Nutritional Value of Diets Containing Distiller's Dried Grain with Solubles for Rainbow Trout, *Oncorhynchus mykiss*

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ABSTRACT. A feeding trial using corn distiller's dried grain with solubles (DDGS) was conducted to evaluate the nutritional value of DDGS and effects of lysine and methionine supplementation in DDGS-based diets on the performance of rainbow trout, *Oncorhynchus mykiss*. The DDGS-based diets were made such that DDGS was used at 7.5% (diet 2), 15% (diet 3), and 22.5% (diet 4) inclusion levels without supplementing crystalline L-lysine-HCl and DL-methionine, and was used at 7.5% (diet 5), 15% (diet 6), and 22.5% (diet 7) with lysine and methionine supplementation in a 2 × 3 factorial design. A fish meal-based diet (diet 1) was used as a control. The control and lysine and methionine supplemented diets contained 2.2% lysine and 1.1% methionine. A total of 630 fish (initial body weight 49.8 g) were randomly stocked into twenty-one, 150-L tanks with 30 fish/tank and 3 tanks/diet. Fish were fed to apparent satiation 3 times/day and 6 days/week. After a 6-week growth period, weight gain (WG) of fish fed diets 1 to 7 was: 48.9, 43.9, 46.5, 42.9, 51.3, 54.3, and 46.4 g, respectively. Feed conversion ratio (FCR) of fish fed diets 1 to 7 was: 1.21, 1.35, 1.25, 1.34, 1.20, 1.11, and 1.29 g diet/g gain, respectively. Survival was 100% for fish fed all diets. Two-way ANOVA showed that lysine and methionine supplementation improved

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WG ($P = 0.0002$) and FCR ($P = 0.0011$). Fish fed diets containing 15% DDGS, or replacing 50% of fish meal on isonitrogenous and isocaloric basis, were not significantly different from fish fed the fish meal-based diet in terms of WG, FCR, and survival ($P > 0.05$), indicating that DDGS could be used at the 15% inclusion level, or replace up to 50% of fish meal. Furthermore, DDGS could be used at the 22.5% inclusion level, or replace up to 75% of fish meal in rainbow trout diets with lysine and methionine supplementation.

KEYWORDS. Distiller's dried grain with solubles, lysine, methionine, rainbow trout, *Oncorhynchus mykiss*

INTRODUCTION

Aquaculture diet costs generally account for up to 60% of total farm production costs (Tan and Dominy 1997), and two of the expensive nutrients are protein and phosphorus. Furthermore, concerns about water pollution from aquaculture activities have increased tremendously during the last decade. Excessive excretion of nutrients, especially phosphorus, is the main cause of freshwater pollution. Therefore, it is important for researchers to search for ways to reduce phosphorus excretion and minimize aquaculture production costs. One way of reducing phosphorus excretion is to supplement commercial phytase in plant protein-based fish diets (Cain and Garling 1995; Rodehutscord and Pfeffer 1995; Storebakken et al. 1998; Vielma et al. 1998, 2000; Papatryphon et al. 1999; Sugiura et al. 2001; Cheng and Hardy In press). Another way is to reduce dietary phosphorus levels. Diet ingredients from plant sources, such as distiller's dried grain with solubles (DDGS), contain less phosphorus than animal proteins, such as fish meal. Therefore, substituting DDGS for fish meal in trout diet formulations reduces the total phosphorus level of the diet, thereby lowing the levels of total phosphorus discharged into water, and thus minimizing water pollution.

In a previous study, Cheng and Hardy (unpublished data) reported that apparent digestibility coefficients of nutrients in DDGS were high for rainbow trout, *Oncorhynchus mykiss*. Apparent digestibility coefficients of crude protein was 90.4%, essential amino acids were greater than 90% except that of threonine (87.9%), and non-essential amino acids were greater than 86% except that of cystine (75.9%). Results indicated that DDGS could be a good protein source to replace portions of fish meal in trout diets. However, one disadvantage of using DDGS in fish diets is that the most limiting amino acids, lysine and methionine, are usually lower than in fish meal. Therefore, when using DDGS as fish diet ingredient, supplementing lysine and methionine is necessary. The objectives of this trial were to evaluate the nutritional value of corn DDGS and effects of supplementing crystalline L-lysine-HCl and DL-methionine in DDGS-based diets on weight gain (WG), feed conversion ratio (FCR), survival, body composition, and apparent nutrient (crude protein and phosphorus) retention for rainbow trout.

MATERIALS AND METHODS

Experimental Design and Diet Preparation

This feeding trial was designed as a 2 × 3 factorial arrangement, with two formulation strategies (supplementing with and without lysine and methionine) and three DDGS inclusion levels (7.5, 15, and 22.5%), accordingly 25, 50, and 75% of fish meal was replaced on an isonitrogenous and isocaloric basis. A fish meal-based diet containing 30% herring meal served as a control. All diets were formulated to contain 44% crude protein and a calculated digestible energy of 3600 kcal/kg diet. L-lysine-HCl and DL-methionine supplemental levels were based on the analytic values of lysine and methionine levels in DDGS and other ingredients used in this trial (data not shown). Analyzed crude protein value was close to expected values (Table 1). Experimental diets were manufactured without steam using a laboratory pellet mill (California Pellet Mill Co., San Francisco, California[1]) with a 2.4-mm die. Diets were air-dried for 48 hours and stored at room temperature (approximately 20°C) until used. Duplicate samples of each diet were taken for chemical analyses. The trial was designed as completely randomized arrangements for statistical evaluation of data, and diets were assigned randomly with respect to location to three tanks per diet within the fish rearing laboratory. This trial was conducted from November 12 to December 26, 2001, at the Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho.

1. Use of trade or manufacturer's name does not imply endorsement.

TABLE 1. Diet composition and chemical analyses (%, as is basis).

	Diets						
Ingredients[1]	1	2	3	4	5	6	7
Herring meal	30.0	22.5	15.0	7.5	22.5	15.0	7.5
Distiller's dried grain with solubles	0.0	7.5	15.0	22.5	7.5	15.0	22.5
Fish oil, herring	17.6	17.7	17.8	17.9	17.7	17.8	18.1
Whole wheat	18.1	12.7	7.3	1.9	13.15	7.91	2.51
Soybean meal, 48%	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Corn gluten meal (white)	16.9	22.2	27.5	32.8	21.2	25.8	30.36
L-lysine-HCl, 98.5%, feed grade	0.0	0.0	0.0	0.0	0.41	0.82	1.23
DL-methionine	0.0	0.0	0.0	0.0	0.14	0.27	0.40
Vitamin C (Stay-C)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Trace mineral premix[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix[3]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Chemical analyses (%, as is basis, n = two samples)							
Moisture	6.0	5.8	5.8	6.3	6.2	6.4	6.9
Crude protein	43.4	43.7	43.8	43.7	43.9	44.2	43.5
Crude fat	19.4	19.9	18.9	17.1	19.3	18.5	18.8
Ash	8.6	6.8	6.2	4.9	6.9	5.7	4.7
Phosphorus	0.9	0.8	0.7	0.6	0.8	0.7	0.6

[1]Origin of ingredients is as follows: herring meal, whole wheat, corn gluten, choline chloride, trace mineral premix, and vitamin premix were purchased from Nelson & Sons, Inc., Murray, Utah; fish oil, soybean meal, and vitamin C were from Rangen, Inc., Buhl, Idaho; Distiller's dried grain with solubles was from Pro-Corn, LLC, Preston, Minnesota; L-lysine-HCl was from Heartland Lysine Inc., Chicago, Illinois; DL-methionine was from Sigma Chemical Co., St. Louis, Missouri.
[2]Composition of trace mineral premix (mg/kg): Zn (as $ZnSO_4 \cdot 7H_2O$), 75; Mn (as $MnSO_4$), 20; Cu (as $CuSO_4 \cdot 5H_2O$), 1.54; I (as KIO_3), 10.
[3]Composition of vitamin premix (mg/kg of premix, unless otherwise listed): D Calcium pantothenate, 26,840; pyridoxine (pyridoxine HCl), 7,700; riboflavin, 13,200; niacinamide, 55,000; folic acid, 2,200; thiamine (thiamine mononitrate), 8,800; biotin, 88; vitamin B_{12}, 5.5; menadione sodium bisulfite complex, 2.75; vitamin E (DL α-tocopherol acetate), 88,000 IU; vitamin D_3 (stabilized), 110,000 IU; vitamin A (vitamin A palmitate, stabilized), 1,650,000 IU.

Fish Culture

Rainbow trout (average weight 49.84±0.05 g) were selected from a large population, counted in groups of 30 fish per tank with 3 tanks per diet (treatment). Fish were weighed and placed into twenty-one, 150-L fiberglass tanks. Fish tanks were supplied with 4 L/minute of untreated, constant temperature (14.5°C) spring water. Fish were fed 3 times per day and 6 days per week to apparent satiation for a period of 6 weeks. At the end of the trial, all fish were starved for 48 hours, and 5 fish from each tank were sacrificed and pooled for whole-body composition analyses.

Sampling and Analyses

Fish in each tank were bulk-weighed and counted at the beginning and the end of the experiment. From the initial population of fish, and from each tank at the end of the experiment, 5 fish were sacrificed, processed into a puree using a Robot Coupe food processor (Robot Coupe R-2, Ridgeland, Mississippi), and subsampled for proximate and phosphorus analyses. Analyses of diet samples were conducted using the following methods: moisture by oven drying at 105°C for 2 hours (AOAC 1990), crude protein by nitrogen determination (total nitrogen × 6.25) using a LECO FP-428 nitrogen analyzer (LECO Instruments, St Joseph, Michigan), crude fat by using a soxhlet extraction apparatus (Soxtec System HT, Foss Tecator AB, Hoganas, Sweden) using methylene chloride as the extracting solvent, ash by incineration at 550°C in a muffle furnace, and phosphorus by the method of Taussky and Shorr (1953). The methods used for analyzing fish samples were the same as those for diet samples except that fish moisture was determined by oven drying overnight. The trial followed the guidelines approved by the Animal Care and Use Committee of the University of Idaho, Moscow, Idaho.

Calculations and Data Analyses

Fish weight gain (g) was determined by fish final weight minus initial weight; feed conversion ratio was calculated as diet consumed (g)/weight gain (g); and apparent nutrient retention was calculated as [nutrients in whole fish body (final)–nutrients in whole fish body (initial)]/nutrient intake from diet. Differences among fish fed all diets were analyzed using one-way analysis of variance (ANOVA) with Prism, version 3.0 (GraphPad Software, Inc., San Diego, California). A significance level of $P < 0.05$ was used, and tank mean values were considered units of observation for statistical analysis. Two-way ANOVA was used to evaluate the effects of lysine and methionine supplementation and DDGS supplemental levels on fish WG, FCR, survival, body composition, and apparent nutrient retention.

RESULTS AND DISCUSSION

Initial weight, final weight, WG, and FCR of fish fed experimental diets for 6 weeks are presented in Table 2. There were no significant differences in fish initial weight ($P = 0.99$). Fish were selected from a large population, and on the basis of no visible disease sign or physical defect. After a 6-week growing period, significant differences occurred among fish fed different diets. Fish fed the fish meal-based control diet (diet 1) grew faster than those fed the 22.5% DDGS-based diet (diet 4, 75% of fish meal replacement diet), but were not different from fish fed the 7.5 and 15% DDGS-based diets (diets 2 and 3, respectively), indicating that DDGS could be used at up to a 15% inclusion level, or to replace up to 50% of fish meal in rainbow trout diets.

There were no significant differences among fish fed DDGS-based diets supplemented with lysine and methionine (diets 5, 6, and 7) and fish fed the fish meal-based control diet, meaning that lysine and methionine supplementation improved the nutritional value of DDGS, and DDGS could be used at 22.5% inclusion level, or replace up to 75% of fish meal in rainbow trout diets with lysine and methionine supplementation.

FCR followed a pattern similar to fish WG. Fish fed the fish meal-based control diet had lower FCR than fish fed the 7.5 and 22.5% DDGS-based diets, but were not different from fish fed other diets. Fish fed diet 3 had lower FCR than fish fed diets 2 and 4. Fish fed DDGS-based diets with lysine and methionine supplementation (diet 5) had lower FCR than those fed DDGS-based diets without amino acid supplementation (diet 2), indicating that DDGS was deficient in lysine and methionine. There were no significant differences in survival among fish fed different diets; survival was 100% for fish fed all diets.

Two-way ANOVA (Table 2) showed that there were no significant interactions between lysine and methionine supplementation and DDGS inclusion levels in WG ($P = 0.2913$) and FCR ($P = 0.2541$). Lysine and methionine supplementation improved WG ($P = 0.0002$) and FCR ($P = 0.0011$) significantly. DDGS inclusion levels also had significant effects on WG ($P = 0.0057$) and FCR ($P = 0.0035$). When DDGS inclusion level was 15%, fish had lower FCR than fish fed diets containing 7.5 and 22.5% DDGS. This may be due to the fact that amino acids in fish meal and DDGS complement each other in the 50% fish meal replacement diets, and thus providing an optimum amino acid profile for fish growth.

TABLE 2. Initial weight, final weight, weight gain and feed conversion ratio of rainbow trout fed experimental diets for 6 weeks (Mean±S.D., n = 3 tanks). Means in the same row that do not share a common letter differ significantly ($P < 0.05$).

	Diets							
Items	1	2	3	4	5	6	7	P values
Initial weight (g)	49.9±0.5a	49.9±0.8a	49.9±0.2a	49.9±0.3a	49.8±0.6a	49.8±0.7a	49.8±0.5a	0.9999
Final weight (g)	98.8±1.8a	93.8±2.8ab	96.4±0.9ab	92.8±1.3b	101.1±2.6a	104.1±3.9a	96.2±2.1ab	0.0005
Weight gain (g)	48.9±1.9a	43.9±2.4ab	46.5±0.6ab	42.9±1.3b	51.3±2.0a	54.3±4.6a	46.4±1.7ab	0.0005
Feed conversion ratio (g diet/g gain)	1.21±0.04a	1.35±0.06b	1.25±0.02a	1.34±0.02b	1.20±0.05a	1.11±0.09a	1.29±0.04ab	0.0010

Two-way ANOVA (P value summary)

	Lysine and methionine supplementation	DDGS inclusion level	Interactions
Weight gain	0.0002	0.0057	0.2913
Feed conversion ratio	0.0011	0.0035	0.2541

Whole-body composition of rainbow trout and apparent nutrient retention are presented in Table 3. Two-way ANOVA showed significant interactions between lysine and methionine supplementation and DDGS inclusion levels in fish body moisture, crude protein, crude fat, ash and phosphorus ($P < 0.05$), and significant interactions between lysine and methionine supplementation and DDGS inclusion levels in apparent retention of crude protein and phosphorus ($P < 0.05$). Thus, all data were analyzed using one-way ANOVA. There were significant differences in moisture and crude fat contents among fish fed different diets, but no differences in crude protein, ash, and phosphorus levels were found. Fish body moisture levels decreased but crude fat level increased as DDGS levels in diets increased (diets 2, 3, and 4).

Fish fed the 15% DDGS-based diet with lysine and methionine supplementation (diet 6) had the highest apparent protein retention. Fish fed the fish meal-based control diet had significantly higher apparent protein retention than fish fed the 7.5% DDGS-based diet (diet 2), but were not significantly different than fish fed other diets except for fish fed diet 6. Fish fed the DDGS-based diets (except diet 2) had significantly higher apparent phosphorus retention than fish fed the fish meal-based control diet. Fish fed diets containing higher DDGS inclusion levels had higher apparent phosphorus retention than fish fed diets containing lower DDGS inclusion levels (diet 4 versus diet 3 versus diet 2); a similar trend existed when fish were fed DDGS-based diets supplemented with lysine and methionine (diet 7 versus diet 6 versus diet 5). This was due to the fact that higher inclusion levels of DDGS in diets had lower dietary phosphorus (Table 1). When diets were made to meet the phosphorus requirement of 0.6% (NRC 1993), apparent phosphorus retention was the highest (diets 4 and 7). Results of this trial demonstrate that by replacing high phosphorus fish meal with low phosphorus DDGS, dietary phosphorus levels are reduced, and the apparent phosphorus retention is increased.

Traditionally, DDGS was used in diets for ruminant animals as an energy and protein source. The use of DDGS in fish diets started in late 1940s (Phillips 1949). Earlier studies indicated that warmwater fish such as channel catfish, *Ictalurus punctatus*, could utilize 7.5-15% DDGS (Hastings 1967; Robinette 1984). In the early 1990s, researchers had successfully used DDGS in winter diets for adult channel catfish (515 g) at an inclusion rate up to 90% (Webster et al. 1992b). Tidwell et al. (1990) reported that there were no significant differences in individual fish weight, percentage survival, or feed conversion for channel catfish fed diets containing 0, 10, 20, and 40% DDGS for 11 weeks. In two 12-week studies, Webster et al. (1991, 1992a) found that juvenile chan-

TABLE 3. Rainbow trout whole body composition (%, Mean ± S.D., as is basis, n = 15 pooled fish) and apparent nutrient retention. Means in the same row that do not share a common letter differ significantly ($P < 0.05$).

	Diets							
Items	1	2	3	4	5	6	7	P values
Moisture	72.3±0.4a	73.2±0.7b	71.6±0.9a	69.8±1.8c	71.9±0.4a	71.5±0.3a	71.1±0.4a	< 0.0001
Crude protein	16.0±0.1a	15.8±0.3a	15.8±0.8a	16.2±1.6a	15.7±0.5a	16.3±0.6a	15.8±0.5a	0.7406
Crude fat	9.8±0.4a	9.0±0.5b	10.8±0.8c	12.1±0.8d	10.5±0.4ac	10.4±0.3ac	10.8±0.4c	< 0.0001
Ash	3.2±0.7a	2.6±0.2a	2.5±0.1a	3.1±0.3a	2.7±0.6a	3.1±0.4a	2.8±0.8a	0.1781
Phosphorus	0.15±0.02a	0.14±0.01a	0.15±0.01a	0.16±0.02a	0.15±0.00a	0.15±0.01a	0.15±0.02a	0.7566
Apparent nutrient retention (%, Mean±S.D., n = 15 pooled fish)								
Crude protein	31.5±1.1a	27.6±1.3b	29.7±0.4ab	29.3±0.5ab	30.4±1.4ab	35.0±2.8c	28.9±0.8ab	0.0005
Phosphorus	26.4±0.9a	26.6±1.2a	32.8±0.4b	46.6±0.6c	29.6±1.3d	38.9±2.9e	42.0±1.1f	< 0.0001

nel catfish (10-11 g) could utilize 35% DDGS and, if lysine was supplemented, fish could utilize a diet containing 70% DDGS. Webster et al. (1993) further reported that channel catfish raised in floating cages could utilize up to 30% DDGS. It was reported that rainbow trout could utilize 3% DDGS (Sinnhuber 1964) and lake trout, *Salvelinus namaycush*, could use 8% DDGS (Hughes 1987). Results in the present trial indicate that DDGS can be used at the 15% inclusion level, or replace up to 50% of fish meal. With supplemental lysine and methionine, DDGS can be used at the 22.5% inclusion level, or replace up to 75% of fish meal in rainbow trout diets.

Amino acid nutrition, especially lysine and methionine in aquaculture nutrition, has received increased attention during the last two decades (Robinson et al. 1980; Murai et al. 1986; Robinson 1992; Bai and Gatlin 1994; Zarate and Lovell 1997; Robinson and Li 1998; Mukhopadhyay and Ray 1999; Coyle et al. 2000; Webster et al. 1992a, 1992b, 2000; Cheng et al. In press). Lysine is the most limiting amino acid in fish diets; when lysine is deficient, fish growth and FCR will be affected (Wilson 1991). Results from the present trial show that crystalline L-lysine-HCl and DL-methionine supplementation in DDGS-based diets improves WG and FCR for rainbow trout. DDGS is deficient in lysine and methionine. Therefore, when lysine and methionine are supplemented, the performance of rainbow trout is improved.

Results of the present trial show that DDGS is a good protein source in rainbow trout diets. It can be used at the 15% inclusion level in diets to replace up to 50% of fish meal on an isonitrogenous and isocaloric basis. When lysine and methionine are supplemented, DDGS can be used at the 22.5% inclusion level to replace up to 75% of fish meal in rainbow trout diets. Future research should focus on the entire growing stages of the fish to elucidate the advantages of feeding DDGS to rainbow trout, and on the economic analyses comparing fish meal and DDGS in rainbow trout diets.

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