

Replacement of Fish Meal with High Protein Distillers Dried Grain in Juvenile Rainbow Trout Diets

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Abstract: The inclusion of High Protein Distillers Dried Grains (HPDDG) in Juvenile rainbow trout (*Oncorhynchus mykiss*) diets was evaluated in a 73 day feeding trial. Three experimental diets contained 0, 10, 20% HPDDG, with the HPDDG, directly replacing fish meal as a protein source. With each incremental increase in HPDDG, there was a significant decrease in total and percent weight gain as well as an increase in feed conversion ratio, among the treatments. No significant differences in mortality, gut inflammation or any fish health measures were observed among the diets. The hepatosomatic index did not significantly change with changes in dietary HPDDG. Fillet crude protein levels were significantly lower in trout receiving the highest amount of dietary HPDDG. There were no differences in fillet lipid concentrations among any of the dietary treatments.

Key words: HPDDG, rainbow trout, *Oncorhynchus mykiss*, alternative proteins, feeding

INTRODUCTION

Fish meal has been the primary protein source in the high protein diets of carnivorous aquaculture fish species; such as, rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) (Satia, 1974; Kim *et al.*, 1991; NRC, 2011; Cheng and Hardy, 2004b). However, the rapid growth in global aquaculture (FAO, 2009), combined with use of fish meal in other agricultural livestock feeds, has greatly increased the demand and market prices for limited fish meal stocks (Tacon and Metian, 2008; Hardy, 2010). Thus, lower-cost, plant-based protein sources such as Distillers Dried Grains with Solubles (DDGS) will likely be needed to either partially or totally replace the fish meal component in salmonid diets (Hardy, 2010).

As the corn-based ethanol biofuel industry has grown, so has the availability of the DDGS coproduct (Rosentrater and Muthukumarappan, 2006). DDGS are relatively high protein (Wu *et al.*, 1997; Rosentrater and Muthukumarappan, 2006) and contain few if any, anti-nutritional factors found in other plant protein sources (Jauncey and Ross, 1982; Wilson and Poe, 1985;

Shiau *et al.*, 1987; Robinson, 1991). Compared to other corn products nutrients are more concentrated in DDGS (Chevanan *et al.*, 2008), although compared to fish meal, the essential amino acids lysine and methionine are present in lower concentrations (Cheng and Hardy, 2004b).

Average protein levels in conventional DDGS are near 30% (Spiehs *et al.*, 2002), although there is often considerable variation from batch to batch (Belyea *et al.*, 1998; Rosentrater and Muthukumarappan, 2006) due to processing variations (Belyea *et al.*, 2004) or the characteristics of the source grain (Abo-State *et al.*, 2009). High protein distillers dried grains HPDDG are produced by fractionating the corn and removing the non-fermentable fractions prior to ethanol production (Singh *et al.*, 2005). The protein levels in HPDDG are approximately 50% greater than those of DDGS produced by conventional processing and phosphorous availability may also be increased (Robinson *et al.*, 2008).

The earliest incorporation of conventional DDGS in rainbow trout *Oncorhynchus mykiss* diets was performed by Phillips (1949), with 3% dietary DDGS successfully

used by Sinnhuber (1964). Similar to DDGS, distillers dried solubles were used by Phillips (1949). Other distillers grain products showed no ill effects when incorporated into salmonids diets at low concentrations (Fowler and Banks, 1976; Hughes, 1987). Cheng and Hardy (2004b) stated that in comparison to diets containing 30% fish meal, DDGS could replace up to 50% the fish meal component when fed to 50 g rainbow trout. They also observed that with lysine and methionine supplementation, DDGS could replace 75% of the fish meal component (22.5% of the total diet). However, the diets used in their study including the control, contained 15% soybean meal. A true fish meal only control was not included in that study; Stone *et al.* (2005) noted that rainbow trout fed fish meal control diets performed significantly better than trout receiving dietary DDGS. Cheng *et al.* (2003) indirectly examined DDGS in rainbow trout diets. In their study, diets containing 18.5% DDGS, 17.5% soybean meal and 17.5% fish meal, in conjunction with the use of a methionine hydroxyl analogue, produced similar rearing results as diets with 18.5% DDGS and 35% fish meal (Cheng *et al.*, 2003). Cheng and Hardy (2004a) evaluated the use of phytase in rainbow trout diets containing DDGS and found that apparent digestibility coefficients were improved for total-phosphorous and other minerals with phytase supplementation, whereas Barnes *et al.* (2012a) noted decreased growth in Juvenile rainbow trout fed dietary DDGS even with phytase and amino acid supplements.

There is a relative lack of published information specific to the use of HPDDG in rainbow trout diets with only one short-term study (Barnes *et al.*, 2012b) indicating that HPDDG supplemented with essential amino acids could be included at dietary concentrations up to 20%. The objective of this study was to evaluate the direct replacement of fish meal with HPDDG in Juvenile rainbow trout diets without the inclusion of additional amino acids.

MATERIALS AND METHODS

The feeding trial was conducted at Cleghorn Springs State Fish Hatchery, Rapid City, South Dakota, USA, using spring water at a constant temperature of 11°C (total hardness as CaCO₃, 250 mg L⁻¹; alkalinity as CaCO₃, 170 mg L⁻¹; pH, 7.5; total dissolved solids, 150 mg L⁻¹; dissolved O₂, 9 mg L⁻¹). Flows in each tank were set at 40 L min⁻¹. Approximately, 200 McConaughy strain rainbow trout (Mean±SE initial weight 10.5±0.6 g, length 8.1±0.2 mm) were placed into each of 16 fiberglass circular tanks (1.8 m diameter, 0.6 m depth) on July 13, 2009. Tank were loaded based on weight (to the nearest g) and fish numbers were estimated. Feeding commenced the

following day and continued for 72 days until the end of the trial. Feeding amounts for the tanks were determined by the Hatchery Constant (HC) method with a planned feed conversion of 1.1 and a maximum growth rate of 0.065 mm day⁻¹ which was based on the historical performance of the McConaughy strain at Cleghorn Springs State Fish Hatchery. Feed amounts were updated daily. Feed was uniformly dispensed from 07:00-17:00 h in each tank using automatic Sweeney vibrating feeders (Sweeney Enterprises, Inc., Boerne, Texas) electronically programmed to release feed at 60 min intervals. All feed dispensed and fish mortalities were recorded daily for each tank. Percent mortality was determined by dividing the number of fish that died during the trial by the total number of fish (200) initially present in each tank.

The 28 tanks were randomly assigned to one of four different diets (Table 1), with 4 tanks receiving the same diet. About 3 experimental diets contained 0, 10 or 20% HPDDG (Poet Dakota Gold HPDDG, Glenville East, South Dakota, USA, 41.7% protein, 4.5% fat), with the HPDDG incrementally replacing fish meal as the primary protein source. In addition, a commercial diet (Silvercup®, Nelson and Sons, Inc., Murray, Utah) was included in the design as a reference diet. Feed manufacturing of the experimental diets is described in Ayadi *et al.* (2011). The resulting extruded feed pellets were analyzed according to AOAC (2009) methodology for protein (method 2001.11) and crude lipid (method 2003.5, modified by substituting petroleum ether for diethyl ether) and ash

Table 1: Percent composition and chemical analysis of the diets used in the Juvenile rainbow trout feeding trial

Ingredients	Reference ^a	Diet		
		1	2	3
Herring meal ^b	-	50.0	40.00	30.00
HPDDG ^c	0.00	0.0	10.00	20.00
Whole wheat flour ^d	-	15.0	15.00	15.00
Corn gluten meal ^e	-	15.0	15.00	15.00
Menhaden oil ^f	-	12.0	12.50	13.00
Celufil ^g	-	5.5	5.00	4.50
Vitamin and mineral mix ^h	-	2.0	2.00	2.00
Vitamin C (Stay-C) ⁱ	-	0.5	0.50	0.50
Total	-	100.0	100.00	100.00
Chemical analysis (dry basis %)				
Crude protein	49.6	52.6	50.80	46.40
Crude lipid	19.6	15.8	15.70	15.10
Ash	-	9.0	7.80	6.40
GE (MJ kg ⁻¹ dry matter)	-	17.8	17.48	16.47

^aSilvercup, Nelson and Sons, Inc., Murray, Utah, USA; ^bLortscher Agri Service, Inc., Bern, Kansas, USA; ^cPoet Nutrition, Sioux Falls, South Dakota, USA; ^dBob's Red Mill Natural Foods, Inc., Milwaukie, Oregon, USA; ^eConsumers Supply Distributing Company, Sioux City, Iowa, USA; ^fOmega Protein, Inc., Houston, Texas, USA; ^gUSB Corporation, Cleveland, Ohio, USA; ^hLasi Fish Premix, NB-8055, Lortscher Agri Service, Inc., Bern, Kansas, USA; ⁱDSM Nutritional Products France SAS, Village-Neuf, France; ^jAnalysis conducted on post-extrusion pellets

content by AACC (2000) method 08-03. The protein and lipid amounts obtained by these methods were multiplied by their respective physiological fuel values of 23.6 and 39.5 joules (NRC, 2011) to obtain estimated gross energy values.

At the end of the trial, total tank weights were recorded to the nearest g with weight gain calculated by subtracting the initial weight from the final weight for each tank. Feed conversion ratio for each tank was calculated by dividing the total amount of food fed by the total weight gain. In addition to total tank measurements, 5 fish from each tank were randomly selected from each tank and individually weighed to the nearest g and measured (total length) to the nearest mm. Fish health profiles, based on a modification of Goede and Barton (1990), Adams *et al.* (1993) and Barton *et al.* (2002) were completed using the score sheet described in Table 2. Liver weights were also recorded to the nearest mg and the Hepatosomatic Index (HSI) determined using the formula (Strange, 1996):

$$\text{HSI (\%)} = 100 \times \text{Liver weight} / \text{whole fish weight}$$

Apparent protein digestability was determined using a direct method (Windell *et al.*, 1978). Digesta was removed from 5 fish per tank at the end of the trial. Each fish was dissected and the last cm of the distal end of the intestine was gently squeezed to remove the contents. Digesta from 5 fish per tank was pooled and flash frozen on dry ice prior to analysis. Protein analysis was

conducted using AOAC (2009) method 990.03. Apparent protein digestability was calculated using the formula:

$$\text{Apparent protein digestability} = \frac{\text{Protein in the diet} - \text{protein in the digesta}}{\text{Protein in the diet}}$$

At the end of the experiment, 5 whole fish per tank were euthanized; muscle fillets were then removed and flash frozen for determination of carcass composition. The fillets from each tank were pooled and analyzed for crude protein levels with a TruSpec CNS combustion analyzer (LECO Corp., St. Joseph, Michigan, USA) using AOAC (2009) method 992.15. AOAC (2009) acid hydrolysis method 948.15 with a 50:50 mix of diethyl ether and petroleum ether for extraction was used for fat analysis and moisture was determined by drying loss using AOAC (2009) method 952.08.

Data were analyzed using the SPSS (9.0) Statistical analysis Program (SPSS, Chicago, Illinois, USA) with significance predetermined at $p < 0.05$. One-way Analysis of Variance (ANOVA) was conducted and if the treatments were significantly different, pairwise mean comparisons were performed using the Tukey HSD test (Kuehl, 2000). All mortality percentage data were arcsine-square root transformed prior to analysis to stabilize the variances (Kuehl, 2000).

RESULTS

With each incremental increase in the amount of dietary HPDDG, there was a significant decrease in total weight gain among the tanks of rainbow trout (Table 3). There was no significant difference in weight gain between those tanks receiving the reference diet and the formulated diet that did not contain any HPDDG. Percent

Table 2: Criteria used at the end of the study for fish health observations based on Goede and Barton (1990), Adams *et al.* (1993) and Barton *et al.* (2002)

Structure or tissues	Rating criteria	Numeric rating
Eyes	Normal	0
	Abnormal	1
Fat	None	0
	<50% of gut covered	1
	>50% of gut covered	2
Fins	100% of gut covered	3
	No erosion	0
	Light erosion	1
	Moderate erosion	2
Gills	Severe erosion	3
	Normal	0
	Clubbed, frayed or discolored	1
Gut	Normal	0
	Slight inflammation	1
	Moderate inflammation	2
	Severe inflammation	3
Kidney	Normal	0
	Abnormal	1
Liver	Normal	0
	Abnormal	1
Pseudobranchs	Normal	0
	Abnormal	1
Opercles	Normal	0
	Short	1
Spleen	Normal	0
	Cysts or enlarged	1

Table 3: Total tank rearing data (Means±SE) including Feed Conversion Ratio (FCR^a) and estimated Apparent Protein Digestibility (APD^b) for tanks of rainbow trout receiving one of 7 different diets containing incremental amounts of High Protein Distillers Dried Grains (HPDDG)

Parameters	Reference (0%)	Diet (HPDDG (%))		
		1 (0)	2 (10)	3 (20)
Tanks	4	4	4	4
Start weight (g)	1.665	1.665	1.665	1.665
End weight (g)	7.591±102 ^a	7.595±37 ^a	7.123±31 ^b	6.639±38 ^c
Gain (g)	5.926±102 ^a	5.930±37 ^a	5.528±31 ^b	4.974±38 ^c
Gain (%)	356±6 ^a	356±2 ^a	332±2 ^b	299±2 ^c
Food fed (g)	4.494	4.494	4.494	4.494
FCR	0.76±0.01 ^a	0.76±0.01 ^a	0.81±0.01 ^b	0.90±0.01 ^c
Mortality (%)	1.00±0.54	0.50±0.20	0.38±0.13	0.38±0.24
APD	-	88.1±0.4 ^a	88.9±0.2 ^{ab}	89.3±0.2 ^b

^aFCR = Total food fed/total weight gain; ^bAPD = (Dietary protein-protein in digesta)/dietary protein; means with different letters across a row are significantly different ($p < 0.05$)

weight gain followed the same pattern as that observed with total weight gain with the tanks receiving the reference and non-HPDDG diets increasing in weight over 350% during the course of the trial.

Feed conversion ratio was <1.0 for all of the diets with significant differences with each increase in dietary HPDDG. Mortality was not significantly different among any of the diets, ranging from a high 1.0% in commercial diet to 0.5% or less in the experimental diets. Apparent protein digestibility was significantly greater in the 20% dietary treatment compared to the diet that did not contain any HPDDG.

Individual fish lengths and weights generally decreased with increasing amounts of dietary HPDDG although these trends were not significantly different (Table 4). There was no significant difference in HSI among the treatments. No abnormalities in the eyes, kidneys, pseudobranchs or spleen were observed in any of the fish, regardless of diet. Some light fin erosion and

opercle shortening was commonly observed in fish from all of the tanks. Abdominal fat covered nearly all of the viscera in nearly all of the fish examined.

Crude protein was over 18% in fillets from trout fed either the experimental diet void of any HPDDG or the diet containing 10% HPDDG (Table 5). Protein levels were significantly lower in those fish receiving the diet with 20% HPDDG however. Crude lipid, ash and water percentages were not significantly different among any of the dietary treatments.

DISCUSSION

The decrease in growth with increasing concentrations of dietary HPDDG observed in this study are different than the results obtained by Barnes *et al.* (2012b) where diets containing HPDDG also contained additional amino acids and phytase. These studies differed in dietary formulation however. In this study, HPDDG directly replaced fish meal with a corresponding decrease in fish meal with the inclusion of increasing amounts of HPDDG. In Barnes *et al.* (2012b), fish meal levels did not decrease in diets containing 10% HPDDG and only decreased from by 25% in the diets containing 20% HPDDG. Amino acid supplementation may explain the different results. It is possible that the diets containing 20% HPDDG diets may have possibly been methionine deficient (NRC, 2011) in the present study, although it cannot be stated with any certainty because dietary amino acid compositions were not determined. However, the relatively high levels of fish meal used in the 10% HPDDG diets likely provided enough essential amino acids.

The decrease in growth with increasing concentrations of dietary HPDDG observed in this study are different than the results obtained by Cheng and Hardy (2004b). They stated that DDGS could replace up to 50% of the fish meal component in trout diets. There are substantial differences between the two studies, however, the control diet used in this study was a fish-meal only control, whereas the control and DDGS-containing diets used by Cheng and Hardy (2004b) all contained soybean meal as an additional protein source. The feed conversion ratio of their control diet at 1.21 was also much greater than that observed in this study at 0.76. Stone *et al.* (2005) also noted that fish meal control diets also performed better than diets containing DDGS during rainbow trout rearing. Cheng and Hardy (2004b) used lower protein DDGS, versus the HPDDG used in this study. The results of this study are consistent with those of other experiments involving dietary DDGS and rainbow trout (Phillips, 1949; Barnes *et al.*, 2012a).

Table 4: Ending mean (±SE) lengths (mm), weights (g), condition factors (K)^a, liver weights (g), hepatosomatic index values (HSI)^b and fish health assessments^c of rainbow trout fed diets containing incremental amounts of High Protein Distillers Dried Grains (HPDDG)

Variables	Reference (0%)	Diet (HPDDG (%))		
		1 (0)	2 (10)	3 (20)
Length	15.40±0.30	15.30±0.40	14.90±0.50	14.70±0.30
Weight	43.20±2.80	41.40±2.90	37.60±3.70	35.30±3.30
K	1.18±0.02	1.15±0.02	1.13±0.01	1.10±0.03
Liver weight	0.89±0.08	0.74±0.04	0.58±0.04	0.78±0.11
HSI	2.09±0.24	1.80±0.15	1.57±0.13	2.20±0.14
Eyes	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Fat	2.80±0.20	2.60±0.20	2.80±0.10	2.90±0.10
Fins	1.00±0.10	0.80±0.10	0.90±0.10	0.80±0.10
Gills	0.70±0.10	0.40±0.10	0.50±0.10	0.60±0.10
Gut	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Kidney	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Liver	0.00±0.00	0.00±0.00	0.10±0.10	0.20±0.20
Pseudobranchs	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Opercles	0.40±0.10	0.60±0.10	0.60±0.20	0.60±0.10
Spleen	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

^aCondition factor (K) = 10³ × (weight)/(length³); ^bHepatosomatic Index (HSI) = 100×(Liver weight/body weight); ^cFish health assessments rating system described in Table 2; Means in a row with different letters are significantly different (N = 4, p<0.05)

Table 5: Mean (±SE) percent water, crude protein, crude lipid and ash concentrations from fillets of rainbow trout fed diets containing incremental amounts of High Protein Distillers Dried Grains (HPDDG)

Parameters	Diet (HPDDG (%))		
	1 (0)	2 (10)	3 (20)
Water (%)	75.2±0.5	73.8±0.4	73.1±0.7
Crude protein (%)	18.4±0.4 ^a	18.8±0.2 ^a	17.0±0.2 ^b
Crude lipid (%)	5.1±0.5	5.4±0.3	7.5±1.0
Ash (%)	1.4±0.1	1.4±0.1	1.4±0.1

Means in a row with different letters are significantly different (N = 4, p<0.05)

Feed production techniques can affect diet digestibilities in fish (Jeong *et al.*, 1991; Cheng and Hardy, 2003; Glencross *et al.*, 2011). Feed for this study was prepared by extrusion, likely the most common process for commercial feed manufacturing, while Cheng and Hardy (2004b) used a pellet mill to manufacture their feed. This study used rainbow trout that were approximately 80% smaller than those used by Cheng and Hardy (2004b) hence, the diets contained higher protein because of the smaller sized fish. Due to the variability in the nutritional composition from batch to batch of conventional DDGS (Belyea *et al.*, 1998; Rosentrater and Muthukumarappan, 2006; Abo-State *et al.*, 2009), it may be difficult to replicate the results of trials using conventional DDGS. Lastly, differences in water temperature are known to affect digestible energy and protein (Watanabe *et al.*, 1996a, b; Azevedo *et al.*, 1998) and the water temperatures used in the present study were over 3°C colder than that used by Cheng and Hardy (2004b). The fish size, feed production technique and other methods used by Cheng and Hardy (2004a) which are similar to those of Cheng and Hardy (2004b), may also make comparisons to this study difficult.

Although, the diets were formulated to be as isonitrogenous and isocaloric as possible, there was a slight decrease in protein, lipid and gross energy with each increase in dietary HPDDG. However, the protein concentration of the experimental diets with either 0 or 10% HPDGG was greater than the commercial diet and all diets were within the range recommended for rainbow trout (Satia, 1974; Wilson, 1989; Kim *et al.*, 1991; NRC, 2011). Even though menhaden oil amounts were increased in the dietary formulations in conjunction with HPDDG increases, this was not enough to compensate for the loss of lipid resulting from the decrease in fish meal. In addition, the increase in menhaden oil in the HPDDG-containing diets was not enough to induce any protein sparing effects (Kim, 1997; Yigit *et al.*, 2002; Chaiyapechara *et al.*, 2003; Morrow *et al.*, 2004).

The nearly identical results observed between the control and reference (commercial) diet used in this study indicates the validity of the control as a comparison to the experimental diets containing HPDDG. The relatively low feed conversion ratios for both the control and reference diet are not unusual for production rainbow trout at this size at hatcheries in South Dakota (Barnes *et al.*, 2011) or elsewhere (Figueiredo-Silva *et al.*, 2005) and could also be explained by the low rearing densities used in the trial (Holm *et al.*, 1990; Procarione *et al.*, 1999). Although, significantly different than that observed in the control diet, the poorer feed conversion ratios in the diets containing HPDDG may still produce an acceptable cost-benefit ratio during trout production.

Apparent protein digestibilities were very similar to those reported by Cheng and Hardy (2004a) for rainbow trout fed diets containing fish meal, soybean meal and DDGS with varying amounts of phytase. However, apparent protein digestibilities were slightly lower than that observed by Barnes *et al.*, (2012b) for rainbow trout fed HPDDG-containing diets with phytase and additional amino acids as well as that reported by Gao *et al.* (2011) for rainbow trout fed either diets entirely of fish meal or diets with partial fishmeal replacement by various plant protein sources. The rainbow trout used in both of these studies were considerably larger at initial weights of 129 and 940 g respectively, compared to those used in this study which were only 10 g at the start of the trial. The different techniques used to estimate protein digestibility in fish fed fish meal diets in this study compared to those in Gao *et al.* (2011) may also explain the different results (Rawles *et al.*, 2010).

Changes in dietary protein component and lipid concentrations can influence the resulting composition of fish fillets (Gatlin *et al.*, 2007; Tobin *et al.*, 2006; Sealey *et al.*, 2011a). The decreased crude protein fillet proximate analysis percentages observed with increasing dietary HPDDG in this study are different than that reported for fish fed HPDDG with supplemental amino acids (Barnes *et al.*, 2012b) but similar to that reported for channel catfish (*Ictalurus punctatus*) fed either fish meal or DDGS-containing diets (Li *et al.*, 2010, 2011). Although not statistically significantly different, the increase in fillet lipid concentration in this study with increasing dietary HPDDG appears to follow the pattern observed by Lim *et al.* (2009) and Li *et al.* (2010). Both Lim *et al.* (2009) and Li *et al.* (2010) reported increased fat concentrations in channel catfish with increasing amounts of DDGS in the feed. While, it is possible that HPDDG influenced fillet lipid composition, Barnes *et al.* (2012b) did not observe any significant difference in rainbow trout fed diets containing HPDDG and Johnson *et al.* (2011) did not observe increased lipid concentrations in Atlantic salmon (*Salmo salar*) fed low fish meal, high fat diets in comparison to those receiving high fish meal, low fat feeds. The percent moisture and crude protein of fillets from the trout receiving the control, fish-meal-only diet were very similar to that reported by Yildiz (2004) but less than that reported by Sealey *et al.* (2011b). However, the rainbow trout fillets analyzed by Sealey *et al.* (2011b) came from fish that were fed a 29% fish meal control diet that also contained 16% soybean meal.

The lack of difference in HSI among the diets was unexpected. Hepatosomatic index is positively related to carbohydrate levels in the diet (Daniels and Robinson, 1986; Kim and Kaushik, 1992). Higher HSIs have also been associated with slower growth rates and decreased feed

efficiency (Takeuchi and Watanabe, 1982) both of which describe the fish receiving dietary DDGS. The hepatosomatic index either slightly decreased or showed no effect, from dietary DDGS in tilapia (*Oreochromis niloticus*) (Schaeffer *et al.*, 2009, 2010) and was also unaffected by dietary protein in common carp (*Cyprinus carpio*) (Fine *et al.*, 1996).

Although, specific feeding trial durations are not universally specified, they generally need to last long enough for any potential significant differences among the diets to materialize (Weatherup and McCracken, 1999). In a study by De Francesco *et al.* (2004), differences in trout rearing performance between fish meal and plant-based diets did not become apparent until after 12 weeks. The present study lasted over 10 weeks which was long enough for significant differences to occur.

CONCLUSION

The results of this study indicate that the direct replacement of fish meal in juvenile rainbow trout diets may only be suitable at dietary concentrations of 10% or less HPDDG. Juvenile rainbow trout diets containing HPDDG likely need to be supplemented with amino acids to produce acceptable fish growth and feed conversion ratios (Cheng and Hardy, 2004a; Barnes *et al.*, 2012b). In addition, trials should be conducted involving the *ad libitum* feeding of HPDDG-containing diets to different sizes of rainbow trout in contrast to the production-based methodology used with juvenile-sized fish in this study.

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