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## Abstract

An initial investigation into the inclusion of high protein distillers dried grain with solubles (HPDDG) in juvenile rainbow trout *Oncorhynchus mykiss* diets was conducted during a 36-day feeding trial. Four experimental diets containing either 10% or 20% HPDDG with supplemental amino acids, and either with or without phytase, were compared to a fish-meal-based, non-HPDDG, diet. There was no significant difference among any of the diets in total weight gain, percent weight gain, feed conversion ratio, or percent mortality. There was also no significant difference in length, weight, condition factor, hepatosomatic index, viscerosomatic index, or any fish health parameter in fishes fed any of the diets. Fillet composition, as determined by crude protein, crude lipid, water, and ash were also not significantly different from fish reared on any of the diets. Estimated protein digestibility coefficients were significantly less in the fish receiving the diet void of HPDDG compared to any of the other diets. The addition of phytase had no effect on any rearing parameters. The results suggest that HPDDG, if supplemented with essential amino acids, may be included at concentrations of at least 20% (dry weight) in rainbow trout diets and that more detailed investigation into the use of HPDDG is warranted.

## Keywords

Alternative proteins, Fish feed, HPDDG, *Oncorhynchus mykiss*, Salmonid

## Disciplines

Agriculture | Aquaculture and Fisheries | Bioresource and Agricultural Engineering

## Comments

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# Initial Observations on the Inclusion of High Protein Distillers Dried Grain into Rainbow Trout Diets

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**Abstract:** An initial investigation into the inclusion of high protein distillers dried grain with solubles (HPDDG) in juvenile rainbow trout *Oncorhynchus mykiss* diets was conducted during a 36-day feeding trial. Four experimental diets containing either 10% or 20% HPDDG with supplemental amino acids, and either with or without phytase, were compared to a fish-meal-based, non-HPDDG, diet. There was no significant difference among any of the diets in total weight gain, percent weight gain, feed conversion ratio, or percent mortality. There was also no significant difference in length, weight, condition factor, hepatosomatic index, viscerosomatic index, or any fish health parameter in fishes fed any of the diets. Fillet composition, as determined by crude protein, crude lipid, water, and ash were also not significantly different from fish reared on any of the diets. Estimated protein digestibility coefficients were significantly less in the fish receiving the diet void of HPDDG compared to any of the other diets. The addition of phytase had no effect on any rearing parameters. The results suggest that HPDDG, if supplemented with essential amino acids, may be included at concentrations of at least 20% (dry weight) in rainbow trout diets and that more detailed investigation into the use of HPDDG is warranted.

**Keywords:** Alternative proteins, Fish feed, HPDDG, *Oncorhynchus mykiss*, Salmonid.

## INTRODUCTION

In response to increased demand for biofuels, the fuel-based ethanol industry has grown dramatically, with over 200 ethanol plants in the USA alone producing 54 billion L of ethanol in early 2011 [1]. A result of this increase in ethanol production has been a substantial increase in the amount of distillers dried grains with solubles (DDGS) [2]. DDGS is a valuable, relatively high protein source for animal feeds [3, 4], that do not contain anti-nutritional factors found in other plant protein sources fed to fish [5-8]. Compared to other corn products, nutrients are more concentrated in DDGS [9]. However, in comparison to fish meal, the essential amino acids lysine and methionine are present in lower concentrations [10] which may require supplementation when DDGS are incorporated into fish feeds.

Conventional DDGS contains protein levels of approximately 30% [11]. However, this value varies substantially, even from batch to batch [2, 12, 13]. Higher protein distiller dried grains with solubles (HPDDG) is produced by fractionating the corn and removing the nonfermentable fractions prior to ethanol production [14], resulting in protein levels approximately 50% greater than those of DDGS pro-

duced by conventional processing [15]. HPDDG nutritional values are much more consistent than conventional DDGS [15].

DDGS has been incorporated in rainbow trout *Oncorhynchus mykiss* diets for some time. The earliest experimentation was performed by Philips [16], and 3% dietary DDGS was successfully used by Sinnhuber [17]. Similar to DDGS, dried distillers solubles were used by Phillips *et al.* [18]. Other distiller grain products produced acceptable results when incorporated into salmonids diets at low concentrations [19, 20]. Dietary DDGS concentrations of 15% were used successfully by Cheng and Hardy [10] when fed to juvenile rainbow trout, while concentrations of 22.5% were acceptable with lysine and methionine supplementation. However, Stone *et al.*, [21] noted that rainbow trout on fish meal control diets performed significantly better than trout receiving dietary DDGS. Cheng *et al.*, [22] 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 [22]. Rainbow trout diets including plant ingredients may benefit from the inclusion of phytase to catalyze indigestible phosphorous (phytic acid) found in plant products to a digestible organic form of phosphorous [23-26]. However, not all studies have indicated a positive effect of phytase supplementation on trout growth [27, 28].

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Little research has been conducted on the incorporation of HPDDG into fish diets, particularly with rainbow trout. In addition, little research has been conducted on the use of phytase in HPDDG-containing diets. Thus, the objective of this study was to provide an initial evaluation of the use of HPDDG, supplemented with amino acids and phytase, in juvenile rainbow trout diets.

## METHODS

The trial occurred at McNenny State Fish Hatchery (Spearfish, South Dakota, USA) using degassed and aerated well water at a constant temperature of 11°C (total hardness as CaCO<sub>3</sub>, 360 mg/L; alkalinity as CaCO<sub>3</sub>, 210 mg/L; pH, 7.6; total dissolved solids, 390 mg/L). Flow rates in each tank were set at 40 L/min. Shasta strain rainbow trout (initial weight 33.6 ± 1.5 g, length 146.7 ± 2.1 mm, mean ± SE) were randomly placed into each of 15 fiberglass circular tanks (1.8 m diameter, 0.6 m depth) on September 2, 2010. Tanks were loaded with 40 fish, and total tank weights were measured to ± 1 g. Feeding commenced the following day and continued for 36 days until the end of the trial. Feeding amounts for the tanks were determined by the hatchery constant (HC) method [29], with a planned feed conversion of 1.1 and a maximum growth rate of 0.066 cm/day, which was based on the historical performance of the Shasta strain at McNenny State Fish Hatchery. Feed amounts were updated daily. Fish were hand fed once per day. All feed fed and mortality were recorded daily for each tank.

The 15 tanks were randomly assigned to one of five different diets (Table 1), with three tanks receiving the same diet (N=3). In addition to a fish-meal-based, non-HPDDG diet, four other diets contained either 10% or 20% HPDDG (Poet Dakota Gold HP, Glenville East, South Dakota, USA, 41.7% protein, 4.5% lipid). To make the essential amino acid profiles similar in all of the diets and potentially improve the acceptability of dietary HPDDG [22, 30], the HPDDG-containing diets were supplemented with lysine, methionine, isoleucine, and histidine. Dietary amino acid analysis was conducted according to AOAC [31] method 982.30 (Table 2). In addition, phytase was added to one-half of the HPDDG-containing diets. Pelleted diets were produced by extrusion processing. Experimental diets were analyzed according to AOAC [31] methodology for protein (method 2001.11) and crude lipid (method 2003.5, modified by substituting petroleum ether for diethyl ether), and ash content by AACC [32] 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 mJ [33] to obtain estimated digestible energy values.

At the end of the trial, total tank weights were measured to ± 1 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, five fish from each of the 15 tanks (75 total) were randomly selected, euthanized, and individually weighed to ± 1 g and measured (total length) to ± 1 mm. Fish health profiles, based on a modification of Goede and Barton [34], Adams *et al.* [35], and Barton *et al.* [36], were completed using the score sheet described in (Table 3). To

obtain information on the energy reserve status, as well as the general health condition of the fish, hepatosomatic index (HSI) and viscerosomatic index (VSI) were calculated. To determine HSI, liver weights were recorded to ± 1 mg for inclusion in the formula: HSI (%) = 100 x (liver weight [g]/whole fish weight [g]) [37]. Similarly, viscera weights were also recorded to the nearest mg and VSI determined using the formula: VSI (%) = 100 x (viscera weight [g]/whole fish weight [g]). Condition factor was calculated as  $K = 10^5 \times (\text{weight [g]} / (\text{length}^3 [\text{mm}]^3))$ .

To quantify the digestibility of dietary protein, apparent protein digestibility was determined using a direct method [38]. Digesta was removed from five fish per tank at the end of the trial. Each fish was dissected and the last 1 cm of the distal end of the intestine was gently squeezed to remove the contents. Digesta from five fish per tank was pooled and flash frozen on dry ice prior to analysis. Protein analysis was conducted using AOAC [31] method 990.03. Apparent protein digestibility was calculated using the formula: apparent protein digestibility (%) = (protein in the diet – protein in the digesta) / protein x 100 in the diet.

Muscle fillets were 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 [31] method 992.15. AOAC [31] acid hydrolysis method 948.15 with a 50:50 mix of diethyl ether and petroleum ether for extraction was used for fat analysis. Moisture was determined by loss on drying using AOAC [31] method 952.08.

Data were analyzed using the SPSS (9.0) 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 [39]. Mortality (%) data were arcsine transformed prior to analysis to stabilize the variances [39].

## RESULTS

There was no significant difference in fish rearing performance measures among any of the diets evaluated (Table 4). Mean weight gain, percent weight gain, and food conversion ratio were nearly identical in the diets containing HPDDG compared to the fish-meal-based diet. The mean estimated digestion coefficient of protein was significantly different among the different diets. It ranged from 93.4% to 94.1% in the fish fed diets with HPDDG, which was significantly greater than the 91.4% value for fish receiving the fish-meal-based diet. Dietary phytase had no significant effect on weight gain, conversion, or protein digestibility. No differences in mortality were detected as only one mortality was observed during the experiment.

Individual fish parameters were also very similar among the diets (Table 5). No significant differences were detected among treatments for length, weight, or condition factor. The viscerosomatic index and hepatosomatic index were also not significantly different. None of the fish health values varied significantly among the fish receiving any of the diets. There was no gut inflammation observed in any fish.

**Table 1. Ingredients Composition (%) and Chemical Analysis of the Diets Used in the Trial**

Diet	1	2	3	4	5
HPDDG (%)	0	10	10	20	20
Phytase supplement	no	no	yes	no	yes
Ingredients					
Menhaden meal <sup>a</sup>	40.0	40.0	40.0	30.0	30.0
HPDDG <sup>b</sup>	0.0	10.0	10.0	20.0	20.0
Whole wheat <sup>c</sup>	20.0	11.0	11.0	11.0	11.0
Yellow corn gluten <sup>d</sup>	25.0	20.0	20.0	20.0	20.0
Menhaden oil <sup>e</sup>	11.5	11.5	11.5	12.0	12.0
CMC <sup>f</sup>	0.6	0.0	0.0	0.0	0.0
Vitamin premix <sup>g</sup>	0.5	0.5	0.5	0.5	0.5
Mineral premix <sup>h</sup>	0.0	0.1	0.1	0.1	0.1
Vitamin C (Stay-C) <sup>i</sup>	0.5	0.5	0.5	0.5	0.5
Phytase <sup>j</sup>	0.0	0.0	0.037	0.0	0.037
Yeast <sup>k</sup>	0.125	0.125	0.125	0.125	0.125
L-Lysine <sup>l</sup>	0.0	0.5	0.5	0.5	0.5
L-Isoleucine <sup>l</sup>	0.0	0.3	0.3	0.3	0.3
L-Histidine <sup>l</sup>	0.0	0.1	0.1	0.1	0.1
L-Methionine <sup>l</sup>	0.0	0.5	0.5	0.5	0.5
Sodium chloride	0.5	0.9	0.9	0.9	0.9
Potassium chloride	0.6	0.7	0.7	0.7	0.7
Magnesium oxide	0.0	0.1	0.1	0.1	0.1
Calcium phosphate dibasic	0.0	2.3	2.3	2.3	2.3
Chemical analysis (% dry basis) <sup>l</sup>					
Crude protein	45.3	46.8	44.9	44.2	46.8
Crude lipid	10.7	14.6	14.3	15.4	15.7
Crude fiber	1.3	1.0	1.4	1.3	1.0
Ash	10.5	11.8	10.2	10.1	11.6
DE (MJ kg <sup>-1</sup> dry matter)	14.92	16.81	16.24	16.51	17.25

<sup>a</sup> IPC 740, Scoular, Minneapolis, Minnesota, USA.

<sup>b</sup> BPX-HP, Poet Nutrition, Sioux Falls, South Dakota, USA.

<sup>c</sup> Bob's Red Mill Natural Foods, Milwaukie, Oregon, USA.

<sup>d</sup> Consumers Supply Distributing, Sioux City, Iowa, USA.

<sup>e</sup> Omega Protein, Inc., Houston, Texas, USA.

<sup>f</sup> Carboxymethyl cellulose.

<sup>g</sup> ARS 702, [87].

<sup>h</sup> ARS 640, [87].

<sup>i</sup> DSM Nutritional Products France SAS, Village-Neuf, France.

<sup>j</sup> Ronozyme P-CT, DSM Nutritional Products, Basel, Switzerland.

<sup>k</sup> Diamond V, Cedar Rapids, Iowa, USA.

<sup>l</sup> PureBulk, Roseburg, Oregon, USA

Analysis conducted on post-extrusion pellets.

**Table 2. Amino Acid Composition (% Dry Weight) of the Diets Used in the Trial, and of the Dietary Ingredient High Protein Distillers Dried Grain (HPDDG)**

Diet	1	2 and 3	4 and 5	HPDDG
HPDDG (%)	0	10	20	
Phytase	no	no	yes	
<u>Essential amino acids</u>				
Arginine	2.19	2.16	2.07	1.41

Table 2. cont....

Diet	1	2 and 3	4 and 5	HPDDG
Histidine	1.05	1.10	1.03	0.97
Isoleucine	1.83	2.10	2.10	1.52
Leucine	4.79	4.62	4.77	5.20
Lysine	2.32	2.75	2.57	1.14
Methionine	1.04	1.50	1.48	0.72
Phenylalanine	2.15	2.07	2.09	1.98
Threonine	1.57	1.60	1.53	1.36
Tryptophan	0.40	0.39	0.40	0.21
Valine	2.13	2.13	2.15	2.01
<u>Nonessential amino acids</u>				
Alanine	3.10	3.10	3.16	2.92
Aspartic acid	3.24	3.29	3.26	2.48
Cysteine	0.52	0.52	0.52	0.65
Glutamic acid	7.91	7.46	7.26	6.56
Glycine	2.36	2.46	2.40	1.41
Hydroxylysine	0.08	0.09	0.07	0.00
Hydroxyproline	0.37	0.41	0.00	0.00
Lanthionine	0.12	0.11	0.14	0.26
Orthonine	0.03	0.03	0.04	0.02
Proline	3.11	2.93	2.98	3.25
Serine	1.75	1.74	1.53	1.56
Taurine	0.18	0.19	0.18	0.03
Tyrosine	1.66	1.63	1.62	1.54

Table 3. Criteria Used at the End of the Study for Fish Health Observations [based on Goede and Barton [34], Adams *et al.* [35], and Barton *et al.* [36]

Structure or Tissues	Rating Criteria	Numeric Rating
Eyes	Normal Abnormal	0 1
Fat	None < 50% of gut covered > 50% of gut covered 100% of gut covered	0 1 2 3
Fins	No erosion Light erosion Moderate erosion Severe erosion	0 1 2 3
Gills	Normal Clubbed, frayed, or discolored	0 1
Gut	Normal Slight inflammation Moderate inflammation Severe inflammation	0 1 2 3
Kidney	Normal Abnormal	0 1
Liver	Normal Abnormal	0 1

Table 3. cont...

Structure or Tissues	Rating Criteria	Numeric Rating
Pseudobranchs	Normal	0
	Abnormal	1
Opercles	Normal	0
	Short	1
Spleen	Normal	0
	Cysts or enlarged	1

**Table 4. Total Tank Rearing Data (Means  $\pm$  SE), Including Feed Conversion Ratio (FCR) and Estimated Digestion Coefficient of Protein (DCP) for Tanks of Rainbow Trout Receiving One of five Different Diets Containing Either 10% or 20% High Protein Distillers Dried Grain (HPDDG), with or Without Phytase. Means in a Row with Different Letters are Significantly Different ( $N = 3, P < 0.05$ )**

Diet	1	2	3	4	5
HPDDG (%)	0	10	10	20	20
Phytase supplement	no	no	yes	no	yes
Start Weight (g)	1,218 $\pm$ 77	1,306 $\pm$ 16	1,234 $\pm$ 33	1,315 $\pm$ 59	1,309 $\pm$ 19
End Weight (g)	2,229 $\pm$ 76	2,335 $\pm$ 41	2,244 $\pm$ 46	2,367 $\pm$ 54	2,347 $\pm$ 37
Gain (g)	1,011 $\pm$ 17	1,029 $\pm$ 26	1,010 $\pm$ 70	1,051 $\pm$ 7	1,038 $\pm$ 18
Gain (%)	83.2 $\pm$ 5.4	78.8 $\pm$ 1.0	82.0 $\pm$ 7.5	80.1 $\pm$ 4.1	79.3 $\pm$ 0.3
Food fed (g)	834	834	834	834	834
FCR	0.82 $\pm$ 0.01	0.81 $\pm$ 0.02	0.83 $\pm$ 0.06	0.79 $\pm$ 0.05	0.80 $\pm$ 0.01
% mortality	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.8 $\pm$ 1.4	0.0 $\pm$ 0.0
DCP	91.4 $\pm$ 0.1 <sup>a</sup>	94.1 $\pm$ 0.1 <sup>b</sup>	93.4 $\pm$ 0.1 <sup>b</sup>	93.7 $\pm$ 0.1 <sup>b</sup>	93.4 $\pm$ 0.1 <sup>b</sup>

**Table 5. Ending Mean ( $\pm$  SE) Lengths, Weights, Condition Factors (K)<sup>a</sup>, Liver Weights, Hepatosomatic Index Values (HSI), Viscera Weights, Viscerosomatic Index (VSI) and Fish Health Assessments<sup>b</sup> of Rainbow Trout Fed Diets Containing Either 10% or 20% High Protein Distillers Dried Grains (HPDDG), with or without Phytase ( $N = 3$ )**

Diet	1	2	3	4	5
% HPDDG	0	10	10	20	20
Phytase supplement	no	no	yes	no	yes
Length (mm)	179 $\pm$ 3	176 $\pm$ 3	169 $\pm$ 2	175 $\pm$ 1	179 $\pm$ 2
Weight (g)	59.6 $\pm$ 3.6	62.0 $\pm$ 1.1	51.0 $\pm$ 2.1	55.1 $\pm$ 0.5	59.0 $\pm$ 4.9
K	1.00 $\pm$ 0.01	1.11 $\pm$ 0.09	1.01 $\pm$ 0.01	1.01 $\pm$ 0.01	0.99 $\pm$ 0.01
Viscera weight (g)	6.12 $\pm$ 0.36	6.00 $\pm$ 0.23	4.84 $\pm$ 0.13	5.42 $\pm$ 0.05	5.82 $\pm$ 0.34
VSI	10.32 $\pm$ 0.24	9.73 $\pm$ 0.26	9.45 $\pm$ 0.11	9.93 $\pm$ 0.09	12.62 $\pm$ 2.90
Liver weight (g)	0.73 $\pm$ 0.01	0.63 $\pm$ 0.01	0.55 $\pm$ 0.01	0.61 $\pm$ 0.03	0.60 $\pm$ 0.04
HSI	1.26 $\pm$ 0.06	1.03 $\pm$ 0.02	1.07 $\pm$ 0.05	1.12 $\pm$ 0.03	1.27 $\pm$ 0.26
Eyes	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Fat	1.6 $\pm$ 0.1	1.7 $\pm$ 0.1	1.9 $\pm$ 0.1	2.0 $\pm$ 0.1	1.7 $\pm$ 0.1
Fins	1.2 $\pm$ 0.2	1.3 $\pm$ 0.1	1.1 $\pm$ 0.2	1.2 $\pm$ 0.2	1.2 $\pm$ 0.2
Gills	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
Gut	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Kidney	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Liver	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Pseudobranchs	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

Table 5. cont.....

Diet	1	2	3	4	5
Opercles	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Spleen	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

<sup>a</sup> Condition factor (K) = 10<sup>5</sup> x (weight [g])/(length<sup>3</sup> [mm])

<sup>b</sup> Fish health assessments rating system described in Table 2.

**Table 6. Mean (± SE) Percent Water, Crude Protein, Crude Lipid, and Ash Concentrations from Fillets of Rainbow Trout Fed Diets Containing Either 10% or 20% High Protein distillers Dried Grains (HPDDG), with or Without Phytase (N = 3)**

Diet	1	2	3	4	5
HPDDG (%)	0	10	10	20	20
Phytase supplement	no	no	yes	no	yes
Water (%)	77.3 ± 1.5	76.2 ± 0.8	76.4 ± 0.3	75.9 ± 0.3	75.3 ± 0.3
Crude protein (%)	18.6 ± 0.5	19.1 ± 0.2	19.0 ± 0.3	19.1 ± 0.2	19.1 ± 0.2
Crude lipid (%)	4.6 ± 0.2	4.2 ± 0.9	4.4 ± 0.5	3.6 ± 0.2	3.8 ± 0.1
Ash (%)	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1

Fillet composition was not significantly affected by any of the diets used in this study (Table 6). Fillet protein percentages ranged from 18.6% in fish fed the fish meal control to 19.0% and 19.1% in fish receiving feed with HPDDG. Although not significantly different, mean fillet lipid percentages were 4.6% in the fish-meal-based diet and were less than 4.0% in the fish fed the highest dietary concentrations of HPDDG.

## DISCUSSION

The inclusion of up to 20% dietary HPDDG with no negative effects on growth or conversion is similar to that reported by Cheng and Hardy [10] with lower protein, conventional DDGS. They noted no deleterious effects of up to 22.5% inclusion in rainbow trout diets containing conventional DDGS, with lysine and methionine supplementation. In contrast, Stone *et al.* [21] noted deficiencies in diets with conventional DDGS compared to a fish meal control.

Phytase supplementation had no effect on growth or feed conversion in the fish fed diets containing HPDDG. However, these results are difficult to interpret because it is unknown how much phytate (phytic acid salts or esters that interfere with phosphorous absorption) was present in the HPDDG, the experimental diets were likely not phosphorous-limited, the efficiency of phytase may be somewhat dependent on the method used to incorporate it into the feed [40], and the phytase may have been deactivated during extrusion [41]. Cheng and Hardy [25] also observed no improvements in growth or feed conversion in rainbow trout receiving diets containing 15% DDGS and varying amounts of phytase. Rainbow trout growth was also unaffected by phytase supplementation in diets containing soy [28] or canola protein concentrate [27]. However, phytase did enhance rainbow trout growth when included in soybean-based diets [23, 24, 26]. Dietary phytase supplementation in higher quality diets of fish species other than rainbow trout has generally had no positive effect on growth and feed conversion

ratio [40, 42-44], except in common carp *Cyprinus carpio* [45, 46] and Nile tilapia *Oreochromis niloticus* [47]. Although it was not measured in this study, phytase has been repeatedly shown to increase the availability of phosphorous in fish feeds containing plant (primarily soybean) ingredients [48-52].

Similar to the results obtained by Cheng and Hardy [25] who used lower protein conventional DDGS, phytase supplementation did not improve the estimated digestibility of protein. Cheng and Hardy [25] suggested that this was due to the relatively high protein digestibility of the DDGS observed in their study. The over 93% estimated protein digestibility in this study was even greater than that observed by Cheng and Hardy [25]. This significant improvement in protein digestibility of the diets containing HPDDG compared to the fish meal control can likely be attributed to amino acid supplementation [53-56].

Fillet lipid concentrations did not increase with increasing HPDDG in this study. In contrast, increased lipid levels were observed with the dietary DDGS inclusion by Lim *et al.* [57] and Li *et al.* [58]. However, Johnsen *et al.* [59] 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. Fillet protein concentrations were also unaffected by diet in this study. Li *et al.* [58] and Li *et al.* [60] also reported decreased fillet protein composition in channel catfish *Ictalurus punctatus* fed either fish meal or DDGS-containing diets. 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 [61], but less than that reported by Sealey *et al.* [62]. However, the rainbow trout fillets analyzed by Sealey *et al.* [62] came from fish that were fed a 29% fish meal control diet that also contained 16% soybean meal. Additional differences between this study and Sealey *et al.* [62], such as feeding rates and fish sizes, may also explain the differences in fillet composition.

Although the hepatosomatic index is positively related to carbohydrate levels in the diet [63, 64], there was no increase in HSI as dietary HPDDG levels increased. Hepatosomatic index either slightly decreased, or showed no effect, from dietary HPDDG in tilapia *Oreochromis niloticus* [65, 66], and was also unaffected by dietary protein in common carp *Cyprinus carpio* [67]. Because dietary phosphorus is inversely related to liver lipid levels and HSI, [68], the lack of difference in HSI among the diets would appear to indicate no deficiencies in phosphorus availability from any of the diets, regardless of phytase supplementation.

VSI was unaffected by changes in diet, even though the experimental diets contained lipid levels greater than that of the control. Other studies have noted an increase in VSI with increasing dietary lipid levels [69-71].

The relatively low feed conversion ratios for both the control and treatment diets are not unusual for production rainbow trout of this size at hatcheries in South Dakota [72] or elsewhere [73]. They could also be explained by the low rearing densities used in the trial [74, 75], which were due to the size of the tanks available for experimentation and the limited quantity of feed that could be manufactured. Feeding rates may also have influenced feed conversion ratio results.

Because of the often dramatic differences in conventional DDGS nutritional composition [2, 12, 13], it may be difficult to compare the results between studies examining DDGS use in rainbow trout diets. In comparison to conventional DDGS, HPDDG is more nutritionally consistent [15]. Other factors, such as feed production techniques [76-78], water temperature [79-81], other dietary ingredients, such as soybean meal [10], and fish size, may also potentially effect diet performance and contribute to differing results in DDGS-related studies.

In conclusion, the results from this introductory short-term study indicate that HPDDG with amino acid supplementation may be successfully incorporated at up to 20% in juvenile rainbow trout grower diets, thereby warranting additional, longer-term, studies. With the rapid growth in global aquaculture [82] and the increased demand and market prices for limited fish meal stocks [83, 84] and other agricultural commodities, HPDDG could provide an additional and effective lower-cost protein source for the diets of carnivorous aquaculture fish species, such as trout and perhaps other salmonids [10, 33, 85, 86].

#### CONFLICT OF INTEREST

None declared.

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