EVALUATION OF THE NUTRITIONAL VALUE OF ETHANOL YEAST IN PRACTICAL-TYPE DIETS AS AN ALTERNATIVE PROTEIN SOURCE FOR RAINBOW TROUT *Oncorhynchus mykiss*

by

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ABSTRACT

Ethanol yeast (EY) is a single-cell protein obtained as a co-product during the production of fuel ethanol and may have potential as an alternative protein source for rainbow trout. The objective of the current study was to determine if EY could replace fish meal (FM) without negatively impacting growth performance of juvenile rainbow trout. Three experiments were conducted to evaluate the use of EY. In Exp. 1 a digestibility trial was done to determine EY apparent digestibility coefficients (ADCs) for protein, lipid, energy, DM, and apparent availability coefficients (AACs) for amino acids. In Exp. 2 a feeding trial was conducted where a control diet (42% digestible protein and 20% crude lipid) was compared to diets where FM digestible protein was replaced by EY at varying levels (25, 37.5, 50, 62.5, 75, 87.5, and 100%). Diets were fed twice daily to rainbow trout to apparent satiation in a 15°C recirculating system. There were 4 replicate tanks per diet (30 fish/tank). Experiment 3 was conducted to determine if a mycotoxin inhibitor (Biofix Plus) could improve performance of rainbow trout when fed higher levels of EY. The experiment was a 2x3 factorial where FM was replaced with EY (0, 50 and 100%) with or without Biofix Plus. There were three replicate tanks per diet (15 fish/tank). Results from Exp. 1 showed that Ethanol yeast ADCs for protein, DM, lipid, energy and AAC for and sum of amino acids were quantified at 98, 65, 100, 70, 81 and 81%, respectively. Results from Exp. 2 showed that fish growth was not different from the control diet at the 25% and 37.5% replacement levels. However, reduced growth ($P < 0.001$) and poorer feed conversion ($P < 0.001$) were measured when EY replaced more than 37.5% of dietary FM (11.2% EY inclusion). Results from Exp. 3 found no effect of Biofix Plus on performance of rainbow trout. There was reduced growth ($P=0.001$) in the 50 and 100% replacement diets. Apparent digestibility coefficients suggested that EY nutrients were highly digestible. However, growth was reduced at EY inclusion levels that were greater than 11.2%.

INTRODUCTION

Aquaculture is a rapidly growing industry, but faces many unique challenges. Fish meal, the most common source of protein in aquafeeds, dramatically increased in price from $800/ton in 2006 to almost $2000/ton in 2010 (Figure 1) due, in part, to disasters such as the Gulf of Mexico oil spill in January 2010 and the major earthquake in Chile where much of the fish meal supply was located. Although, fish meal prices have dropped recently ($1350/ton in December 2011; http://www.indexmundi.com/commodities/?commodity=fish-meal), the inconsistency in these prices and vulnerability to sharp supply decreases makes fish meal a less economical feed ingredient for the aquaculture industry to solely rely upon for feeding farm-raised fish. Recently, there has been an increased emphasis on using sustainable plant products in aquafeeds (Gatlin et al., 2007) including cereal grains such as corn, wheat, and barley and legumes like soybean meal. There also has been interest in utilizing terrestrial animal protein sources such as blood meal and poultry by-products. However, the use of any alternative proteins in fish diets not only must be sustainable and cost-effective, they must provide equivalent or enhanced growth, fillet quality, and health of the fish.

Rainbow trout cultured as a food fish is a rapidly growing industry (Figure 2). Production of rainbow trout expanded in the 1950s as pelleted feeds became commercially available and was further facilitated by the U.S. trout industry's adoption of the cooking-extrusion process as the primary method of feed manufacture. As a result of this increased ability to produce higher quality and energy-dense pellets, trout feeds

have undergone a shift from being relatively high in protein content and low in total lipid to being lower in protein content and higher in lipid (Hardy, 2002). Over the same period, the percentage of digestible protein has increased making modern trout feeds much more efficient and less polluting (FAO, 2012). When rainbow trout are fed appropriately formulated feeds with these nutritional levels, they can often exhibit a better than 1:1 feed to gain conversion ratio (FCR), and in production situations, the industry average is similarly very good.

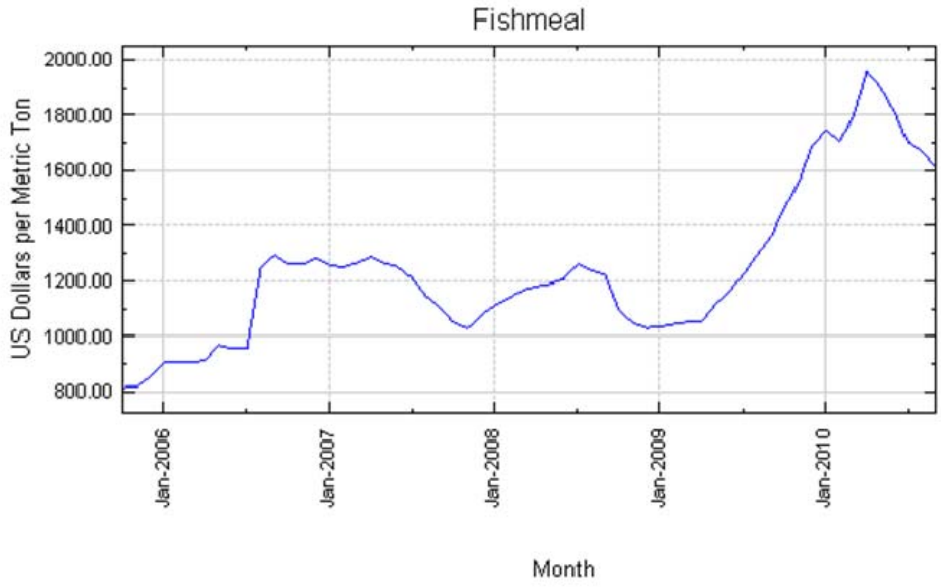

Figure 1. The rise in fish meal prices from January 2006 to January 2010.

Current feed formulations for rainbow trout diets include fish meal, fish oil, grains and other ingredients (FAO, 2012). However, fish meal can be a primary source of protein for rainbow trout in these commercially manufactured diets. Alternative proteins have had some effect on reducing the amount of fish meal in rainbow trout diets,

decreasing the total amount of fish meal included in the diet to about 50% of the dietary protein sources. However, further increasing plant-based ingredient inclusion levels, has resulted in slightly reduced growth and poorer FCRs (Barrows et al., 2007; Gomes et al., 1995). Possible reasons to explain this reduced performance likely include that the essential amino acid (EAA) profile in plant proteins differs substantially from that of fish meal (Lansard et al., 2010). Thus, rainbow trout that are fed plant-based diets without supplemental amino acids are generally not able to obtain the required amount of EAAs from their diet resulting in reduced protein synthesis and subsequently the building of skeletal muscle and overall growth of the fish.

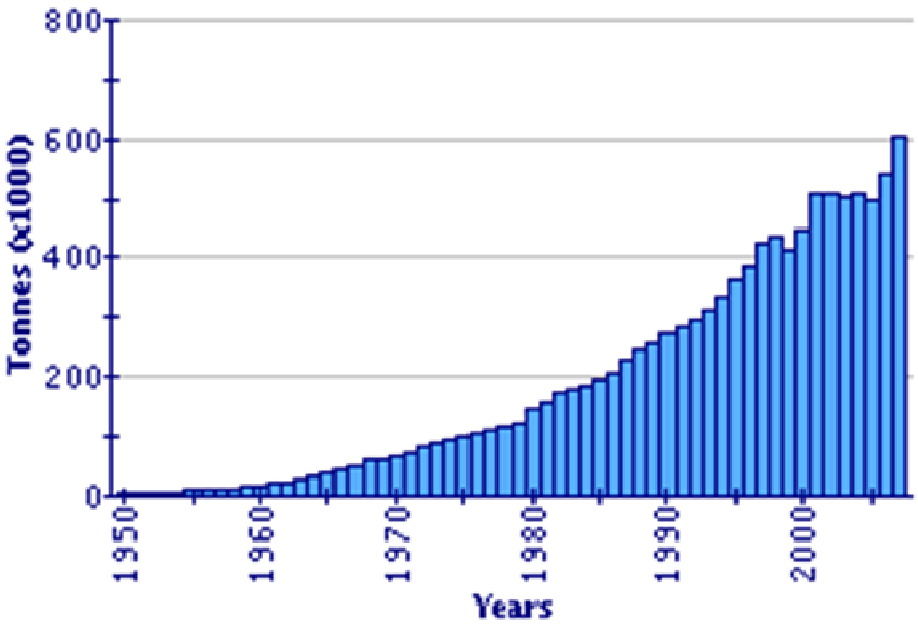

Figure 2. Global aquaculture production of rainbow trout *Onchorhynchus mykiss* from the year 1950 to 2010.

Examples of amino acid imbalances have been well documented in fish fed diets in which plant proteins have been substituted for fish meal (Gatlin et al., 2007). The usage of synthetic amino acids in the last two decades has demonstrated that with appropriate amino acid balance through synthetic amino acid supplementation, plant-based protein diets can provide growth of fish, equal to FM supplementation (Aksnes et al., 2007; Cheng et al., 2003; Gaylord and Barrows, 2009; Snyder et al., 2012). Often times a single amino acid has been supplemented to alleviate part or most of the growth reduction observed when high levels of plant feedstuffs are utilized instead of fish meal. Providing multiple amino acids have shown more success by addressing the potential second, third or even fourth limiting amino acids simultaneously in alternative protein diets (Davies and Morris, 1997).

The inability to provide a balanced amino acid profile has historically created an obstacle for fish nutritionists attempting to replace dietary fish meal with plant-based alternatives. However, an additional caveat to successful fish meal replacement is that success today is still dependent on the use of high quality, highly processed plant-based ingredients and concentrates such as corn gluten meal and soy protein concentrates because when lower quality protein sources are used, growth can be reduced regardless of amino acid balance.

Another probable explanation for reduced performance of trout fed alternative ingredients even when amino acid profile is balanced includes anti-nutritional factors that cause carnivorous fish to experience intestinal distress if low-quality plant ingredient inclusion levels are too high. Plant ingredients such as soybean meal can induce chronic

intestinal inflammatory conditions characterized by increased mucosal leukocyte accumulations and epithelial cell proliferation. Dale et al. (2009) found, through histological examination, that adenocarcinomas evolving through progressive epithelial dysplasia were associated with severe chronic inflammation due to the feeding of high levels of plant products to rainbow trout and Atlantic salmon. The histological progression was analogous to that of human colorectal cancer associated with inflammatory bowel disease. Similar results of inflammation in the liver and intestine of Atlantic salmon were found in a separate study by Thorsen et al. (2008). To prevent these and other anti-nutritional effects of plant ingredients, many are only used at lower levels in fish diets.

Glencross et al. (2007) concluded that the development of alternative protein-based diets for fish depended on the nutritional quality of the ingredients used, as well as understanding the nutritional requirements of the fish being cultured. When investigating the potential of a novel ingredient in aquafeeds it is necessary to have an understanding of the amino acid requirements of the specific fish species and the amino acid content and availability of the potential ingredient as well as knowing if anti-nutritional characteristics of that novel ingredient are present.

Novel animal feed ingredients have been developed as co-products of the ethanol fuel production process. Barrows et al. (2008a) described the potential interest in utilizing these co-products as aquafeeds from the alternative fuels industry. One specific co-product that has been recently developed and may have potential as an aquaculture feedstuff is Ethanol yeast (EY; Registered AAFCO name is Grain Distillers Dried Yeast).

Ethanol yeast is a single-cell protein obtained as a co-product from the ethanol fuels industry process during which this yeast culture converts the carbohydrates that are in corn into fuel ethanol (Gause and Trushenski, 2011a,b). Once the yeast life cycle is completed, the harvested protein then becomes a protein biomass that has the potential to be used as a feed source in the animal diets. Ethanol yeast is relatively high in protein content (~52%) and may be a possible alternative source of protein for fish meal in rainbow trout diets.

Therefore, the overall objective of this thesis research was to investigate factors that affect the use of EY in aquafeeds, including protein concentration, protein digestibility, digestible energy value, and the presence and level of anti-nutrients or contaminants.

LITERATURE REVIEW

Current Status of the Utilization of Alternative-Protein Diets for Rainbow Trout

Results from digestibility trials with novel feed ingredients can be valuable sources of information for researchers and commercial feed manufacturers, when formulating new diets. For example, EY, has a protein content of approximately 52%. Most fish meal sources have crude protein levels somewhere between 60 and 70% making it appear that EY is a more inferior source of protein. However, fish only utilize those nutrients that are available to them. Therefore, when comparing EY to fish meal as a protein source, it is more important to analyze how digestible the protein and individual amino acids are within the ingredient and compare these results to other feed ingredients rather than simply comparing chemically determined nutrient contents.

Evidence shows that balancing diet formulations for rainbow trout and other species of fish can be difficult with the limited nutritional data on different types of feed ingredients. Gaylord et al. (2008 and 2010) published the apparent digestibility of gross nutrients and apparent availability of amino acids of rainbow trout for ingredients that included different fish meal sources, terrestrial animal by-products, plant protein concentrates, plant meals and low-protein plant ingredients. Results from those studies indicated that fish meal sources varied widely in protein digestibility, reaching a low of 86% for fair and average quality fish meal and a high of 97% for anchovy fish meal. Within the terrestrial animal ingredients, poultry blood meal, feather meal and poultry by-product were found to have protein digestibility values ranging from 86% to 88%. With

regard to plant protein concentrate sources, protein availability ranged from 89% for rice protein concentrate to 100% for wheat gluten meal. In contrast, the plant meals showed slightly lower protein digestibility averaging around 73%, except for soybean meal which was 89% available. Lower-protein plant sources which included rice bran, wheat middlings, wheat millrun, barley, whole wheat, wheat flour and whole corn showed a higher degree of variability in protein digestibility. A low of 57% was found in barley versus a high of 85% for whole wheat. Similar trends were measured for each of these individual ingredients for their apparent amino acid availability.

To date, researchers have observed both positive and negative results with alternative protein ingredients even when balancing for digestible protein and amino acids. De Francesco et al. (2004) concluded that long-term feeding of diets based on plant-protein mixtures to rainbow trout resulted in reduced growth and altered the body and fillet quality traits of these fish. Barrows et al. (2007) found that rainbow trout fed fish meal-free diets with plant meals or plant protein concentrates had slightly reduced rate and efficiency of gain when compared to fish fed commercial formulations. Barrows et al. (2008b) did observe improved results when plant-based diets were supplemented with vitamins above the previously recommended requirements (NRC, 1993). However, fish meal diets supplemented with the same vitamin-premix still grew faster than the supplemented plant-based diets. Similar responses were found when Barrows et al. (2010) supplemented macro-minerals and inositol in a plant-based diet; improved growth was measured in an all plant-based diet but rainbow trout showed better growth and had lower FCRs when fish meal was included as the main source of dietary protein.

Cheng et al. (2003) observed no differences in growth and feed conversion of rainbow trout fed a plant-based diet supplemented with 0.4% or higher lysine compared to fish meal diets. Plant diets with lower lysine supplementation resulted in poorer performance. In a more recent study conducted by Snyder et al. (2012), no differences were found in growth or FCR when rainbow trout were supplemented with the additional amino acids; lysine, methionine, threonine, glycine and carnosine compared to a diet based on fish meal.

Alternative ingredients have been shown to perform better than fish meal supplemental diets. Sealey et al. (2011) reported total replacement of fish meal with a poultry by-product meal did not result in differences in growth when fed to rainbow trout. In the same study, chicken concentrate and chicken and egg concentrate improved growth and performance of fish when these products replaced all of the fish meal included in the control diet. Similar results were found by Saadiah et al. (2010), when studying cobia, reporting no significant differences when poultry by-product replaced 100% of the dietary fish meal protein, and found improved specific growth rates in some of the intermediate replacement levels.

The Potential Impacts of Mycotoxin Contamination on the Performance of Fish Fed Alternative Protein Diets

The previously described inconsistencies regarding successful fish meal replacement by alternative protein sources could be due, at least in part, to mold contamination of the feed ingredients. Mycotoxins are among the most common contaminants in animal feed, causing great economic loss in the livestock industry and,

especially in aquaculture (Jantrarotai et al., 1990; Jantrarotai and Lovell, 1990a; Jantrarotai and Lovell, 1990b). Mycotoxins are structurally diverse, potentially highly toxic, secondary metabolites produced by filamentous fungi frequently contaminating agricultural commodities used as animal feedstuffs (Hussein and Brasel, 2001). Mycotoxins can be deleterious in animals, especially during stressful periods when their immune systems are suppressed. This depression may result in a toxic response when animals consume mycotoxin-contaminated feeds (Whitlow et al., 2004). It is estimated that worldwide about 25% of farmed crops are contaminated annually with mycotoxins (CAST, 2003). Yearly forecasts of mycotoxin damage to the US agricultural industry are estimated at approximately $1.4 billion (CAST, 2003).

Mycotoxins are carcinogenic and responsible for the wide spread occurrence of hepatic carcinoma of farmed rainbow trout when consumed at a high rate (Carlson et al., 2001). The most common mycotoxin affecting rainbow trout is aflatoxin. Aflatoxin-contaminated cottonseed meal was recognized as the primary cause of high mortality rates of rainbow trout, in Idaho trout hatcheries in the early 1960s (Ashley and Halver, 1963). Symptoms of aflatoxicosis in fish can include; poor growth and feed efficiency, cancerous tumors, reduced feed consumption, kidney abnormalities, gastric gland damage, damage to liver, immune suppression, and high or spiking mortality. These symptoms were often reported in the results of experiments which were unsuccessful in replacing fish meal with alternative protein sources.

Further studies have explored mycotoxins other than aflatoxin that have had harmful effects in fish. Hooft et al. (2010) examined the effects of the mycotoxin,

deoxynivalenal (DON), and found that it can reduce performance of rainbow trout when present within their diet. Specifically, Hooft et al. (2010) reported decreased weight gain, feed intake, poorer FCR, energy retention efficiency (ERE), and protein retention efficiency (PRE) of juvenile rainbow trout due to increased levels of DON.

With the increased utilization of alternative proteins in aquaculture, many research efforts have focused on removing or binding contaminants that were present in feedstuffs. Some of these approaches have included mycotoxin separation from contaminated feeds, detoxification and inactivation. Detoxification and inactivation methods include the use of binders or sequestering agents added to feed. This is achieved by reducing the reactivity of bound mycotoxins and reducing intestinal absorption (Whitlow et al., 2004). Substances used as mycotoxin binders include indigestible adsorbent materials such as silicates, activated carbons, and complex carbohydrates (Whitlow et al., 2004). Deactivation by enzymatic degradation of mycotoxins that are not easily bound can also be achieved by dietary supplementation or ingredient pretreatment with specific enzymes.

Limited research has investigated the use of mycotoxin binder and/or deactivator products in fish diets; however, recent studies have shown some success in lessening the effects of mycotoxins in aquafeeds. Agouz and Anwer (2010) reported increases in both growth rate and survivability when using a probiotic (Biogen®) or a binder (Myco-Ad®) in the diets of common carp when compared to an un-contaminated control diet. Higher survivability, better growth and feed efficiency were reported by Abdelaziz et al. (2010) when tilapia were fed diets using clay as a mycotoxin binder compared to the control diet.

In both these studies, aflatoxin and ochratoxin were the specific contaminating mycotoxins present within the treatment diets.

Yeast Products as a Potential Alternative Protein Source for Fish

Research, measuring the effects of yeast products in the diets of rainbow trout, has focused on its role as an immune-stimulant and most inclusion rates were less than 5% of diet DM (Gatesoupe, 2007). However, single cell proteins have also shown potential as dietary protein sources. Rumsey et al. (1991a) analyzed the digestibility of brewer's dried yeast (BDY) processed by different methods and then measured growth performance of rainbow trout when BDY was included as a main portion of the diet. When BDY was processed into a protein isolate, protein digestibility increased to 87.3% compared to 63.2% for the non-processed intact BDY. In a subsequent study, Rumsey et al. (1991b) found that BDY could be included at up to 25% of the total diet (Casein was primary protein source; no fish meal included in diet) without measuring decreases in weight gain or FCR. However, when BDY was included beyond that inclusion rate, the fish experienced poor growth and FCR.

Other work examined the utilization of yeast proteins as a fish meal replacement in rainbow trout diets. Martin et al. (1993) found that yeast biomass *Candida utilis* could effectively replace up to 35% of dietary fish meal without significant decreases in growth performance of rainbow trout.

More recently, EY was examined for its potential to act as a fish meal replacement in sunshine striped bass diets (Gause and Trushenski, 2011a, b). Results

suggested that EY could replace up to 75% of the protein provided by fish meal without having a negative impact on growth rate and FCR. Another commercial yeast product (NuPro) could only replace 25% of fish meal in cobia diets without a resultant decrease in growth and performance (Lunger et al., 2006). However, NuPro did show promise when fed to tilapia, an omnivorous fish, by replacing all of the dietary fish meal without affecting growth (Craig and McLean, 2005).

One primary limitation of these studies was that dietary substitutions were made on crude nutrient (protein) basis without balancing or ensuring that the essential amino acid needs were met. Numerous studies have shown that formulating fish diets on an available amino acid basis improves alternative ingredient utilization (Yamamoto et al., 1997, 1998; Cheng and Hardy, 2003; Thiessen et al., 2003, 2004; Gaylord et al., 2009). Additionally, in those studies, utilization of the ideal amino acid profile as dietary amino acid targets improved both growth and protein retention over that of fish fed the recommended levels of EAA (NRC, 1993). The evolving understanding of the amino acid needs of trout through analysis of the amino acid content and availability in EY combined with an increased understanding of and mitigation of potential anti-nutrient factors such as mycotoxin contamination may provide additional insight into the suitability to further advance the use of this protein source as a fish meal replacer.

MATERIALS AND METHODS

Experiment 1

Objective

The objective of experiment one was to determine nutrient composition and the digestibility of EY.

Animals

Experiments were conducted at the Bozeman Fish Technology Center, Bozeman, MT. All fish were handled and treated in accordance with guidelines approved by the US Fish and Wildlife Service. Fish averaged approximately 300g each and were stocked at a rate of 20 fish per 200-L poly tank. Water temperature was maintained at 14 ºC and lighting was maintained on a 13:11 h diurnal cycle.

Design and Treatments

Nutrient and energy availability were first determined for EY utilizing an *in-vivo* digestibility study. The methods of Cho et al. (1982) and Bureau et al. (1999) were used to estimate ADCs for EY. A reference diet (Table 1) which met or exceeded known nutritional requirements of rainbow trout was blended with EY in a 70:30 ratio (dry-weight basis) to form the test diet. This ratio was chosen as an acceptable level that could be used to determine the digestibility of the test ingredient (Kleiber 1961; Forster 1999). Yttrium oxide was included at 0.1% of the reference diet, to determine fecal output, and nutrient retention was determined relative to the inert marker.

Table 1. Composition of reference diet on a dry wt. basis

Ingredients, %	
Wheat Flour[1]	28.3
Squid Meal	25.0
Soy Protein Concentrate[2]	17.1
Fish Oil[3]	13.4
Corn Gluten Meal[4]	8.3
Soybean Meal[5]	4.3
Vitamin Premix ARS[6]	1.0
Chromic Oxide[7]	1.0
Choline Chloride[7]	0.6
Taurine	0.5
Stay-C 35	0.2
Trace Mineral Premix[8]	0.1
Yttrium Oxide[7]	0.1

[1]Archer Daniels Midland (Decatur, IL, USA) 4 g/kg protein
[2]Solae Profine VP (St. Louis, MO, USA) 693 g/kg crude protein
[3] Omega Proteins Inc.
[4] Cargill, 601.0 g/kg protein
[5] Archer Daniels Midland (Decatur, IL, USA), 480 g/kg protein
[6] Contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 gm: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inostitol 600
[7]Sigma-Aldrich Company (St Louis, MO, USA)
[8]Contributed in mg/kg of diet; zinc 40; manganese 13; iodine 5; copper 9

Diets were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18-s exposure to an average of 127 ^{o}C in the sixth extruder barrel section. The die plate was water cooled to an average temperature of 60 ^{o}C. Pressure at the die head varied from 200 to 400 psi, depending on test diet. Four mm pellets were

produced then dried in a pulse-bed drier (Buhler AG, Uzwil, Switzerland) for 25 minutes at 102 ºC with a 10-minute cooling period. Final moisture levels were less than 10%. The final step was top-coating the dried and cooled feed with fish oil using a vacuum coater (A.J. Mixing, Ontario, Canada).

Diets were randomly assigned to each tank and fed to three replicate tanks of fish. Fish were fed twice a day to apparent satiation 7d prior to fecal collection.

Measurements and Collections

Fecal Collections: Manual stripping of all fish in each tank was accomplished by netting and anesthetizing the fish, followed by gently drying and then applying pressure to the lower abdominal region to express fecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. Fecal samples for a given tank were pooled, freeze-dried and stored at -20 ^{0}C until chemical analyses were performed.

Analytical Methods: Dry matter analysis of ingredients, diets and feces were performed according to standard methods (AOAC, 1995). Yttrium and P were determined in diets and feces by inductively coupled plasma atomic absorption spectrophotometry. Crude protein (N x 6.25) was determined in ingredients, diets and feces by the Dumas method (AOAC, 1995) on a Leco Truspec N nitrogen determinator (LECO Corporation, St. Joseph, Michigan, USA). Total energy was determined by isoperibol bomb calorimetry (Parr Instrument Company Inc., Moline, Illinois, USA).

Calculation of Apparent Nutrient Digestibility: Apparent availability coefficients of each amino acid in the test diet and ingredients were calculated according to the following equations (Kleiber, 1961; Forster, 1999):

$ADCN_{diet} = 100\text{-}100 \{ \frac{\%\ Yt\ in\ diet}{\%\ Yt\ in\ feces} \times \frac{\%\ nutrient\ in\ feces}{\%\ nutrient\ in\ diet} \}$

$ADCN_{ingredient} = \{(a+b)\ ADCN_t - (a)\ ADCN_r\}\ b^{-1}$

where,

$ADCN_{ingredient}$ = apparent digestibility coefficient of the nutrient in the test ingredient

$ADCN_t$ = apparent digestibility coefficients of the nutrient in the test diets

$ADCN_r$ = apparent digestibility coefficients of the nutrient in the reference diet

a = (1-p) x nutrient content of the reference diet

b = p x nutrient content of the test ingredient

p = proportion of test ingredient in the test diet.

Experiment 2

Objective

The objective of this experiment was to determine if EY could replace fish meal without negatively impacting rate and efficiency of gain, and proximate composition of rainbow trout.

Animals

Rainbow trout eggs from a single lot were obtained from Troutlodge Inc., Sumner, Washington, US and cultured at the Bozeman Fish Technology Center,

Bozeman, MT until the start of the experiment. All fish were handled and treated in accordance with guidelines approved by the US Fish and Wildlife Service. Fish were stocked at a rate of 30 fish/tank (initial average fish weight + standard deviation = 22.1g ± 0.26g). Water temperature was maintained at 14 ºC. Lighting was maintained on a 13:11 h diurnal cycle. Fish were acclimated to tanks for one week prior to the beginning of feeding trial.

Design and Treatments

A nine-week feeding trial with juvenile rainbow trout was conducted to examine the effects of replacing fish meal with eight graded levels of EY (0, 25, 37.5, 50, 62.5, 75, 87.5, and 100% replacement; Table 2) on growth performance, nutrient retention and diet digestibility. Treatments consisted of four replicate tanks per diet.

Diets (Table 2) were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18-s exposure to an average of 127 ^{o}C in the sixth extruder barrel section. The die plate was water cooled to an average temperature of 60 ^{o}C. Pressure at the die head was varied from 200 to 400 psi, depending on diet. Pellets of 4 mm were produced then dried in a pulse-bed drier (Buhler AG, Uzwil, Switzerland) for 25 minutes at 102 ºC with a 10-minute cooling period. Final moisture levels were less than 10%. The final step was to top-coat the dried and cooled feed with fish oil using a vacuum coater (A.J. Mixing, Ontario, Canada). Diets were randomly assigned to each of the 32 tanks. Fish were fed twice a day to apparent satiation, six days a week for nine weeks.

Measurements and Collections

Pellet Durability: Pellet durability was assessed using a NHP100 portable pellet durability tester (Holmen, Norfolk, UK). The NHP 100 operates by loading approximately 50 g of pellets into the test chamber which cascades them in an air stream causing the pellets to collide with each other and with the perforated hard surfaces within the test chamber. Each test cycle was run for a duration of 60 s. After the test cycle, the subject pellets were ejected for manual weighing. The 'pellet durability index' (PDI) was calculated as the difference between pellet weight before and after the test recorded as a percentage. PDIs were determined on duplicate pellet samples from each diet within seven days of diet manufacture.

Table 2. Rainbow trout diets formulated to have 42% digestible protein, 20% crude lipid and balanced for methionine, threonine, lysine, and phosphorus

Diets[1]	0%	25%	37.5%	50%	62.5%	75%	87.5%	100%
Ingredients[2]				%DM				
CPC[3]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
PBM[6]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
EY[3]	0.0	7.6	11.2	14.9	18.6	22.3	25.9	29.6
SBM[3]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
PBP	16.3	16.3	16.3	16.3	16.3	16.3	16.3	16.3
FM[5]	25.0	18.8	15.6	12.5	9.4	6.3	3.1	0.0
WF[3]	14.5	10.8	9.5	8.3	6.9	5.7	4.4	3.1
FO[7]	14.6	14.8	14.9	15.0	15.2	15.3	15.4	15.9
Lecithin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay-C 35	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
VP[8]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
TM[9]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
NaCl	0.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3
MgO	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
KCl	0.0	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Ca_2P	0.0	0.9	1.4	1.9	2.4	2.8	3.3	3.8
Choline Cl	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DL-Met	0.4	0.5	0.5	0.5	0.5	0.6	0.6	0.6

Table 2 – Continued

Lysine HCl	1.9	2.1	2.2	2.2	2.3	2.4	2.5	2.6
Threonine	0.4	0.5	0.5	0.6	0.6	0.6	0.7	0.7
Taurine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Yttrium	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sum w/o oil	85.5	89.1	90.4	91.6	93.0	94.2	95.5	96.8

[1]Percent of fish meal that is replaced by Ethanol yeast
[2]CPC-corn protein concentrate, PBM-poultry blood meal, spray-dried, EY-Ethanol yeast, SBM-soybean meal, PBP-poultry by-product, pet food grade, FM-fish meal, WF-wheat flour, FO-fish oil, VP-vitamin premix ARS, TM-trace mineral ARS 640
[3]Archer Daniels Midland (Decatur, IL, USA)
[4]MGP Ingredients, Inc. (Atchison, KS, USA)
[5]Peruvian anchovy, Silver Cup Fish Feeds (Murray, UT, USA)
[6]Gavilon LLC,(Omaha, NE, USA)
[7]Nelson & Sons Inc. (Murry, UT, USA)
[8] Contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 gm: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inostitol 600
[9]Contributed in mg/kg of diet; zinc 40; manganese 13; iodine 5; copper 9

Growth Performance: Throughout the study, fish were weighed every two weeks and feed intake was recorded weekly. Feed conversion ratio (FCR), feed intake as a percent body weight and weight gain were calculated according to the following formulas:

Feed Intake=((feed consumed*100/fish weight)/2)/number of days on feed

Weight Gain=final weight-initial weight

FCR=feed consumed/weight gain

Proximate Composition: Ten fish from the initial population were sacrificed for determination of initial whole-body proximate composition. At the conclusion of the study, three fish from each tank were taken for whole body composition.

Body Condition Indices: At the conclusion of the study, three additional fish per tank were randomly selected, individually weighed and measured. Viscera, liver and muscle samples were also obtained for determination of hepatosomatic index (HSI), visceral somatic index (VSI), fillet ratio (FR), according to the following formulas:

VSI=gut weight/fish weight*100

HSI=liver weight/fish weight*100

FR=fillet weight*2/fish weight*100

Statistical Analysis

Tanks of fish were the experimental unit. Proc GLM of SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to perform linear regression analysis. Treatment effects were considered significant at $P<0.05$.

Experiment 3

Objective

The objective of Exp. 3 was to determine if a mycotoxin binder enhanced the ability of EY to replace fish meal by maintaining growth performance and proximate composition of rainbow trout.

Animals

Rainbow trout eggs from a single lot were obtained from Troutlodge Inc., Sumner, Washington, US and cultured at the Bozeman Fish Technology Center, Bozeman, MT until the start of the experiment. Fish were stocked at a rate of 15

fish/tank (average initial BW 26.4±0.86). Water temperature was maintained at 14 ºC. Lighting was maintained on a 13:11 h diurnal cycle and fish were acclimated to tanks for one week prior to the beginning of feeding trial.

Design and Treatments

A 12-week 2 x 3 factorial experiment was conducted to measure the growth performance of rainbow trout when fed diets with or w/o mycotoxin inhibitor and replacing none, half or all of the dietary protein provided by fish meal, with the ingredient EY. The mycotoxin inhibitor investigated was Biofix Plus (Biomin USA, Inc, San Antonio, TX). It was mixed and applied post-extrusion in the oil portion at 0.1% inclusion level of the diet. There were three replicate tanks for each of the treatments. All fish were handled and treated in accordance with guidelines approved by the US Fish and Wildlife Service.

Table 3. Rainbow trout diets formulated to have 42% digestible protein, 20% crude lipid and balanced for methionine, threonine, lysine, and phosphorus with Biofix Plus

Diets[1]	0% w	0% w/o	50% w	50% w/o	100% w	100% w/o
Ingredients[2]			%DM			
CPC[3]	5.0	5.0	5.0	5.0	5.0	5.0
PBM[6]	3.0	3.0	3.0	3.0	3.0	3.0
EY[3]	0.0	0.0	14.9	14.9	29.6	29.6
SBM[3]	15.0	15.0	15.0	15.0	15.0	15.0
PBP	16.3	16.3	16.3	16.3	16.3	16.3
FM[5]	25.0	25.0	12.5	12.5	0.0	0.0
WF[3]	14.5	14.5	8.3	8.3	3.1	3.1
FO[7]	14.6	14.6	15.0	15.0	15.9	15.9
Lecithin	1.0	1.0	1.0	1.0	1.0	1.0
Stay-C 35	0.2	0.2	0.2	0.2	0.2	0.2
VP	1.0	1.0	1.0	1.0	1.0	1.0
TM	0.1	0.1	0.1	0.1	0.1	0.1
NaCl	0.0	0.0	0.3	0.3	0.3	0.3
MgO	0.0	0.0	0.1	0.1	0.1	0.1

Table 3 – Continued

KCl	0.0	0.0	0.6	0.6	0.6	0.6
Ca_2P	0.0	0.0	1.9	1.9	3.8	3.8
Choline Cl	1.0	1.0	1.0	1.0	1.0	1.0
DL-Met	0.4	0.4	0.5	0.5	0.6	0.6
Lysine HCl	1.9	1.9	2.2	2.2	2.6	2.6
Threonine	0.4	0.4	0.6	0.6	0.7	0.7
Taurine	0.5	0.5	0.5	0.5	0.5	0.5
Yttrium	0.1	0.1	0.1	0.1	0.1	0.1
Biofix®	0.1	0.0	0.1	0.0	0.1	0.0
Sum w/o oil	85.6	85.5	91.7	91.6	96.9	96.8

[1]Percent of fish meal that is replaced by Ethanol yeast; w=with Biofix Plus, w/o=without Biofix Plus
[2]CPC-corn protein concentrate, PBM-poultry blood meal, spray-dried, EY-Ethanol yeast, SBM-soybean meal, PBP-poultry by-product, pet food grade, FM-fish meal, WF-wheat flour, FO-fish oil, VP-vitamin premix ARS, TM-trace mineral ARS 640
[3]Archer Daniels Midland (Decatur, IL, USA)
[4]MGP Ingredients, Inc. (Atchison, KS, USA)
[5]Peruvian anchovy, Silver Cup Fish Feeds (Murray, UT, USA)
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[8] Contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 gm: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inostitol 600
[9]Contributed in mg/kg of diet; zinc 40; manganese 13; iodine 5; copper 9

Diets (Table 3) were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18-s exposure to an average of 127 ^{o}C in the sixth extruder barrel section. The die plate was water cooled to an average temperature of 60 ^{o}C. Pressure at the die head was varied from 200 to 400 psi, depending on test diet. Pellets of 4 mm were produced then dried in a pulse-bed drier (Buhler AG, Uzwil, Switzerland) for 25 minutes at 102 ºC with a 10-minute cooling period. Final moisture levels were less than 10%. The final step was to top-coat the dried and cooled feed with fish oil using a

vacuum coater (A.J. Mixing, Ontario, Canada). Diets were randomly assigned to each of the 20 tanks. Fish were fed twice a day to apparent satiation, 6d/wk.

Measurements and Collections

Growth Performance: Throughout the study, fish were weighed every three weeks and feed conversion ratio (FCR), feed intake and weight gain were calculated.

Proximate Composition: Ten fish from the initial population were sacrificed for determination of initial whole-body proximate composition. At the conclusion of the study, four fish from each tank were taken for whole body composition.

Body Condition Indices: Three additional fish were sampled at the conclusion of the feeding trial for determination of hepatosomatic index (HSI), visceral somatic index (VSI), muscle ratio, and blood chemistry analysis.

Statistical Analysis

Tanks of fish were the experimental units. Proc GLM of SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was utilized for analysis of variance (ANOVA) with Tukey's means separations to determine differences within main effects. Treatment effects were considered significant at $P<0.05$.

RESULTS

Experiment 1

The analyzed proximate composition values of EY as compared to fish meal averages were lower in total protein content (Table 4) and lower in certain individual amino acids, specifically, the three most common limiting amino acids for rainbow trout, lysine, methionine, and threonine (Table 5).

Table 4. Chemical analyses of the macronutrients in Ethanol yeast and fish meal on a dry wt. basis

Item, %	Ethanol yeast	Fish meal[1]
DM	91.5	92.7
Crude Protein	52.0	68.0
Fat	3.9	8.0
Energy (cal/g)	5945.0	4709.3

[1]Mean values of menhaden fish meal, special select, USDA-ARS/USFWS Digestibility Database (Barrows et al., 2011)

Table 5. Chemical analyses of the amino acids in Ethanol yeast and fish meal on a dry wt. basis

Item, %	Ethanol yeast	Fish meal[2]
Alanine	3.7	4.7
Arginine	2.2	4.8
Aspartic acid	3.8	6.6
Glutamine	7.7	9.7
Glysine	1.8	5.2
Histidine	1.0	1.5
Isoleucine	2.0	2.9
Leucine	5.9	5.2
Lysine	2.3	4.6
Methionine	1.0	1.7
Phenylalanine	2.8	2.9
Proline	3.4	3.6
Serine	2.7	3.2
Threonine	2.2	3.3
Tyrosine	2.5	2.4

Table 5 – Continued

Valine	2.2	3.7
Sum AA	48.0	66.1

[1]Apparent availability coefficients
[2]Mean values of menhaden fish meal, special select, USDA-ARS/USFWS Digestibility Database (Barrows et al., 2011)

However, EY apparent digestibility coefficients and AACs compared to fish meal resulted in intriguing differences. Ethanol yeast ADCs for protein, DM, fat, and energy were 97.6, 65.4, 100.0, 69.7, respectively (Table 6). Ethanol yeast AACs for sum of amino acids, methionine, lysine, and threonine were 80.7, 88.1, 75.5, and 70.8%, respectively (Table 7).

Table 6. Ethanol yeast and fish meal ADCs[1] for rainbow trout on a dry wt. basis

Item, %	Ethanol yeast	Fish meal[2]
DM	65.4	77.7
Crude Protein	97.6	85.9
Fat	100.0	92.7
Energy	69.7	94.8
Phosphorus	80.7	43.8

[1]Apparent digestibility coefficients
[2]Mean values of menhaden fish meal, special select, USDA-ARS/USFWS Digestibility Database (Barrows et al., 2011)

Table 7. Ethanol yeast and fish meal AACs[1] for rainbow trout on a dry wt. basis

Item, %	Ethanol yeast	Fish meal[2]
Alanine	82.4	89.4
Arginine	80.8	91.4
Aspartic acid	72.1	87.6
Glutamine	83.6	95.3
Glysine	78.7	75.0
Histidine	78.9	92.1
Isoleucine	79.0	95.2
Leucine	84.0	97.2

Table 7 – Continued

Lysine	75.5	92.9
Methionine	88.1	94.9
Phenylalanine	89.4	89.5
Proline	83.0	84.8
Serine	76.2	91.6
Threonine	70.8	92.3
Tyrosine	85.0	95.9
Valine	79.2	93.4
Sum AA	80.7	90.9

[1]Apparent availability coefficients
[2]Mean values of menhaden fish meal, special select, USDA-ARS/USFWS Digestibility Database (Barrows et al., 2011)

Experiment 2

Results indicated that EY was unable to effectively replace 100% of the fish meal in the diets of juvenile rainbow trout. Weight gain decreased (Table 8 and Figure 3) when EY replaced more than 37.5% of dietary fish meal (11.2% EY inclusion level).

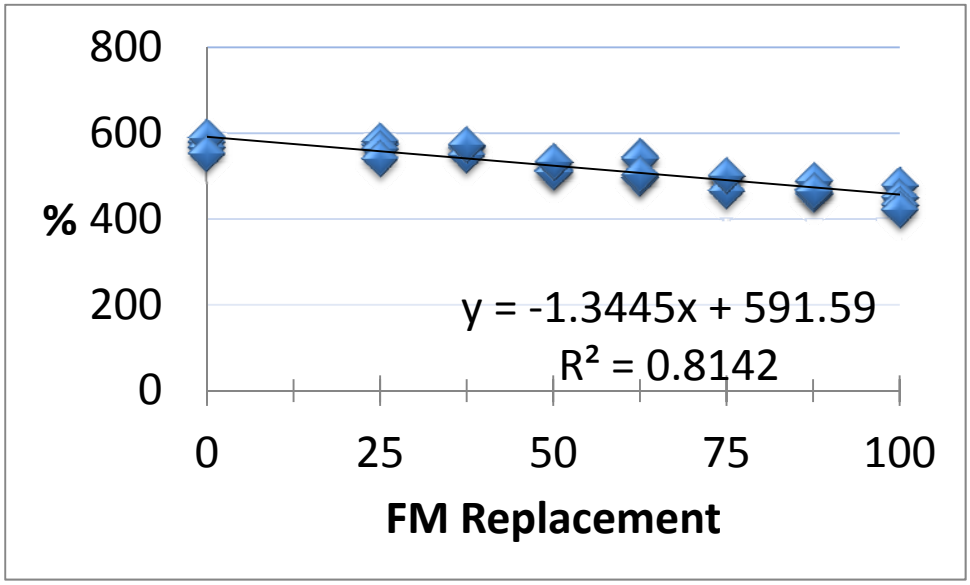

Figure 3. Weight gain (% increase) of rainbow trout fed diets with increasing levels of Ethanol yeast.

Feed intake as a percentage of BW was significantly greater in fish that were fed higher inclusion levels of EY (Table 8 and Figure 4).

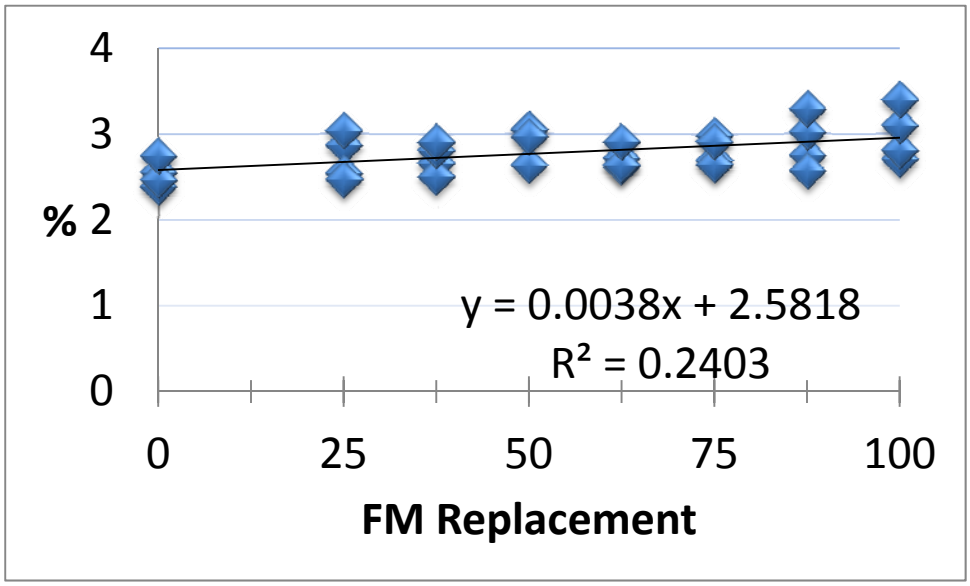

Figure 4. Feed intake (% BW) of fish fed diets with increasing levels of Ethanol yeast.

Reduced weight gain and increased feed intake therefore resulted in poorer feed conversion when trout were fed increasing amounts of EY (Table 8 and Figure 5).

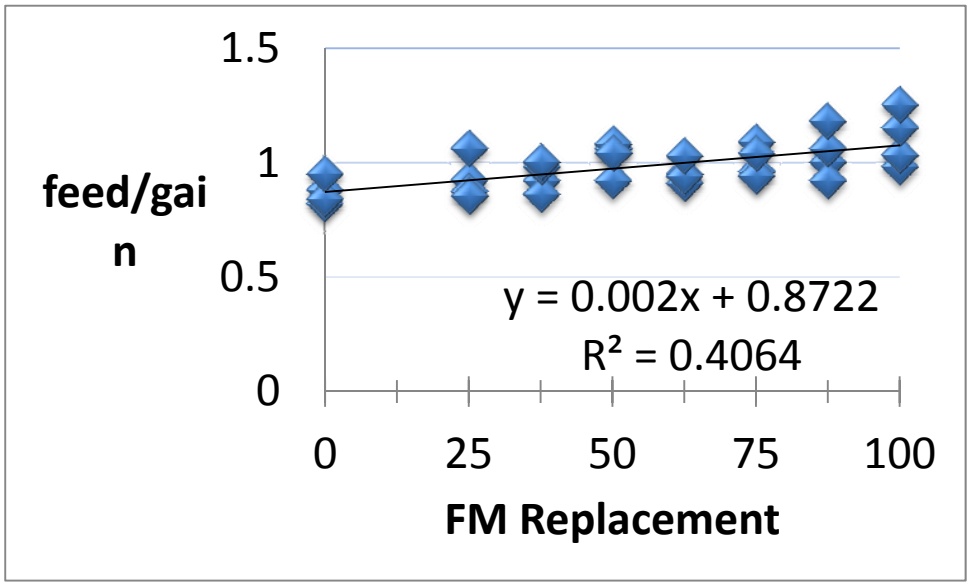

Figure 5. Feed conversion ratio (feed/gain) of fish fed diets with increasing levels of Ethanol yeast.

In contrast, no significant effects of EY inclusion were observed when measuring HSI and FR (Table 9). There was a significant difference in VSI (Table 8 and Figure 6).

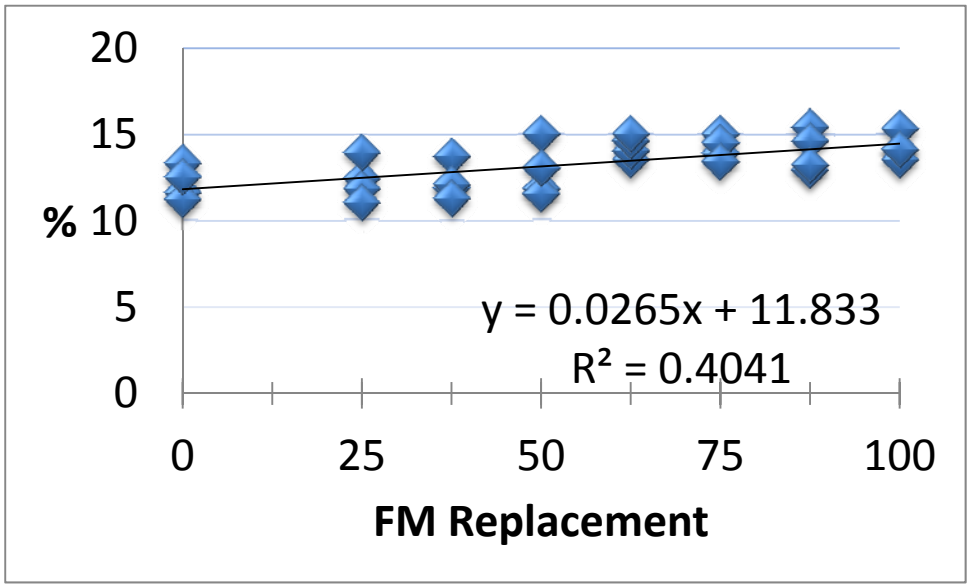

Figure 6. Comparison of the visceral somatic index of fish fed diets of increasing replacement of fish meal with Ethanol yeast.

No significant effects of EY inclusion were observed for whole body proximate composition of protein, fat, and energy (Table 9). Protein retention efficiency and ERE measurements were not significantly different when rainbow trout were fed diets with increasing inclusion levels of EY (Table 9 and Figure 7).

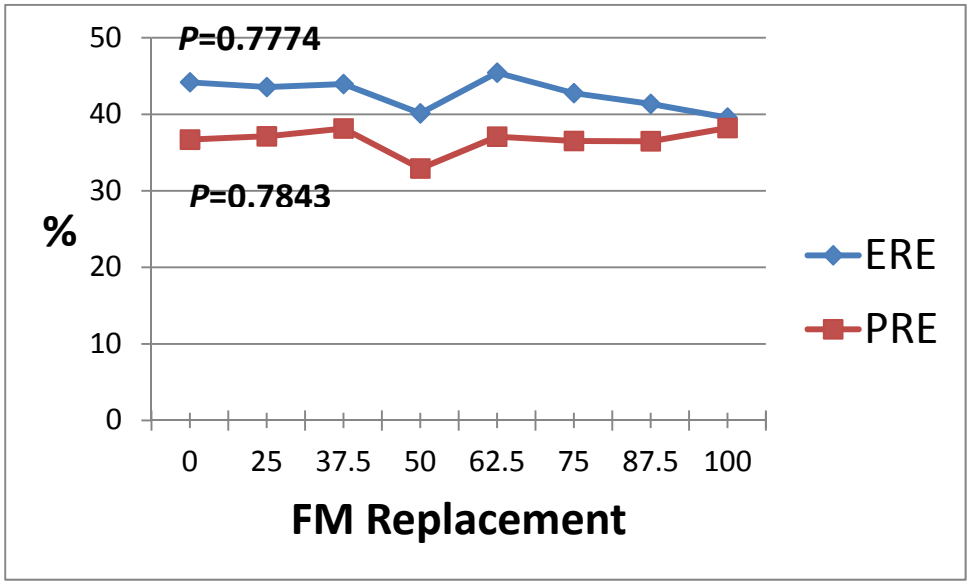

Figure 7. Comparison of protein retention efficiency and energy retention efficiency when fish were fed increasing levels of Ethanol yeast.

Diet digestibility values obtained by collecting fecal samples at the end of the study trial revealed a significant difference in protein digestibility (Figure 8). Protein digestibility increased with higher EY inclusion levels, but it was a very slight increase.

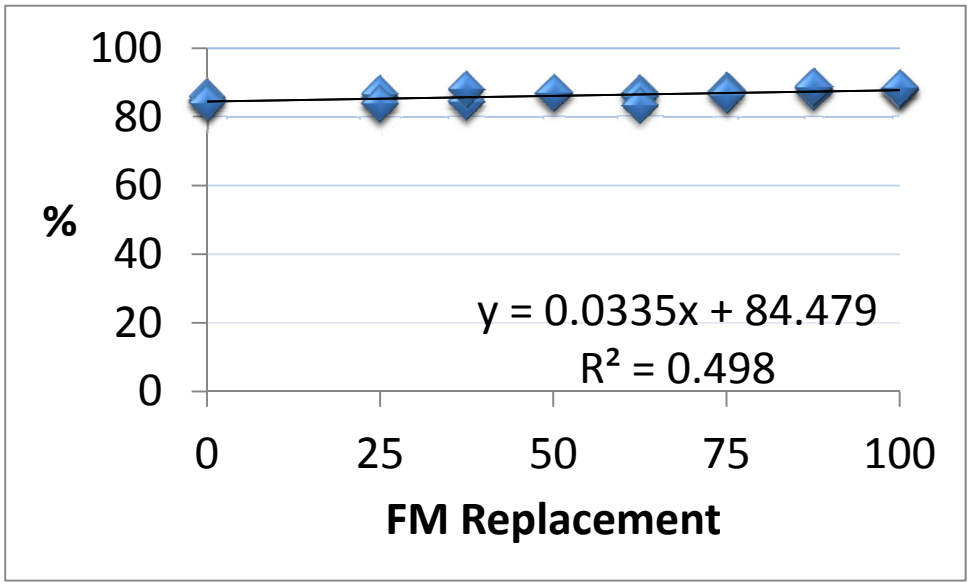

Figure 8. Protein digestibility of individual diets fed to rainbow trout with increasing levels of Ethanol yeast.

Pellet durability demonstrated a quadratic response where as EY was included at greater levels of the diet (Figure 9). There was increasingly more percent loss of the pellets within those diets.

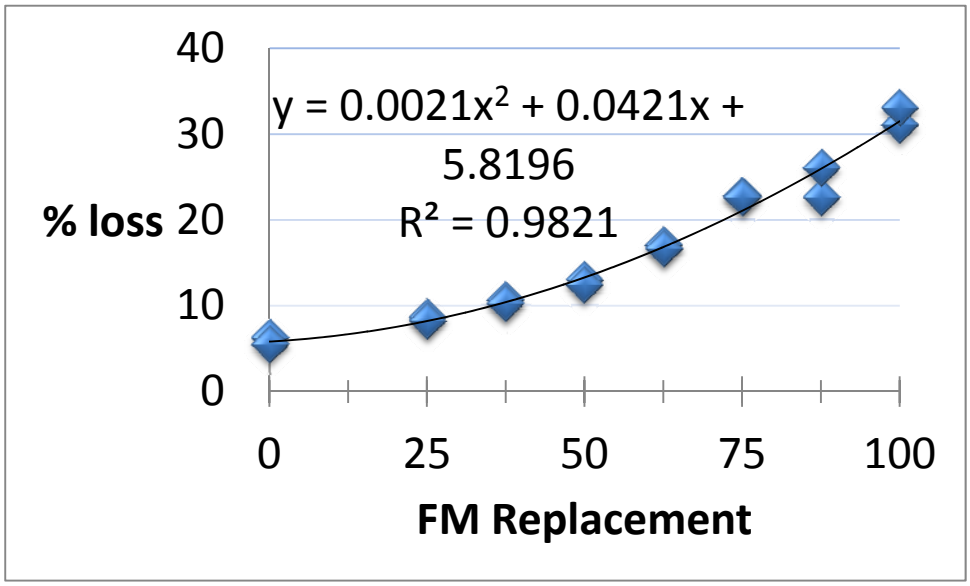

Figure 9. Pellet durability of diets with increasing levels of Ethanol yeast.

Table 8. Growth performance[1] and body indices[2] of rainbow trout fed diets containing 0, 25, 37.5, 50, 62.5, 75, 87.5 or 100% Ethanol yeast for 9 weeks

	Growth Performance[1]			Body indices[2]		
Diet[3]	Weight Gain[4] (% increase)	FCR[5] (g feed/g gain)	Feed intake (%)	Viscera Index (%)	Fillet Ratio (%)	Hepatosomatic Index (%)
0%	570[a]	0.87[c]	2.53	12.2[b]	53.8	1.3
25%	564[a]	0.93[b,c]	2.72	12.3[b]	51.4	1.3
37.5%	564[a]	0.94[b,c]	2.72	12.3[b]	54.1	1.2
50%	522[b]	1.03[a,b]	2.92	12.8[a,b]	53.3	1.2
62.5%	520[b]	0.96[b,c]	2.71	14.3[a]	53.0	1.4
75%	491[c]	1.01[a,b]	2.81	14.2[a]	50.5	1.3
87%	470[c,d]	1.04[a,b]	2.90	14.0[a]	51.7	1.4
100%	444[d]	1.10[a]	3.00	14.2[a]	50.2	1.3
Pooled SE	8.83	0.04	0.11	0.52	1.99	0.08
P>0.05	0.0001	0.0174	0.1525	0.0098	0.7734	0.3555

[1]Means of four replicate tanks (30 fish/tank).
[2]Means of (12 fish/treatment) for replicate tanks per diet.
[3]Percent of fish meal that is replaced by Ethanol yeast
[4](Final wt-initial wt)/100
[5]Feed conversion ratio (final wt-initial wt)/feed consumed

Table 9. Proximate composition and nutrient retention efficiency of rainbow trout fed diets containing 0, 25, 37.5, 50, 62.5, 75, 87.5 or 100% Ethanol yeast for 9 weeks[1]

Diet[2]	Moisture (%)	Fat (%)	Protein (%)	Energy (kcal/g)	Hematocrit (%)	PRE[3] (%)	ERE[4] (%)
0%	67.5	13.5	17.0	2228	41.4	36.7	44.2
25%	68.8	12.5	16.7	2171	46.8	37.1	43.6
37.5%	67.7	13.2	16.7	2267	47.8	38.2	44.0
50%	69.4	11.8	16.1	2087	38.9	32.9	40.1
62.5%	68.3	12.6	17.1	2190	48.3	37.1	45.4
75%	69.3	12.3	16.4	2179	45.8	36.5	42.8
87%	68.4	12.6	16.8	2234	41.6	36.5	41.4
100%	68.3	12.8	16.9	2215	45.9	38.2	39.6
Pooled SE	0.98	0.62	0.32	52.7	2.48	2.21	2.77
P>0.05	0.8371	0.6568	0.4536	0.4164	0.108	0.7843	0.7774

[1]Three fish/tank were sampled for laboratory analysis
[2]Percent of fish meal that is replaced by Ethanol yeast
[3]Protein retention efficiency

Experiment 3

In Exp. 3 we tested the efficacy of a mycotoxin inhibitor (Biofix Plus) and observed similar findings to Exp. 2 where there was reduced growth in fish that were fed higher inclusion levels of EY (Table 10 and Figures 10).

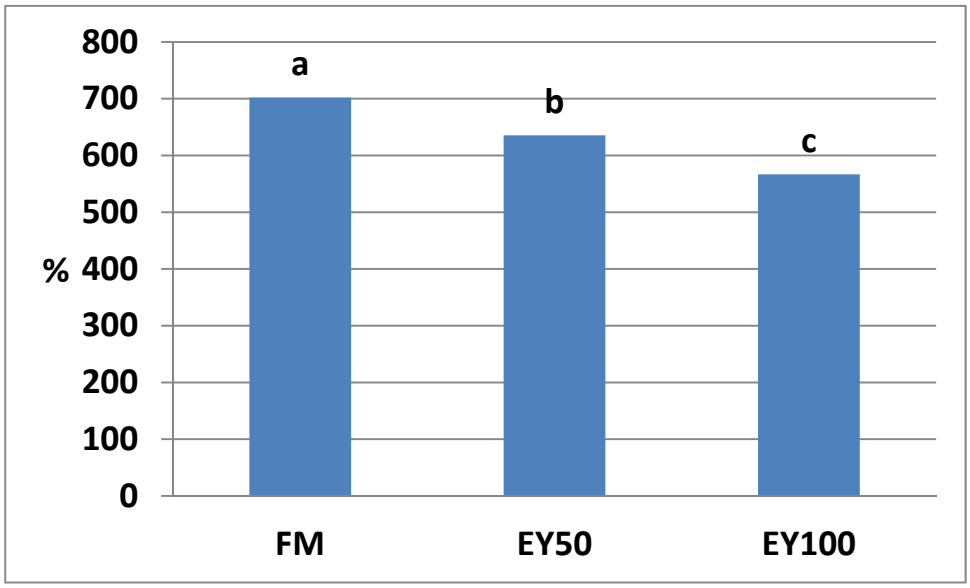

Figure 10. Weight gain (% increase) of fish fed diets replacing either 0, 50, or 100% of fish meal with Ethanol yeast and with or without Biofix Plus.

Findings in regard to feed intake, demonstrated that fish consumed more of the 100% replacement diet when compared to the 50% and 0% diets (Table 10 and Figure 11). This therefore resulted in fish fed the 100% replacement diet exhibiting higher FCRs than fish fed the 50% and 0% diets (Table 10 and Figure 12).

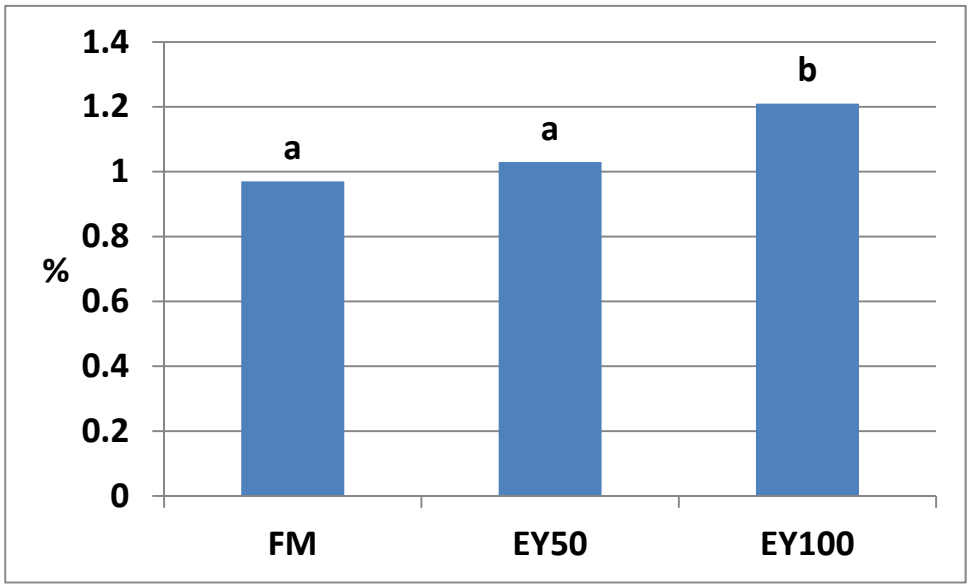

Figure 11. Feed intake (% BW) of fish fed diets replacing either 0, 50, or 100% of fish meal with Ethanol yeast and with or without Biofix Plus.

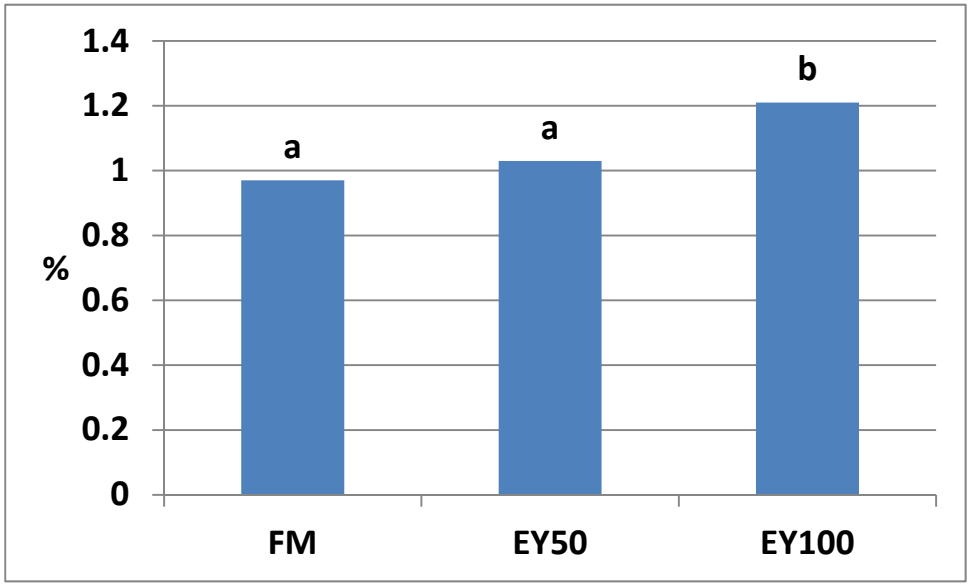

Figure 12. Feed conversion (feed/gain) of fish fed diets replacing either 0, 50, or 100% of fish meal with Ethanol yeast and with or without Biofix Plus.

No statistical benefit was found with the inclusion of the Biofix Plus additive within any of the treatments on growth performance of rainbow trout (Table 10). Neither

were there interactive effects between protein source and Biofix Plus on growth performance of rainbow trout. However, a trend was observed measuring an 8% increase in weight gain of fish fed the Biofix Plus additive (Figure 13).

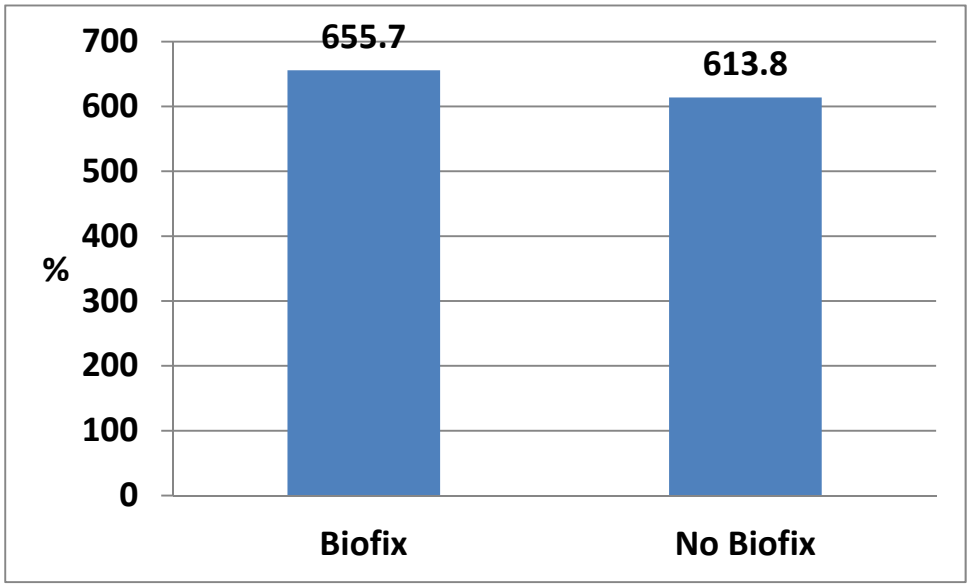

Figure 13. Effect of Biofix Plus on the weight gain of rainbow trout.

When measuring condition indicies, there were differences in HSI, VSI, and FR due to protein source (Table 10; Figures 14, 15, and 16), but no effect was found with supplementation of Biofix Plus and no significant interactions between binder and protein source were observed (Table 10). Hepatosomatic index increased as EY replaced more dietary fish meal protein in contrast to the findings of Exp. 2 where no differences were measured.

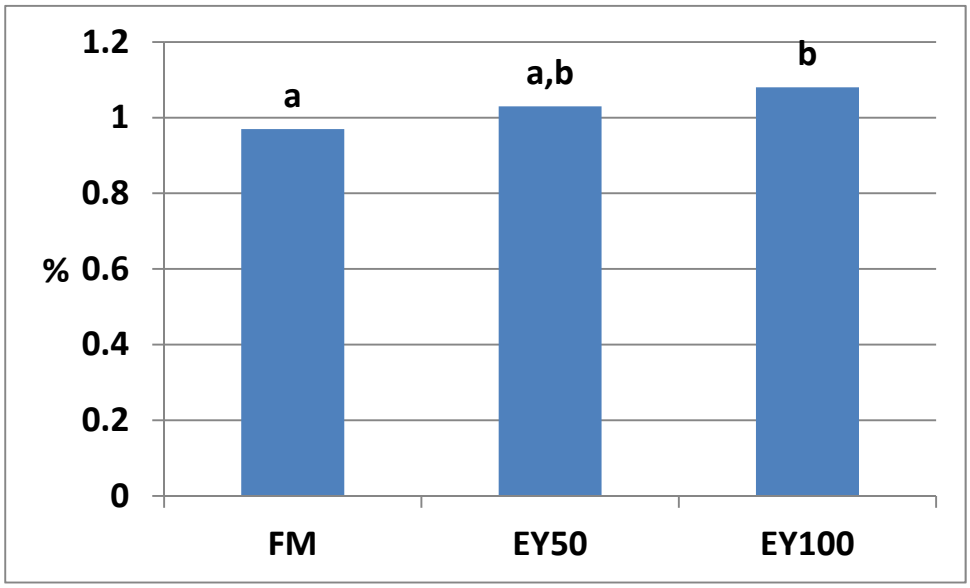

Figure 14. Comparison of hepatosomatic index of fish fed diets replacing 0, 50, or 100% of fish meal with Ethanol yeast and with or without Biofix Plus.

Visceral somatic index increased when EY replaced all dietary protein supplied by fish meal, but was not significantly different in the 50% replacement level. These results were similar to the findings in Exp. 2.

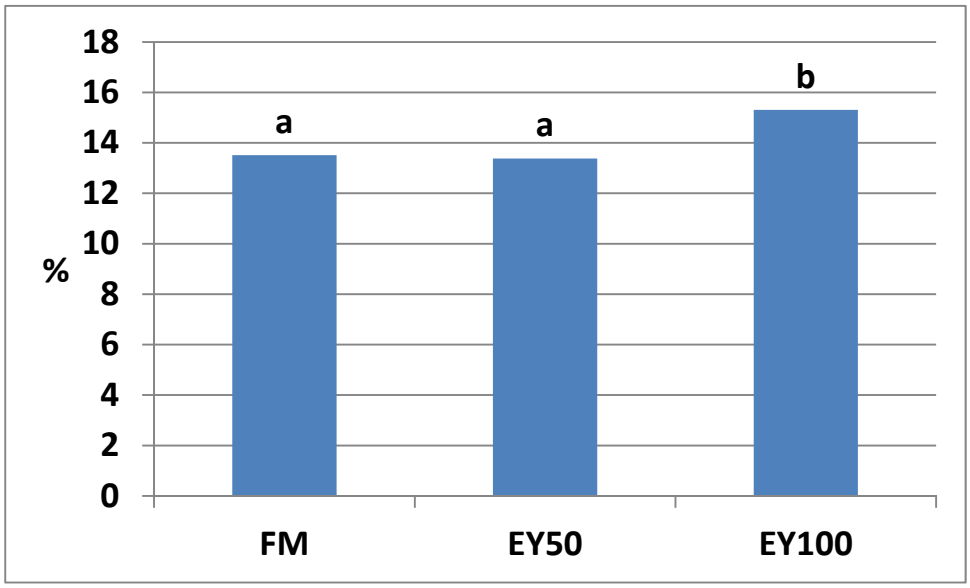

Figure 15. Comparison of the visceral somatic index of fish fed diets replacing 0, 50, or 100% of fish meal with Ethanol yeast and with or without Biofix Plus.

Decreased FRs were observed in fish fed diets where EY completely replaced fish meal, but no statistical differences were observed at the 50% replacement level. These data also were different than in Exp. 2 where no differences were observed in FR with increasing levels of EY.

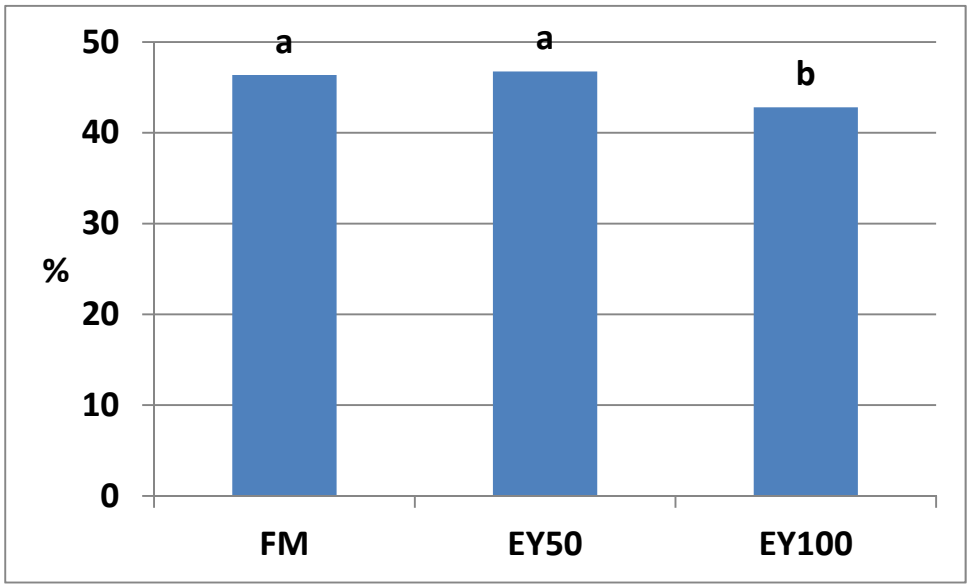

Figure 16. Comparison of fillet ratio of fish fed diets replacing 0, 50, or 100% of Ethanol yeast and with or without Biofix Plus.

No significant differences were found due to protein source, Biofix Plus, or interactions for whole body proximate composition (Tables 11). Whole body protein, lipid, energy and DM ranged from 14.6-17.3%, 10.3-15.2%, 1941.8-2624.8 kcal/g, and 65.2-71.5%, respectively. When looking at PRE and ERE there was only a significant difference found with an interactive effect on protein retention efficiency (Table 11; Figure 17).

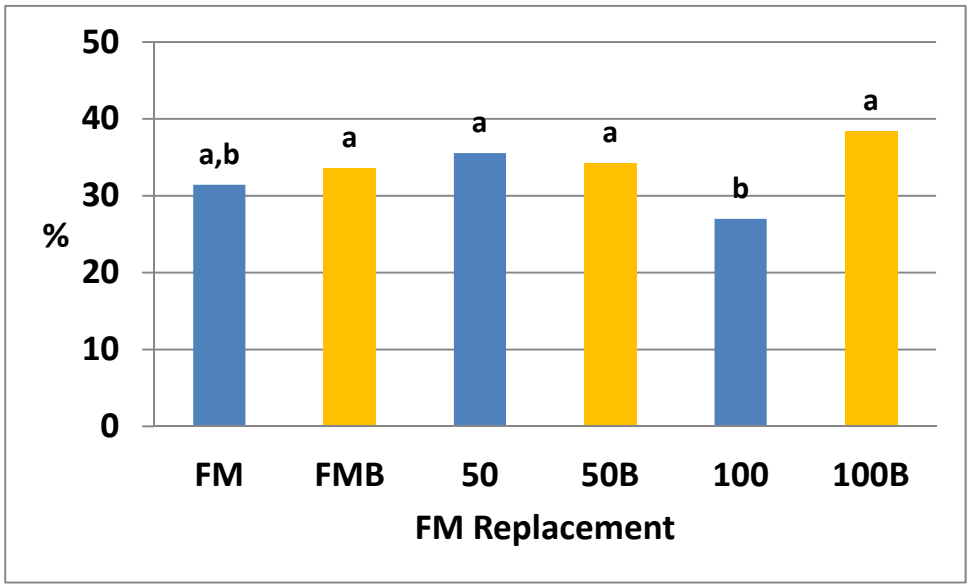

Figure 17. Interaction with protein source and Biofix Plus when measuring protein retention efficiency.

Table 10. Growth performance1 and body indices2 of rainbow trout fed diets containing 0, 50 or 100% Ethanol yeast with or without Biofix Plus for 12 weeks

	Growth Performance			Condition Indices		
Diet[3]	Weight Gain[4] (% increase)	FCR[5] (g feed/g gain)	Feed Intake (%)	Visceral Index (%)	Fillet Ratio (%)	Hepatosomatic Index (%)
0% w/o	734[a]	0.9[a]	2.2[a]	31.5	53.5	2.3
0% w	670[a]	1.0[a]	2.3[a]	28.8	48.5	2.2
50% w/o	672[b]	1.0[a]	2.3[a]	30.9	51.0	2.2
50% w	599[b]	1.0[a]	2.3[a]	29.0	50.5	2.3
100% w/o	561[c]	1.2[b]	2.6[b]	26.2	48.0	2.0
100% w	572[c]	1.2[b]	2.7[b]	27.7	49.8	2.3
Pooled SE	26.568	0.040	0.069	1.089	2.363	0.071
			P-values			
Protein	0.001	0.0002	0.0002	0.0053	0.0099	0.0350
Biofix	0.0773	0.2480	0.3136	0.9371	0.3199	0.5453
Protein*Biofix	0.2604	0.8825	0.7526	0.3460	0.4677	0.3093

Table 10 - Continued

[1]Means of four replicate tanks (30 fish/tank).
[2]Means of (9 fish/treatment) for replicate tanks per diet.
[3]Percent of fish meal that is replaced by Ethanol yeast; w/o=without Biofix Plus, w=with Biofix Plus
[4](Final wt-initial wt)/100
[5]Feed conversion ratio (final wt-initial wt)/feed consumed

Table 11. Proximate composition and nutrient retention efficiency of rainbow trout fed diets containing 0, 50, or 100% Ethanol yeast with or without Biofix Plus for 12 weeks[1]

Diet[2]	Moisture (%)	Fat (%)	Protein (%)	Energy (kcal/g)	PRE[3] (%)	ERE[4] (%)
0% w/o	69.3	12.3	16.2	2073.8	31.4	21.5
0% w	69.3	12.5	16.1	2120.2	33.6	24.1
50% w/o	70.2	11.4	16.1	2069.5	35.6	22.9
50% w	70.1	12.4	16.2	2277.8	34.3	23.0
100% w/o	69.2	13.2	15.5	2118.7	27.0	15.8
100% w	68.0	13.8	15.7	2134.4	38.5	27.6
Pooled SE	0.817	0.669	0.460	89.0	2.357	2.741
			P-values			
Protein	0.2370	0.0968	0.4122	0.6935	0.5404	0.8878
Biofix	0.5321	0.2929	0.8723	0.2385	0.0527	0.0538
Protein*Biofix	0.7598	0.8544	0.9327	0.5270	0.0499	0.1214

[1]Three fish/tank were sampled for laboratory analysis
[2]Percent of fish meal that is replaced by Ethanol yeast; w/o=without Biofix Plus, w=with Biofix Plus
[3]Protein retention efficiency
[4]Energy retention efficiency

DISCUSSION

Ethanol yeast showed improved availability of protein, fat and phosphorus when compared to the average fish meal ADCs found in USDA ARS/USFWS Digestibility Database (Barrows et al., 2011). The protein digestibility of EY was also higher than the average fish meals analyzed by Gaylord et al. (2008) and was comparable to some of the plant concentrate ingredients in that study that were tested. Ethanol yeast protein digestibility was similar to anchovy fish meal (97%), soy protein concentrate (99%) and wheat gluten meal (100%). EY apparent digestibility coefficients for protein were higher than all other ingredients that were reported by Gaylord et al. (2008). Ethanol yeast results also show improved protein digestibility when compared to BDY reported by Rumsey et al. (1991a).

Ethanol yeast AAC values were lower than the fish meals reported by Gaylord et al. (2010). Among the five fish meals tested, the AACs of the ten EAAs for rainbow trout were relatively high ranging from 89% to 101% and the sum of amino acids reached no lower than 92% (Gaylord et al., 2010). Ethanol yeast AACs for the ten EAAs ranged from 79% to 89% with the sum of amino acids totaling to 81%. Ethanol yeast amino acid availability was similar to other alternative proteins AACs reported by Gaylord et al. (2010) including whole wheat (81%), poultry by-product meal (84%) and rice protein concentrate (86%).

The lower AACs for specific amino acids in the digestibility trial may, in part, explain the decreased growth performance of rainbow trout in the feeding trials. The current feeding trial results are similar to the findings of Martin et al. (1993) who

demonstrated that yeast biomass could replace up to 35% of dietary fish meal for rainbow trout. The data from the current study is also consistent with other single-cell protein experiments involving other species of fish (Gause and Trushenski, 2011a; Lunger et al., 2005) where total replacement of fish meal was unattainable. However, the failure of these single-cell protein ingredients as complete fish meal replacers was attributed by the authors as an issue with palatability. In these studies, decreased intakes were observed in diets with higher inclusions of yeast product relative to their control (fish meal) diets. Results from the current study demonstrated that there was no relationship between palatability and the performance of rainbow trout being fed each of the experimental diets.

Similar findings were found with our condition indices and proximate composition measurements as they relate to existing literature (Gause and Trushenski 2011, Snyder et al., 2012). Gause and Trushenski (2011) reported increased liposomatic index's and increased fat proximate composition in fish fed higher inclusion levels of EY. The authors hypothesized that the fish were compensating for amino acid imbalances thus there was reduced accumulation of lean muscle mass and a relative increase in adiposity. Snyder et al. (2012) also reported higher intraperitoneal fat in fish fed isolated soy protein-based diets containing excess branched chain amino acids (BCAAs) and suggested that BCAAs may have caused overeating as fish increased consumption to try and meet EAA requirements. These findings could explain why there was increased consumption and increased VSI responses in fish fed higher levels of EY during the current study trials. The reduction in FR found in Exp. 3 further supports the assumption

that fish may have been putting excess energy into their visceral cavities and not into muscle production.

Findings from the current trial also showed no differences in PRE and ERE even though growth was reduced. One explanation for this discrepancy may be non-protein nitrogen present in EY in the form of the nucleic acid. Rumsey et al. (1991) reported that most BDY products were approximately 20-25% nucleic acid. Rainbow trout utilize nucleic acid nitrogen differently than they utilize nitrogen supplied from amino acids. In monogastric animals, nucleic acid content results in the formation of uric acid in the blood. Uric acid is further catabolized by the enzyme uricase into allatoin which is further degraded into urea and glyoxylic acid which are both excreted in the urine (Rumsey et al., 1991). This may explain why the sum of amino acids in EY are less available than crude protein digestibility and may be the reason for finding no differences in PRE and ERE when fish were fed treatment diets compared to the control. We did not measure the nucleic acid content of EY, but the findings of Rumsey et al. (1991) suggests that EY may be fairly high in nucleic acid and the loss of this nitrogen through the urine and gill excretions was not captured in our digestibility trial.

Alternatively, the decreased growth could be attributable to alterations in pellet quality. Previous authors have suggested that pellet quality alters rate of passage in rainbow trout (Aas et al., 2011) subsequently altering the trout's ability to utilize nutrients present in the diet. Of specific concern in terms of alternative protein is the potential for the crystalline amino acids used in the current study to be up-taken at a faster rate that the plant-based protein amino acids which could potentially create a time-

based imbalance even though the diet was complete. However, the multiple feedings (2X/day) used in the current study likely negated any unequal absorption effects. Gaylord and Barrows (2009) previously demonstrated that plasma amino acids peaked 12-15 hr post-feeding, thus 2X day feeding should have maintained available synthetics at appropriate levels.

Yet another hypothesis to explain the decreased performance observed with increasing amounts of EY in Exp. 2 is the presence of low-level mycotoxin contamination. Ethanol yeast comes from by-product of ethanol fuel production which is derived from corn. Corn is the number one source of mycotoxin contamination (Whitlow et al., 1998). Ethanol yeast was analyzed for mycotoxins (Table 5.1) and found to contain low levels of ochratoxin A, deoxynivalenol, zearalenone, fumonsin B1 and fumonsin B3. Although below those levels previously reported to cause mycotoxicosis in rainbow trout, it is possible that with continued feeding that subclinical decreases in growth performance like those observed in Exp. 2 could result. However, the current mycotoxin inhibitor study's results do not concur with Agouz and Anwer (2010) nor with Abdelaziz et al. (2010) who both found significant increases in performance with dietary inclusion their specific mycotoxin binder products. These contrasting results may be because the levels of mycotoxin contamination in our treatment diets were lower than in the studies of Agouz and Anwer 2010 and Abdelaziz et al. (2010) thus limiting our ability to detect small differences between the treatments. Of note, even though there was no statistical significance of including Biofix Plus on improving growth of rainbow trout fed EY, there still might be some benefit of including a mycotoxin inhibitor in

rainbow trout diets known or suspected to be contaminated with mycotoxins since an interaction with Biofix Plus and protein source was observed for PRE and a similar trend was observed in regards to improvements in growth with Biofix Plus supplementation.

CONCLUSIONS

Identification of appropriate alternative proteins that can mitigate feed price fluctuations and reduce the dependence of commercial rainbow trout production on fish meal is necessary to ensure sustainability of the industry. Towards achieving that goal, the objective of this thesis research was to investigate factors that affect the use of a novel alternative protein, EY, including protein concentration, protein digestibility, digestible energy value, and the presence and level of anti-nutrients.

Results from Exp. 1 demonstrated that EY is moderately high in protein with an acceptable amino acid profile and further that these nutrients were available for rainbow trout (protein ADC of approximately 98%). However, it was observed that the protein source would require supplementation of methionine, lysine and threonine to account for amino acid deficiencies and lower amino acid availability coefficients of these three nutrients which are common limiting amino acids in low or zero fish meal trout diets.

Results from Exp. 2 demonstrated that although nutrients were available, feeding high levels of EY resulted in reduced performance and poorer FCRs. However, this was not associated with palatability because fish actually consumed more of the diets that contained higher inclusion levels of EY. Protein digestibility among diets was the same and all diets were balanced for equal amounts of available methionine, lysine, threonine and phosphorus so reduced performance could likely not be attributed to any of these factors.

Results from Exp. 3 demonstrated no statistical benefit of a mycotoxin inhibitor inclusion. Thus suggesting that the decreased performance observed when EY was most likely not due to low-level mycotoxin contamination.

Results from these experiments might suggest that the reduced growth of fish fed EY with our fish could be the result of an amino acid deficiency. Lending additional support for this theory is the analyzed amino acid of EY diets as compared to their ability to provide the ideal protein balance defined as the rainbow trout muscle amino acid profile. There were several other amino acids in EY that were much lower in content and lower in availability than that of a common menhaden fish meal. Therefore, future research should address whether supplementation of the fourth or fifth limiting amino acids improves growth and performance of rainbow trout fed high levels of EY along with low levels of fish meal.

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