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ABSTRACT

Grain distillers dried yeast (GDDY) is a single-cell protein obtained as a co-product during the production of fuel ethanol that may have potential as a protein replacement for rainbow trout. The goal of this study was to examine the suitability of GDDY as a replacement for fish meal on a digestible protein basis in rainbow trout diets. An *in-vivo* digestibility study was performed to determine the nutrient availability of GDDY. Subsequently, a control diet containing 42% digestible protein and 20% lipid was formulated to replace fish meal protein with GDDY protein at eight different levels (0, 25, 37.5, 50, 62.5, 75, 87.5, and 100%). Diets were fed to juvenile rainbow trout stocked into four replicate tanks per dietary treatment (30 fish/tank) and fed twice daily for nine weeks. High GDDY inclusion rates significantly altered rainbow trout growth and feed conversion but not feed intake. There were no significant differences in production performance in fish fed the 25% GDDY and 37.5% GDDY diets when compared to fish fed the control diet, but further dietary fish meal replacement generally decreased fish performance. Further research is warranted to determine why fish performance decreased with higher inclusion levels of GDDY in spite of similar feed intake among levels.

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1. Introduction

In the past few years, the aquaculture industry has seen an intensive rise in feed costs, particularly for carnivorous species due to competition for limited supplies of fish meal (Tacon and Metian, 2008). This has resulted in renewed vigor for development and examination of alternative feed ingredients, specifically protein sources that may be more sustainable and less expensive (Gatlin et al., 2007). The utilization of by-products from the alternative fuel industries also has been of increasing interest due to potential for increased future volumes of these products (Barrows et al., 2008). Specifically, two feed grade by-products have been investigated and these include distillers dried grains with solubles (DDGS), the grain-based by-product and the spent yeast fraction, grain distillers dried yeast (GDDY). Although, recent studies have address the suitability of DDGS inclusion in rainbow trout diets (Barnes et al., 2012a,b; Cheng and Hardy, 2004; Øverland et al., 2013), the suitability of GDDY for rainbow trout has not been addressed.

Research on the effects of yeast products in the diets of rainbow trout has focused on their role as immune-stimulants rather than macro-nutrient sources at inclusion rates that were predominantly less than 5% of diet (Gatesoupe, 2007). However, selected single-cell proteins have shown potential as dietary protein sources. Rumsey et al. (1991a) determined the digestibility of brewer's dried yeast (BDY) processed by different methods in rainbow trout and then measured growth performance when BDY was included as the primary protein source in 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 a fish meal-free diet without decreases in weight gain or FCR when casein was the primary protein source. When BDY was included above 25%, however, the fish exhibited poor growth and FCR. Similarly, 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. Limited replacement (25%) of fish meal by another commercial yeast product, NuPro™ (Alltech Inc., Lexington, KY, USA), has also been reported in cobia diets (Lunger et al., 2006). Lunger et al (2006) were able to increase NuPro™ inclusion levels in cobia diets

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when amino acid and taurine were supplemented as fish meal declined. NuPro™ also has shown promise when fed to tilapia, an omnivorous fish, by replacing all of the dietary fish meal without affecting growth (Craig and McLean, 2005).

Grain distillers dried yeast (GDDY) is a single-cell protein source produced as a by-product of the renewable fuel industry. Grain distillers dried yeast is a co-product from the wet mill fermentation of ethanol from corn. It is the yeast fraction suspended in the distilled fermentation media and separated from corn gluten meal. The protein and amino acid content make it conducive to inclusion in high protein aquafeeds and potentially as a partial replacement for the protein and amino acids from fish meals (Table 1). Recently, GDDY was examined for its potential to replace fish meal in sunshine bass, *Morone chrysops* × *Morone saxatilis*, diets (Gause and Trushenski, 2011a,b). During a 45-day feeding trial, Gause and Trushenski (2011a) suggested that GDDY could replace up to 75% of the protein provided by fish meal in the control diet of 30% menhaden fish meal without having a negative impact on growth rate and FCR. During a 63-day feeding trial, these authors also found that reducing fish meal to 7.5% of the diet could be accomplished utilizing GDDY (Gause and Trushenski, 2011b).

Although GDDY has shown promise as a protein source in feeds for sunshine bass, no work has been performed with rainbow trout and several aspects need to be addressed in order to confidently utilize the product in formulations. Therefore, the objectives of this study were to determine the digestibility of nutrients in GDDY for rainbow trout and to evaluate GDDY's ability to support production performance of rainbow trout as GDDY replaces fish meal protein in the feeds.

2. Materials and methods

2.1. Experiments

Two studies were conducted to evaluate the suitability of GDDY as a protein source for rainbow trout. The first study consisted of an *in vivo* digestibility assessment to determine apparent digestibility coefficients (ADCs) for protein, lipid and energy and apparent availability coefficients (AACs) for amino acids and phosphorus. The second study consisted of a nine-week feeding trial with growth performance, nutrient retention, feed conversion, diet palatability (determined as relative feed consumption), and diet digestibility as response variables. Fish in these studies were handled and treated in accordance with guidelines approved by the U.S. Fish and Wildlife Service.

Table 1
Composition of digestibility reference diet (% dry-weight) fed to rainbow trout.

Ingredients	%
Wheat flour[a]	28.3
Squid meal	25.0
Soy protein concentrate[b]	17.1
Fish oil[c]	13.4
Corn gluten meal[d]	8.3
Soybean meal[e]	4.3
Vitamin premix ARS[f]	1.0
Chromic oxide[g]	1.0
Choline chloride[g]	0.6
Taurine[h]	0.5
Stay-C 35[i]	0.2
Trace mineral premix[j]	0.1
Yttrium oxide[g]	0.1

[a] Archer Daniels Midland (Decatur, IL, USA) 4 g/kg protein.
[b] Solae Profine VP (St. Louis, MO, USA) 693 g/kg crude protein.
[c] Omega Proteins Inc. (Houston, TX, USA).
[d] Cargill Animal Nutrition (Minneapolis, MN, USA), 601.0 g/kg protein.
[e] Archer Daniels Midland (Decatur, IL, USA), 480 g/kg protein.
[f] Contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 g: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyanocobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inositol 600.
[g] Sigma-Aldrich Company (St. Louis, MO, USA).
[h] Archer Daniel Midlands (Decatur, IL, USA).
[i] Rovimix Stay-C 35 (DSM).
[j] Contributed in mg/kg of diet; zinc 40; manganese 13; iodine 5; copper 9.

2.2. In vivo ingredient digestibility determinations

2.2.1. Study design

The methods of Cho et al. (1982) and Bureau et al. (1999) were used with additional modifications described below to estimate ADCs of GDDY in rainbow trout. A complete reference diet (Table 1) meeting or exceeding all known nutritional requirements of rainbow trout (National Research Council, NRC, 2011) with yttrium oxide as indigestible marker was blended with the test ingredient (GDDY) in a 70:30 ratio (dry-weight basis). Both the reference and test diet were manufactured by cooking extrusion.

2.2.2. Fish

Fish (approximately 300 g ± 23 g, mean ± SD initial weight) were stocked in tanks at 20 fish per 200-L-poly tanks. Water temperature was maintained at 14 °C in a recirculating system. Lighting was maintained on a 13:11 h diurnal cycle. Diets were randomly assigned to three replicate tanks of fish. Fish were fed twice daily to apparent satiation beginning 14 days prior to the first fecal collection.

2.2.3. Feces collections

Feces from fish in each replicate tank were obtained by manual stripping (Austreng, 1978). In brief, all fish in each tank were netted, anesthetized with MS-222 (tricaine methanesulfonate, Western Chemical Company, Ferndale, Washington, USA), dried and gentle pressure was applied 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 freeze-dried, ground with a mortar and pestle, and stored at −20 °C until chemical analyses (described below) were performed.

2.2.4. Analytical methods

Dry matter analysis of ingredients, diets and feces was performed according to standard methods (AOAC, 1995). Yttrium and phosphorus were determined in diets and feces by inductively coupled plasma atomic absorption spectrophotometry following nitric acid digestion (Anderson, 1996). Crude protein (N × 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 6300, Parr Instrument Company Inc., Moline, Illinois, USA). Lipid was determined by petroleum ether extraction using an Ankom XT10 (Ankom Technologies, Macedon, New York, USA). Diet, ingredient and fecal amino acids were quantified following acid hydrolysis utilizing a Beckman 7300 amino acid analyzer and post-column derivitization with ninhydrin (AAA Laboratory, Mercer Island, WA, USA).

2.2.5. Digestibility equations

Apparent digestibility coefficients of each nutrient in the test diet and GDDY were calculated according to established equations (Forster, 1999; Kleiber, 1961):

$$\text{ADCN diet} = 100 \times \frac{(\%\,\text{marker in diet} \times \%\,\text{nutrient in feces})}{(\%\,\text{marker in feces} \times \%\,\text{nutrient in diet})}$$

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

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 $(1 - p) \times$ nutrient content of the reference diet
b $p \times$ nutrient content of the test ingredient
p proportion of test ingredient in the test diet.

2.3. Feeding trial

2.3.1. Study design

A 9-week feeding trial with juvenile rainbow trout was conducted to examine the effects of replacing fish meal protein on a digestible protein basis with increasing levels of GDDY protein (0, 25, 37.5, 50, 62.5, 75, 87.5, and 100% replacement; Table 2) on growth performance, nutrient retention and diet digestibility. All treatments were randomly assigned to four replicate tanks each, making tank the experimental unit. Test diets were formulated to contain 42% digestible protein and 20% crude lipid and were balanced for available lysine (3.82), methionine (1.30), and threonine (2.14), as well as total phosphorus (1.5). Nutrient targets were formulated on a digestible nutrient basis utilizing data from trial 1.

2.3.2. Diet manufacturing

Diets were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18-s exposure to an average of 127 °C in the sixth extruder barrel section. The die plate was water cooled to an average temperature of 60 °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 min at 102 °C with a 10-minute cooling period. Final moisture levels of each diet were less than 10%. Fish oil was top-coated after drying and cooling using a vacuum coater (A.J. Mixing, Ontario, Canada).

2.3.3. Pellet durability testing

Pellet durability was assessed using a NHP100 portable pellet durability tester (Holmen, Norfolk, UK). Briefly, approximately 50 g of pellets were loaded into the test chamber which cascades the pellets in an air stream causing them to collide with each other and the perforated hard surfaces within the test chamber at a test cycle of 60 s. After the test cycle, sample pellets are ejected from the tester for manual weighing. The pellet durability index (PDI) is the difference between pellet weight before and after the test expressed as a percentage of initial weight. PDIs were determined on duplicate pellet samples from each diet within seven days of diet manufacture.

Table 2
Composition of test diets containing grain distillers dried yeast (GDDY) to replace fish meal fed to juvenile rainbow trout (22.1 ± 0.3 g) for 9 weeks.

	Diets[a]							
Ingredients	0%	25%	37.5%	50%	62.5%	75%	87.5%	100%
Grain distillers dried yeast[b]	0.0	7.6	11.2	14.9	18.6	22.3	25.9	29.6
Menhaden fish meal, special select[c]	25.0	18.8	15.6	12.5	9.4	6.3	3.1	0.0
Corn protein concentrate[d]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Blood meal[e]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Soy bean meal, solvent extracted de-hulled[c]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Poultry by-product meal, pet food grade[c]	16.3	16.3	16.3	16.3	16.3	16.3	16.3	16.3
Wheat flour[e]	14.5	10.8	9.5	8.3	6.9	5.7	4.4	3.1
Fish oil, menhaden[c]	14.6	14.7	14.8	14.9	15.0	15.1	15.2	15.3
Lecithin[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay-C 35[f]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix[g]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Trace mineral premix[h]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium chloride	0.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Magnesium oxide	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Potassium chloride	0.0	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Dicalcium phosphate	0.0	0.9	1.4	1.9	2.4	2.8	3.3	3.8
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DL-Met[i]	0.4	0.5	0.5	0.5	0.5	0.6	0.6	0.6
Lysine HCl[i]	1.9	2.1	2.2	2.2	2.3	2.4	2.5	2.6
Threonine[i]	0.4	0.5	0.5	0.6	0.6	0.6	0.7	0.7
Taurine[i]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Yttrium oxide	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Analyzed composition[j]								
% crude protein	50.8	49.8	49.5	49.2	49.1	48.7	48.4	48.5
% lipid	18.0	18.9	19.2	18.7	18.9	19.5	20.1	19.4
Gross energy kcal/g	5533	5606	5629	5633	5679	5727	5762	5804
% moisture	2.3	2.4	2.8	2.9	3.4	3.6	3.9	4.2

[a] Percent of fish meal replaced by GDDY on a digestible protein basis.
[b] Archer Daniels Midland (Decatur, IL, USA).
[c] Nelson & Sons Inc. (Murray, UT, USA).
[d] Gavilon LLC (Omaha, NE, USA).
[e] MGP Ingredients, Inc. (Atchison, KS, USA).
[f] Rovimix Stay-C 35, DSM Products.
[g] Contributed per kg of diet: vitamin A (as retinol palmitate), 30,000 IU; vitamin D_3, 2160 IU; vitamin E (as DL-%-tocopheryl-acetate), 1590 IU; niacin, 990 mg; calcium pantothenate, 480 mg; riboflavin, 240 mg; thiamin mononitrate, 150 mg; pyridoxine hydrochloride, 135 mg; menadione sodium bisulfate, 75 mg; folacin, 39 mg; biotin, 3 mg; vitamin B_{12}, 90 μg.
[h] Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3; selenium, 0.4.
[i] L-Lysine HCL 99% feed grade, DL-methionine 99% feed grade, threonine L-threonine 98.5% feed grade, taurine ADM (Decatur, IL, USA).
[j] Means of two replicate samples per diet on a dry matter basis.

2.3.4. Fish culture

Rainbow trout from a single lot were obtained from a commercial producer (Troutlodge, Inc., Sumner, WA, USA) and cultured at the Bozeman Fish Technology Center, Bozeman, MT. Fish were stocked at 30 fish/tank (22.1 ± 0.26 g, mean ± SD initial weight). 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. Diets were randomly assigned to four tanks per dietary treatment in 32, 200 L tanks. Tanks were configured in a partial recirculating system with biofiltration, solids removal and UV treatment of the water. Approximately 25% makeup water was added to the system daily, and water temperature was maintained at 14 °C. Fish were fed to apparent satiation twice a day, six days a week for nine weeks and feed intake was determined by weighing buckets before and after feeding. Apparent satiation was defined as all the feed the fish will consume in a 30 min period.

2.3.5. Sampling and index calculations

Ten fish from the initial population were sacrificed for determination of initial whole-body proximate composition. During the growth trial, fish were weighed every two weeks for the determination of FCR, feed intake and weight gain. At the conclusion of the study, three fish from each tank were randomly selected for whole body composition and three additional fish were dissected for determination of hepatosomatic index (HSI), visceral somatic index (VSI), and filet ratio (FR).

$$\text{Hepatosomatic Index(HSI)} = \frac{(\text{Liver mass (g)} * 100)}{\text{fish mass(g)}}$$

$$\text{Viscerosomatic Index (VSI)} = \frac{(\text{viscera mass (g)} * 100)}{\text{fish mass (g)}}$$

$$\text{Filet Ratio (FR)} = \frac{(\text{filet mass with ribs (g)} * 100)}{\text{fish mass (g)}}$$

$$\text{Energy retention efficiency(ERE)} = \text{energy gain in fish(g)/energy intake (g)} * 100$$

$$\text{Protein retention efficiency(PRE)} = \text{protein gain in fish(g)/protein intake(g)} * 100$$

2.3.6. Statistical analysis

Response data were subjected to analysis of variance (ANOVA) and regression analysis using the software programs PROC GLM and PROC REG, respectively, in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Additionally, weight gain and FCR data were $\log_e$ transformed ($\text{Ln } Y_T$) using the relationship $\text{Ln } Y_T = \text{Ln } (1 + Y)$ and subjected to spline regression analysis using the SAS software program PROC NONLIN according to Freund and Littell (2000). Following ANOVA, differences among means were separated using the Tukey–Kramer procedure for pairwise comparisons (Kramer, 1956; Tukey, 1953) when treatment effects were considered significant at $P \leq 0.05$. All regressions were considered significant when both $R^2 \geq 0.20$ and $P \leq 0.05$.

Table 3
Chemical analysis (% dry weight) and apparent digestibility coefficients (ADCs) of nutrients in grain distillers dried yeast (GDDY) and menhaden fish meal.

	Chemical analysis		ADCs	
Item, %	GDDY	Fish meal[a]	GDDY	Fish meal[a]
Dry matter	91.5	92.7	65.4	77.7
Crude protein	52.0	68.0	97.6	85.9
Crude fat	3.9	8.0	100.0	92.7
Energy (kcal/g)	5945.0	4709.3	69.7	94.8
Phosphorus	0.7	3.6	80.7	43.8

[a] Mean of values in USDA-ARS/USFWS Digestibility Database for menhaden fish meal, Special Select™ (Barrows et al., 2011).

3. Results

3.1. In vivo digestibility

The analyzed proximate composition of GDDY as compared to fish meal averages was lower in total protein content and lower concentrations of individual amino acids, specifically, the three most common limiting amino acids for rainbow trout, lysine, methionine, and threonine (Table 3; Table 4). Grain distillers dried yeast ADCs for protein, dry matter (DM), fat, and energy were 97.6, 65.4, 100.0, and 69.7%, respectively (Table 3). Grain distillers dried yeast AACs for methionine, lysine, threonine, and the sum of amino acids were 88.1, 75.5, 70.8, and 80.7%, respectively (Table 4).

3.2. Feeding trial

3.2.1. Diet composition and pellet durability

Analyzed dietary macronutrient composition reflected formulation targets (Table 2). Pellet durability was significantly altered by increasing GDDY inclusion such that pellet loss increased quadratically ($y = 0.002x^2 + 0.042x + 5.82$; $R^2 = 0.982$) as function of fish meal replacement (Fig. 1).

3.2.2. Growth performance and body condition indices

Increasing inclusion of GDDY in rainbow trout diets significantly reduced growth and increased feed conversion (Table 5); no effect on feed intake was observed (Table 5; Fig. 4). No significant differences occurred in weight gain and feed conversion ratio of fish fed the 25% and 37.5% FM replacement diets as compared to fish fed the control diet when analyzed by ANOVA. These results were confirmed by spline regression that showed a highly significant ($P < 0.0001$) breakpoint for Ln weight gain when Ln FM replacement level equaled 3.715, *i.e.*, 41% FM replacement or 12% GDDY in the diet (Fig. 2) and when Ln FM replacement level equaled 3.544, *i.e.*, 34% FM replacement or 10% GDDY in the diet for Ln FCR (Fig. 3). There was a linear increase ($P = 0.010$; $R^2 = 0.404$) in VSI with increasing replacement of FM with GDDY in the diet (Table 5; Fig. 5), but no break point could be fit with spline regression analysis. However, FR and HSI were unrelated to GDDY level in the diet (Table 5).

3.2.3. Proximate composition, hematocrit, and retention efficiencies

Replacement of fish meal with GDDY had no significant effect on whole body proximate composition; lipid levels ranged from 11.8 to

Table 4
Amino acids (% dry weight) and their apparent availability coefficients (AACs) in rainbow trout in grain distillers dried yeast (GDDY) and menhaden fish meal.

	Chemical analysis		AACs	
Item, %	GDDY	Fish meal	GDDY	Fish meal[a]
Alanine	3.7	4.7	82.4	89.4
Arginine	2.2	4.8	80.8	91.4
Aspartic acid	3.8	6.6	72.1	87.6
Glutamine	7.7	9.7	83.6	95.3
Glysine	1.8	5.2	78.7	75.0
Histidine	1.0	1.5	78.9	92.1
Isoleucine	2.0	2.9	79.0	95.2
Leucine	5.9	5.2	84.0	97.2
Lysine	2.3	4.6	75.5	92.9
Methionine	1.0	1.7	88.1	94.9
Phenylalanine	2.8	2.9	89.4	89.5
Proline	3.4	3.6	83.0	84.8
Serine	2.7	3.2	76.2	91.6
Threonine	2.2	3.3	70.8	92.3
Tyrosine	2.5	2.4	85.0	95.9
Valine	2.2	3.7	79.2	93.4
Sum AA	48.0	66.1	80.7	90.9

[a] Mean of values in USDA-ARS/USFWS Digestibility Database for menhaden fish meal, Special Select™ (Barrows et al., 2011).

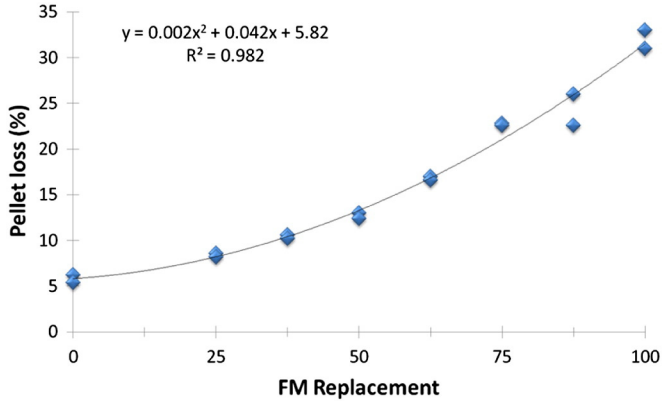

Fig. 1. Pellet durability of diets with increasing levels of FM replacement by GDDY in the diet.

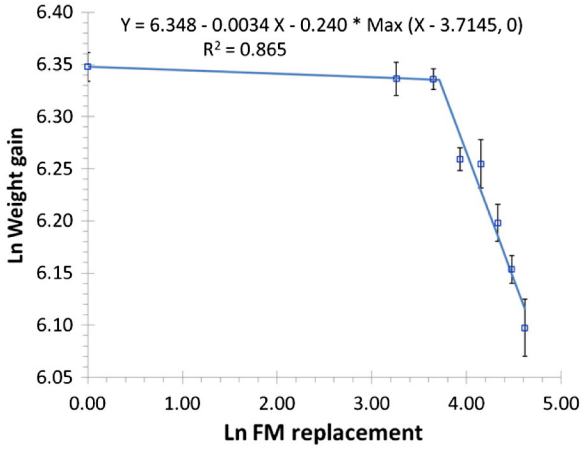

Fig. 2. Spline regression of Ln weight gain in rainbow trout with respect to Ln FM replacement level in the diet.

13.5% and whole body protein ranged from 16.1 to 17.1% (Table 6). Hematocrit ranged from 39 to 48% with no discernible dietary effects. No dietary effects were noted for protein and energy retention efficiencies which ranged between 32.9 to 38.2% and 39.6 to 45.4%, respectively (Table 6).

3.2.4. Diet digestibility post-feeding

No significant effects of diet on lipid or energy ADCs were observed (Table 7). Dietary lipid ADC ranged from 83.6 to 87.9 whereas energy ADC ranged from 77.9 to 81.2. In contrast, significant increases in protein and phosphorus digestibility coefficients were observed with increasing GDDY inclusion. Protein ADCs were relatively high and increased slightly from 84.9 to 88.1 whereas phosphorus ADCs were low and ranged from 31.4 to 51.1.

4. Discussion

Grain distillers dried 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 GDDY was higher than the average fish meals analyzed by Gaylord et al. (2008) and was comparable to some of the plant concentrate ingredients tested in that study. Grain distillers dried yeast protein digestibility was similar to anchovy fish meal (97%), soy protein concentrate (99%) and wheat gluten meal (100%). GDDY apparent digestibility coefficients for protein were higher than all other ingredients that were reported by Gaylord et al. (2008). GDDY results show improved protein digestibility when compared to brewer's dried yeast as reported by Rumsey et al. (1991a). Lower digestibility coefficients for dry matter and energy were observed for GDDY compared to fish meal most likely due to the relatively high nitrogen free extract content of the ingredient.

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

It is possible that 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 included at 20% of the diet could successfully replace up to 35% of dietary fish meal for rainbow trout. The data from the current study are consistent with other reports utilizing single-cell proteins in diets for sunshine bass and cobia (Gause and Trushenski, 2011a;

Table 5
Growth performance and body indices of juvenile rainbow trout (22.1 ± 0.3 g) fed test diets containing grain distillers dried yeast (GDDY) for 9 weeks.

Diet[c]	Growth performance[a]			Body indices[b]		
	Weight gain[d]	FCR[e]	Feed intake[f]	VSI[g]	FR[h]	HSI[i]
	%		% bw day^{-1}	%	%	%
0%	570^{a}	0.87^{c}	2.5	12.2^{b}	53.8	1.3
25%	564^{a}	0.93^{b,c}	2.7	12.3^{b}	51.4	1.3
37.5%	564^{a}	0.94^{b,c}	2.7	12.3^{b}	54.1	1.2
50%	522^{b}	1.03^{a,b}	2.9	12.8^{a,b}	53.3	1.2
62.5%	520^{b}	0.96^{b,c}	2.7	14.3^{a}	53.0	1.4
75%	491^{c}	1.01^{a,b}	2.8	14.2^{a}	50.5	1.3
87%	470^{c,d}	1.04^{a,b}	2.9	14.0^{a}	51.7	1.4
100%	444^{d}	1.10^{a}	3.0	14.2^{a}	50.2	1.3
Pooled SE	8.83	0.04	0.11	0.52	1.99	0.08
Pr > F	<0.001	0.017	0.153	0.010	0.773	0.356

[a] Means of four replicate tanks of fish (30 fish/tank).
[b] Mean determinations in three fish/tank from $N = 4$ replicate tanks/diet.
[c] Percent of fish meal replaced by GDDY on a digestible protein basis.
[d] Weight gain (%) = (final weight − initial weight) * 100 / initial weight.
[e] FCR, feed conversion ratio = g dry feed consumed / g weight gained.
[f] Feed intake (%) = g dry feed consumed/average fish biomass (g) / culture days * 100.
[g] VSI, visceral somatic index (%) = viscera mass × 100 / fish mass.
[h] FR, filet ratio (%) = filet with rib mass * 100 / fish mass.
[i] HSI, hepatosomatic index (%) = liver mass × 100/fish mass.

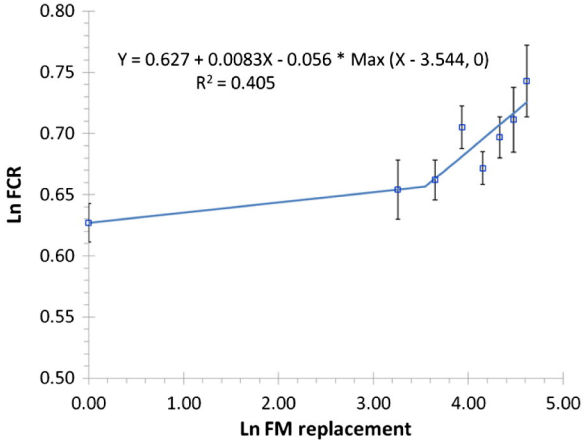

Fig. 3. Spline regression of Ln FCR in rainbow trout with respect to Ln FM replacement level in the diet.

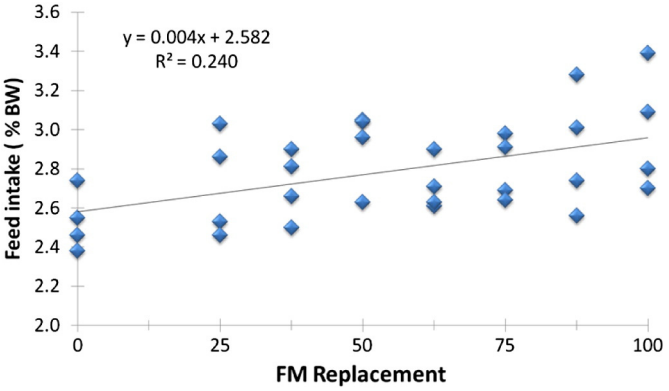

Fig. 4. Feed intake (% BW) of fish fed diets with increasing levels of FM replacement by GDDY in the diet GDDY.

Lunger et al., 2006) where total replacement of fish meal was unattainable. However, the failure of these single-cell protein ingredients as complete fish meal replacers was postulated by those authors to be an issue with palatability. Lunger et al. (2006), although not quantifying feed intake, observed uneaten feed remaining in the tanks when feeding to a percentage of fish biomass. Gause and Trushenski (2011a, 2011b) proposed a palatability issue with increasing ethanol yeast inclusion. In those studies, decreased intakes were observed in diets with highest inclusions of yeast product relative to their control (fish meal) diets. Results from the current study, however, demonstrated that there was no relationship between palatability, as measured by relative intake, and the performance of rainbow trout fed each of the experimental diets.

The effects of dietary GDDY inclusion on rainbow trout condition indices and proximate composition measurements are similar to existing literature regarding observed effects of alternative proteins (Gause and Trushenski, 2011a; Snyder et al., 2012). Gause and Trushenski (2011a) reported increased visceral fat deposits and increased whole body lipid composition in fish fed higher inclusion levels of GDDY, which the authors attributed to reduced accumulation of lean muscle mass and a relative increase in adiposity as fish attempted to compensate for amino acid imbalances. 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 tried to meet EAA requirements. Although, whole body composition was not altered by GDDY inclusion in the current study, the increased consumption and increased VSI found in fish fed higher levels of GDDY during the current study does lend additional credence to the hypothesis of an increasing amino acid deficiency with increasing GDDY replacement of FM in the diet.

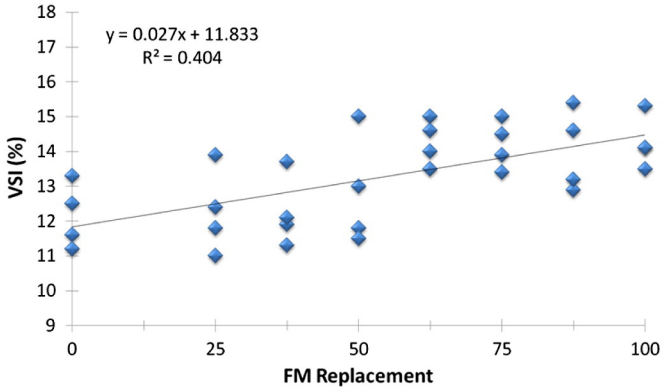

Fig. 5. Visceral somatic index (VSI) of fish fed diets with increasing levels of FM replacement by GDDY in the diet.

Table 6
Whole body proximate composition (wet weight basis) and nutrient retention efficiency of juvenile rainbow trout (22.1 ± 0.3 g) fed test diets containing grain distillers dried yeast (GDDY) for 9 weeks.[a]

Diet[b]	Moisture (%)	Fat (%)	Protein (%)	Energy (kcal/g)	PRE[c] (%)	ERE[d] (%)
0%	67.5	13.5	17.0	2228	36.7	44.2
25%	68.8	12.5	16.7	2171	37.1	43.6
37.5%	67.7	13.2	16.7	2267	38.2	44.0
50%	69.4	11.8	16.1	2087	32.9	40.1
62.5%	68.3	12.6	17.1	2190	37.1	45.4
75%	69.3	12.3	16.4	2179	36.5	42.8
87%	68.4	12.6	16.8	2234	36.5	41.4
100%	68.3	12.8	16.9	2215	38.2	39.6
Pooled SE	0.98	0.62	0.32	52.7	2.21	2.77
Pr > F	0.837	0.657	0.454	0.416	0.784	0.777

[a] Mean determinations in three fish/tank from $N = 4$ replicate tanks/diet.
[b] Percent of fish meal replaced by GDDY on a digestible protein basis.
[c] PRE, protein retention efficiency = g protein gain × 100 / g protein fed.
[d] ERE, energy retention efficiency = kcal energy gain × 100 / kcal energy fed.

One explanation for a potential amino acid deficiency may be the presence of non-protein nitrogen present in GDDY in the form of the nucleic acids. Rumsey et al. (1991a, 1991b) 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 that is further degraded into urea and glyoxylic acid which are both excreted in the urine (Rumsey et al., 1991a,b). Although not analyzed in the current study, the presence of non-protein nitrogen in GDDY is also supported by a lower sum of amino acids in GDDY than the measured crude protein content. In the current study, crude protein digestibility was high at 98% relative to the total amino acid availability which was only 81%. This difference is difficult to reconcile with the fact that we observed no differences in PRE and ERE when fish were fed treatment diets compared to the control, as one would have expected PRE to decrease if the amino acids were less digestible.

Concomitantly, the decreases in growth observed in the current study may be attributable to alterations in pellet quality. Although no major problems with breakage or fines were noted during the feeding trial a significant correlation between GDDY inclusion rate and pellet loss during Holman durability testing was observed. 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 in the diet. These authors observed higher growth rates and increased feed intake potentially due to an increased rate of digestion in the diet with lower hardness and a larger percentage of broken pellets evaluated by DORIS analysis. Baeverfjord et al. (2006) evaluated the effects of feed pellet water stability on protein and fat digestibility with rainbow trout. In that trial, water stability did not affect protein digestibility but as water stability decreased the lipid digestibility tended to decline. Although in the current trial there appears to be a correlation between pellet durability and fish performance, insufficient data exist to clearly define this as the primary restriction to GDDY inclusion on trout performance.

Another potential concern, which has been raised in terms of alternative protein utilization, is the utilization of crystalline amino acids to balance nutritional needs. It has been demonstrated that crystalline amino acids are absorbed from the chyme at a higher rate than amino acids supplied as intact proteins, which could potentially create a time-based imbalance even though the diets were complete (Schuhmacher et al., 1997). Ok et al. (2001) observed a notably shorter time to postprandial peak in most plasma amino acid concentration of 4 h with rainbow trout. In order to overcome the potential limitation of variable amino acid absorption and availability timing in the current trial,

Table 7
Protein, lipid, and energy apparent digestibility coefficients (ADCs) and phosphorus apparent availability coefficient (AAC) values of juvenile rainbow trout (22.1 ± 0.3 g) fed test diets containing grain distillers dried yeast (GDDY) for 9 weeks.[a]

Diet[b]	Crude protein ADC	Crude lipid ADC	Gross energy ADC	Phosphorus AAC
	%			
0%	84.9[c]	87.9	81.2	31.4[e]
25%	85.1[b, c]	86.4	80.6	37.1[d]
37.5%	85.6[b, c]	87.1	79.7	44.3[b, c]
50%	86.8[a, b, c]	86.4	81.0	41.8[c, d]
62.5%	85.6[b, c]	84.3	77.9	44.3[b, c]
75%	87.0[a, b]	83.6	78.6	41.7[c, d]
87.5%	87.9[a]	87.4	80.1	47.8[a, b]
100%	88.1[a]	85.5	79.2	51.1[a]
Pooled SE	0.59	1.29	0.92	1.56
Pr > F	0.0062	0.3467	0.2608	<0.0001

[a] Mean determinations in three fish/tank from $N = 4$ replicate tanks/diet.
[b] Percent of fish meal replaced by GDDY on a digestible protein basis.

multiple feedings (2×/day) were utilized to minimize any unequal absorption effects. The efficient utilization of crystalline amino acids in trout diets has been previously demonstrated by Gaylord and Barrows (2009). In that trial, with the exception of lysine which was postulated to be supplemented in excess, amino acid retention efficiencies of supplemental methionine and threonine were equal to efficiencies from the intact protein diets.

5. Conclusions

Based on the digestibility data, nutrients from GDDY were well digested and absorbed by rainbow trout, yet feeding high levels of GDDY resulted in linear reductions in performance and poorer FCRs. However, these performance factors were not associated with reductions in palatability because fish consumed equivalent amounts of the diets that contained higher inclusion levels of GDDY. Protein digestibilities among diets were essentially equal and all diets were balanced for available methionine, lysine, threonine and phosphorus. Hence, the reduced performance could not be attributed to any of these factors. The factors contributing to the reduced fish performance when 34% (based on FCR) or 40% (based on growth) of the fish meal protein was replaced with GDDY protein are not clear at this point but may be attributable to another limiting amino acid or unknown interactive effect of the high nucleic acid or polysaccharide content of GDDY.

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