFI, visceral fat; SSI, spleen somatic) at Day 112.

Index (%)	Fish Meal	Still Pro 50	Submerged WT28	Raw Wet Cake	SSF PAT 2.4	SSF PAT 2.3	Novita
Fillet/body weight	31.53 ±1.33^{ab}	33.24 ±1.86^{a}	26.50 ±1.39^{b}	29.49 ±1.20^{ab}	30.17 ±1.489^{ab}	31.10 ±0.77^{ab}	32.80 ±0.90^{a}
HSI	1.50 ±0.06^{b}	1.58 ±0.08^{ab}	1.68 ±0.14^{ab}	1.72 ±0.04^{ab}	1.63 ±0.06^{ab}	1.47 ±0.06^{b}	1.89 ±0.06^{a}
VSI	4.00 ±0.12^{b}	4.46 ±0.27^{ab}	4.94 ±0.36^{a}	4.46 ±0.12^{ab}	4.34 ±0.18^{ab}	4.09 ±0.20^{ab}	4.24 ±0.11^{ab}
VFI	4.42 ±0.23^{a}	3.85 ±0.29^{a}	3.41 ±0.31^{a}	3.63 ±0.26^{a}	4.14 ±0.21^{a}	3.79 ±0.20^{a}	3.46 ±0.22^{a}
SSI	0.058 ±0.01^{a}	0.058 ±0.007^{a}	0.059 ±0.006^{a}	0.058 ±0.005^{a}	0.065 ±0.009^{a}	0.089 ±0.031^{a}	0.048 ±0.004^{a}

Hepatosomatic index (HSI) was significantly different between diets (p=0.005). During necropsy, livers of some treatment fish seemed to have a pale color. A pale liver color has been found in other species that have been fed diets with essential fatty acid deficiencies (Takeuchi & Watanabe, 1982; Watanabe et al., 1989; Ruyter et al., 2006). When fish are not utilizing lipids properly or there is imbalance of n-3/n-6 fatty acids. Submerged WT28 had a greater variance than the other diets with HSI's encompassing other treatments. No significant differences existed in spleen somatic (p=0.659) or visceral fat indices (p=0.051).

Objective 3: Determine preliminary mass/energy balance and costs:

The production process has undergone significant changes, which have resulted in substantial reduction in product costs. A comparison of the mass balances can be seen in Table 8.

The Generation 1 data is from a 50 kg process run that produced 33 kg of product resulting in a 66% percent product yield. The loss of mass occurs both from the respiration losses and losses in the centrate. The Generation 2 data is from a 3.5 kg process run that produced 3.0 kg of product resulting in an 86% product yield. The Generation 2 process results in a more efficient mass balance because it does not have the losses associated with the centrate. The loss of non-protein components in the centrate has given increased protein concentrations, but it is anticipated that further optimization of the solid-state process can mitigate this impact. It is anticipated that the product recovery will be further improved as the process is scaled up due to reduced impact of sampling and collection losses.

Table 8. Mass Balance for Generation 1 (Submerged) and Generation 2 (Solid State) Process

Process Generation	Process Step	Process Stream Name	Mass Dry Basis	Moisture Percent	Mass Recovery
Gen 1 Pilot Scale	Fill	Raw Materials	50 kg	90%	
	Incubation	Process Slurry	42 kg	90%	80%
	Incubation	Gases, Vapors	8kg	100%	
	Separation	Wet Grains	33 kg	72%	66%
	Separation	Thin Grains	9 kg	97%	
	Drying	Vapor		100%	
	Drying	Product	33 kg	8%	66%
Gen 2 Lab Scale	Fill	Raw Materials	3.5 kg	50%	
	Incubation	Process Slurry	3.0 kg	50%	86%
	Incubation	Gases Vapors	0.5 kg	100%	
	Drying	Vapor		100%	
	Drying	Product	3.0 kg	8%	86%

The cost reduction realized by the Generation 2 process can be seen in Table 9. The process changes have dramatically reduced the costs associated with the process. Important reductions have occurred in the energy requirements due to lower drying costs, raw product reduction due to greater recovery, and enzyme removal due to improved activity of the organism. Analysis of energy use shows a drop in the cost of energy of over 62%, which is primarily attributed to the reduced drying costs from the solid-state process. Our goals for the next phase of research include further reduction in energy use, increased efficiency in product recovery, and increased protein concentration.

Table 9. Process costs reduction for Generation 2.

Process Cost Reduction Generation 2		
DDGS	23.14%	per ton product
Electric		per ton product
Natural Gas	62.13%	per ton product
Enzyme	100%	per ton product
Organism		per ton product
Antimicrobial		per ton product
Lime		per ton product
Sulfuric Acid		per ton product
Total Var Cost	41.61%	per ton product
CAPEX Service		
Labor		
Total Cost	26.56%	per ton product

Conclusion

The microbial enhancement of DDGS to increase its protein concentration and nutritional value has shown significant potential in this first phase of research. The process has been simplified to reduce cost and increase product performance. The solid state process has brought cost projections to less than half of the cost of fish meal.

The process has demonstrated the ability to increase the protein concentration by over 36% (31.93% to 43.43%) in large scale trials and some bench trials have shown protein levels over 50%. These results are important to the feasibility of using DDGS as an aquafeed ingredient because of the high protein requirements in aquafeeds. The process will benefit from additional optimization to ensure further increases in protein levels and performance. The critical factors for optimization have been identified in this Year-1 research and work has been begun on their implementation.

The performance of the HP-DDGS has been shown to be improved over commodity DDG and even over specialty DDGS products like StillPro or Novameal. The combination of StillPro or Novameal with the microbial conversion process offers potential for further improvement and even higher protein levels. The combination of these processes has begun and will be a part of the process optimization.

The technology to microbially enhance the protein in DDGS to develop a fish meal replacement has been demonstrated to be technically feasible, economically attractive, and a sustainable solution to increased need for quality protein ingredients to replace fishmeal in aquaculture feeds.

Education, Outreach, and Publications

Peer reviewed articles

1. Fallahi, P., K. Muthukumarappan, K.A. Rosentrater, **M.L. Brown**. 2013. Twin-screw extrusion processing of rainbow trout (*Oncorhynchus mykiss*) feeds using graded levels of high protein corn-based distillers dried grains (HP-DDG) and conventional distillers dried grains with solubles (DDGS). Journal of Food Research 2:118-139. URL: http://dx.doi.org/10.5539/jfr.v2n1p118.

Conference/Symposium Proceedings

1. VanMaanen, T. and **W.R. Gibbons**. 2013. Bio-production of high protein feed from ethanol byproducts. CBRD Annual Meeting, Sept 18, Brookings, SD.

Published Abstracts

1. Bishnu, K., K. Muthukumarappan, **W.R. Gibbons**. 2013. Optimization of extrusion processing conditions on enzymatic hydrolysis and fermentation of distillers' low oil wet cake. North Central Branch Am. Soc. Microbiology, 73rd Annual Meeting, Oct. 11-12, Brookings, SD.

Websites Developed

www.PrairieAquaTech.com

Awards

1. 2nd Place South Dakota Governor's Giant Vision April, 2013
2. 1st Place Innovation Award, Kansas City Animal Health Investment Forum, August, 2013
3. Midwest Finalist, Cleantech Open, November, 2013
4. TechConnect National Innovation Award, June, 2014

Scientific/technical honors received

1. Dr. M.L. Brown was promoted to the rank of Distinguished Professor, July 2013
2. Dr. W.R. Gibbons F.O. Butler Award for Excellence in Research, January 2014

Media outreach

1. Jessen, Holly. "Fishing for Profit." *Ethanol Producer Magazine* July 2014:32-37. Print.
2. Bootsma, Jason. (2014, July 17). DDGS on the Hook as an Aquaculture Feed [Webinar] In *Ethanol Producer Magazine* Webinar Series

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Chakraborty P, Gibbons WR, Muthukumarappan K. 2009. Conversion of volatile fatty acids into polyhydroxyalkanoate by *Ralstonia eutropha*. J. Applied Microbiology. Published Online: Mar 25 2009 5:25AM. DOI: 10.1111/j.1365-2672.04158.x (p 1996-2005)

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Fosmer A, Gibbons WR. 2011. Separation of scleroglucan and cell biomass from Sclerotium glucanicum grown in an inexpensive, by-product based medium. Int. J. Agricul. Biol. Eng. (in press).

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