Effects of Microbial Phytase Supplementation in Corn Distiller's Dried Grain with Solubles on Nutrient Digestibility and Growth Performance of Rainbow Trout, *Oncorhynchus mykiss*

Zongjia J. Cheng
Ronald W. Hardy

ABSTRACT. Two experiments were conducted to evaluate the effects of microbial phytase in corn distiller's dried grain with solubles (DDGS) on apparent digestibility coefficients (ADCs) and growth performance of rainbow trout, *Oncorhynchus mykiss*. In experiment 1, DDGS was supplemented with phytase at 0, 300, 600, 900, and 1200 FTU/kg diet. A total of 180 fish (129.1±9.3 g) were stocked into six 140-L tanks with 30 fish/tank. Fish were assigned randomly to a reference diet and each of the 5 diets containing DDGS. Yttrium oxide was used as an inert marker. Results showed that ADCs in DDGS supplemented with different dosages of phytase were: dry matter, 49.1-58.6%; crude fat, 78.9-88.9%; crude protein, 80.0-91.9%; gross energy, 50.5-66.6%; minerals, −7.3-99.7%; and amino acids: 73.9 to 96.8%. In experiment 2, a basal diet containing 15% DDGS supplemented with lysine and methionine was used to determine if trace mineral supplemental levels in rainbow trout diets could be reduced if microbial phytase was supplemented. Six diets were made with trace mineral premix supplementation at 0.1, 0.08, 0.06, 0.04, 0.02, and 0%. Phytase was not supplemented in the basal diet, but supplemented at 500 FTU/kg diet in all other diets. Ten-week results showed that there

Zongjia J. Cheng and Ronald W. Hardy, Hagerman Fish Culture Experiment Station, University of Idaho, 3059F National Fish Hatchery Road, Hagerman, ID 83332.

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were no significant differences in fish weight gain, feed conversion ratio, and survival ($P > 0.05$). There were no significant differences in fish body composition and apparent nutrient retention among fish fed all diets, except that fish fed a diet without trace mineral supplementation had the lowest zinc level and the highest manganese retention. Results indicated that phytase was effective in releasing most of minerals, and that trace mineral supplementation level could be reduced when phytase was used in rainbow trout diets.

KEYWORDS. Distiller's dried grain with solubles, microbial phytase, apparent digestibility coefficients, rainbow trout, *Oncorhynchus mykiss*

INTRODUCTION

Mineral nutrition research in rainbow trout, *Oncorhynchus mykiss*, has increased in the past decade (Wilson 1992; Sugiura et al. 1999, 2000, 2001; Bharadwaj and Brown 2001; Cheng and Hardy In press). Availabilities of minerals, especially phosphorus, are of increasing concern in commercial fish production. Excessive dietary phosphorus will not be retained by the fish, and thus it will cause eutrophication in the fresh water environment (Auer et al. 1986; Hecky and Kilham 1988; Wiesmann et al. 1988; Elser et al. 1990; Pillay 1992; Ketola and Harland 1993; Monteiro et al. 1997). The main source of phosphorus is from fish feed (Bergheim et al. 1984; Cho and Bureau 1997).

Carnivorous fish such as rainbow trout consume high protein diets. Traditionally the protein has been supplied by fish meal. Fish meal production from wild-catches is not growing worldwide (Hardy and Tacon 2002) and, therefore, plant protein meals such as corn distiller's dried grain with solubles (DDGS) will have to be increasingly used in fish diets. However, approximately two thirds of total-phosphorus in plants is present as phytate, the bioavailability of which is very limited to fish (NRC 1993). Phytase is an enzyme specific to phytate hydrolysis. This enzyme is present in the digestive tract of many animals including trout, but the amount is normally too small to digest dietary phytate to a significant extent (Bitar and Reinhold 1972; Sugiura et al. 1999).

Commercial phytase is available in the market. By applying phytase in plant protein-based fish diets, the digestibility of phytate-phosphorus

in fish diets will be increased. Total-phosphorus discharged into water will also be reduced when total-phosphorus levels are lowered by changes in feed formulation. Thus, replacing portions of fish meal with DDGS in fish diets is a promising economical alternative for the aquaculture business. It will not only reduce fish diet costs, but also reduce environmental pollution due to lower discharges of phosphorus and other minerals from fish farms. Furthermore, there is no data on nutrient digestibility and retention in DDGS-based diets for rainbow trout. The objectives of this study were to evaluate effects of phytase supplementation on apparent digestibility coefficients (ADCs) of nutrients in DDGS and to determine the growth performance and apparent nutrient retention of rainbow trout fed diets containing DDGS, phytase, and varying levels of trace mineral premix.

MATERIALS AND METHODS

Experiment 1

Experiment 1 was a digestibility trial in which corn DDGS (Minnesota Energy Ethanol, Buffalo Lake, Minnesota[1]) was combined with a semi-purified, casein-gelatin-based reference diet (Table 1), such that the DDGS constituted 30% of the diet. Yttrium (Y) oxide was added to the diets at 0.01% to serve as an inert indicator for digestibility determination. A reference diet and 5 other diets were made to contain 0, 0, 300, 600, 900, and 1200 microbial phytase (Natuphos 5000 G, BASF Canada, British Columbia, Canada) units (FTU/kg diet). The diets were cold-pelleted using a noodle-making machine, air-dried and stored at room temperature (approximately 20°C) until used. The ADCs for dry matter, crude fat, crude protein, gross energy, minerals, and amino acids were determined. The objectives of experiment 1 were to evaluate effects of phytase supplementation on ADCs of nutrients in DDGS and to determine the optimum dosage of phytase that can be used in DDGS to release nutrients for rainbow trout.

Rainbow trout (average weight 129.1±9.3 g) were selected from a large population, counted in groups of 30 fish, and placed into 140-L digestibility tanks. Each tank was supplied with 5 L/minute of untreated, constant temperature (14.5°C) spring water at the Hagerman Fish Culture

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

TABLE 1. Composition of the reference diet (experiment 1).

Ingredients	%
Casein[1]	44.0
Gelatin[2]	10.5
Dextrin[2]	11.0
Carboxymethyl cellulose[2]	1.0
Alpha-cellulose[2]	4.5
Mineral mixture[3]	3.3
Vitamin mixture[4]	2.0
Amino acid mixture[5]	4.1
Ascorbic acid[6]	0.2
Choline chloride[1]	1.0
Finnstim[7]	1.39
Yttrium oxide[2]	0.01
Herring oil[8]	17.0
Trace mineral solution[9]	(10)
Water	(30)

[1] Purchased from ICN Biomedicals, Inc., Cleveland, Ohio.
[2] Purchased from Sigma Chemical Co., St. Louis, Missouri.
[3] Supplies the following per kg diet: KCl, 12.4 g; $CaHPO_4$, 22 g; MgO, 3 g; NaCl, 2.7 g.
[4] Supplies the following per kg diet: thiamin mononitrate, 62 mg; riboflavin, 71 mg; niacin, 294 mg; calcium pantothenate, 153 mg; pyridoxine hydrochloride, 50 mg; folic acid, 22 mg; vitamin B_{12}, 0.08 mg; d-biotin, 0.8 mg; myoinositol, 176 mg; retinol acetate, 8818 IU; vitamin D_3, 588 mg; α-tocopheryl acetate, 670 mg; menadione sodium bisulfite complex, 37 mg.
[5] Supplies the following per kg dry diet: DL-methionine, 10 g; L-arginine, 10 g; L-histidine, 3 g; L-lysine, 10 g; L-glycine, 10 g; L-threonine, 2 g.
[6] Ascorbate-2-phosphate, Hoffman La-Roche, Basel, Switzerland.
[7] Palatability enhancer, containing 48% betaine. EWOS Canada, LTD, Surrey, B.C., Canada.
[8] Purchased from Rangen Inc., Buhl, Idaho.
[9] Supplies the following per kg dry diet: KI, 1.5 mg; $MnSO_4 \cdot H_2O$, 20 mg; $ZnSO_4 \cdot 7H_2O$, 75 mg; Na_2SeO_3, 2 mg; $CoCl_3 \cdot 6H_2O$, 1.0 mg; $CuSO_4 \cdot 5H_2O$, 3 mg; $FeSO_4 \cdot 7H_2O$, 50 mg.

Experiment Station, University of Idaho, Hagerman, Idaho. The fish culture laboratory was illuminated from 0500 to 1900 hours with fluorescent lighting. Fish were acclimated to experimental conditions for two weeks, during which time all fish received a commercial trout diet (Silver Cup, Murray, Utah). Experimental diets were fed once daily at 1330 hours to apparent satiation for one week before fecal collection. After feeding, tanks were completely cleaned and feces were collected at 1300 hours the next day by the settlement technique (Cho and Slinger 1979).

Fecal collection lasted for two weeks. After collection in the first week, fish were transferred to different tanks and fed their respective diets for 5 days, and then feces were collected again according to the rotational method used by Rawles and Gatlin (2000). Feces collected in

each week were pooled and represented a replicate, and analyzed separately. The experiment protocol was approved by Animal Care Committee, University of Idaho, Moscow, Idaho. During the entire experiment, no fish mortality or disease sign occurred.

Diet samples were dried in a convection oven at 105°C for 2 hours according to the methods of AOAC (1990), and feces were dried overnight. The dried samples were finely ground by mortar and pestle and were analyzed for crude fat using a soxhlet extraction apparatus (Soxtec System HT, Foss Tecator AB, Hoganas, Sweden) using methylene chloride as the extracting solvent. Crude protein (total nitrogen × 6.25) was analyzed using LECO FP-428 nitrogen determinator (Leco Instruments, St. Joseph, Michigan). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, Illinois).

Phytate-phosphorus was determined according to the method of Latta and Eskin (1980). Briefly, 1 g diet or 0.5 g fecal samples were acid extracted using 0.65 N HCl for 3 hours at room temperature with continuous shaking, centrifuged at 12,000 rpm for 5 minutes, and eluted through anion exchange column chromatograph (AG1-X4 resin, 100-200 mesh, Bio-Rad Laboratory, Hercules, California) using the procedure of Harland and Oberleas (1986). Phytate-phosphorus level was measured using sodium phytate (dodecasodium salt, 99% purity, 12% water. Sigma Chemical Co., St Louis, Missouri) as a standard.

Other mineral analyses were conducted by the University of Idaho Analytical Sciences Laboratory, Moscow, Idaho. Briefly, samples were dried at 60°C for 48 hours and ground to pass a 1 mm sieve. A 0.25-g sub-sample was predigested in 3 mL of concentrated, trace-metal grade nitric acid at room temperature overnight followed by digestion at approximately 115°C for 3-4 hours. Digestates were diluted to 10 mL with 18 MΩ•cm water and analyzed for calcium, potassium, magnesium, sulfur, total-phosphorus, copper, manganese, zinc and Y using an Optima 3200 radial inductively-coupled plasma atomic emission spectrometry (Perkin-Elmer Corp., Norwalk, Connecticut). Amino acids were analyzed by University of Missouri-Columbia, Missouri.

The ADCs of dry matter, crude fat, crude protein, gross energy, minerals, and amino acids were calculated as a fractional net absorption of nutrients from diets based on Y as a non-absorbable indicator (Marcus and Lengemann 1962; Sugiura et al. 1998). The digestibility data were subjected to one-way analysis of variance to determine the differences among different treatments. The ADCs of nutrients (or dry matter and energy) for reference and test diets were calculated as: ADCs (%) = 100 ×

[1 − (% marker in diets/% marker in feces) × (% nutrient in feces/% nutrient in diets)] (Maynard and Loosli 1969; NRC 1993), and ADCs of dry matter was calculated as: ADCs (%) = 100 × [1 − (% marker in diets/% marker in feces)].

ADC values for test ingredients supplemented with different dosages of phytase were calculated using the formula below (Cho and Slinger 1979; Cho et al. 1982; Hajen et al. 1993): ADC (%) = [ADC_{test} − (1 − i) × ADC_{ref}]/i, where ADC_{test} and ADC_{ref} were the apparent nutrient (or dry matter and energy) digestibility coefficients of the test and reference diets, respectively, and i was the percentage of ingredient included in the test diets, i = 30% or 0.3 in this experiment. Digestible energy was calculated using the following equation: Digestible energy (mj/kg) = gross energy (mj/kg) × ADCs (%).

Prism, version 3.0 (GraphPad, Inc., San Diego, California), was used to perform statistical calculations. Mean values of experimental variables in each diet were compared using all pair-wise multiple comparison procedures of the Student-Newman-Keuls. $P < 0.05$ was considered statistically significant.

Experiment 2

This experiment was a feeding trial in which 6 diets were made with dietary trace mineral premix levels reduced by 0, 20, 40, 60, 80, and 100% (diets 1 to 6, respectively). Microbial phytase (Ronozyme P (L), Roche Vitamins, France) was supplemented at 500 FTU/kg diet in diets 2 to 6, but not in diet 1. A total of 540 fish were randomly distributed into 18 tanks with 30 fish per tank and 3 tanks per diet. Fish were fed 3 times per day and 6 days per week for 10-week growth period. At the end of the experiment, all fish were starved for 48 hours and 5 fish from each tank were sacrificed and pooled together for processing into a puree using a Robot Coupe food processor (Robot Coupe Co., Ridgeland, Mississippi). This puree was subsampled for analyses of fish body composition. The objectives of experiment 2 were to determine the effects of reducing trace mineral premix supplementation and on apparent nutrient retention in rainbow trout fed diets supplemented with phytase.

Fish culture, and sample and data analyses procedures were the same as those described in experiment 1, except that samples for fish body composition were dried overnight. Diet formulation and composition are presented in Table 2. Diets were formulated to be isonitrogenous and isocaloric, and contained 41% crude protein, 21% crude fat, and a calculated digestible energy of 3.6 kcal/g diet. Diets were pelleted with-

TABLE 2. Diet formulation (%) and composition (experiment 2).

	Diets					
	1	2	3	4	5	6
	Microbial phytase supplemental levels (FTU/kg diet)					
Ingredients[1]	0	500	500	500	500	500
Fish meal (herring)	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal	15.00	15.00	15.00	15.00	15.00	15.00
Distiller's dried grain with solubles	15.00	15.00	15.00	15.00	15.00	15.00
Whole wheat flour	7.91	7.93	7.95	7.97	7.99	8.01
Fish oil (herring)	17.80	17.80	17.80	17.80	17.80	17.80
Corn gluten meal (yellow)	25.80	25.80	25.80	25.80	25.80	25.80
L-lysine-HCl	0.82	0.82	0.82	0.82	0.82	0.82
DL-methionine	0.27	0.27	0.27	0.27	0.27	0.27
Choline chloride (50%)	0.50	0.50	0.50	0.50	0.50	0.50
Stay-C	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix[2]	1.50	1.50	1.50	1.50	1.50	1.50
Trace mineral premix[3]	0.10	0.08	0.06	0.04	0.02	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Composition of diets (as fed basis, n = 2 samples)						
Moisture (%)	7.46	7.65	7.28	7.37	7.18	7.78
Crude protein (%)	41.22	40.88	41.20	41.29	41.01	40.72
Crude fat (%)	21.23	21.74	21.78	21.53	21.52	21.54
Ash (%)	5.34	5.22	5.22	5.13	5.42	5.07
Calcium (%)	1.02	0.94	0.97	0.90	0.88	0.97
Phosphorus (%)	0.97	0.95	0.97	0.93	0.92	1.01
Potassium (%)	0.80	0.79	0.78	0.79	0.78	0.81
Magnesium (%)	0.15	0.15	0.15	0.15	0.15	0.15
Sodium (%)	0.13	0.13	0.13	0.13	0.13	0.14
Sulfur (%)	0.60	0.61	0.63	0.63	0.60	0.62
Copper (mg/kg)	8.33	8.27	8.02	8.29	7.70	7.56
Manganese (mg/kg)	55.53	49.87	51.92	40.30	36.20	31.35
Zinc (mg/kg)	129.56	106.20	97.35	77.81	59.41	46.57

[1]Origin of ingredients: fish meal, whole wheat flour, corn gluten meal and choline chloride 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; Ronozyme P (L) was provided by Roche Vitamins, Inc., France.

[2]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.

[3]Composition of trace mineral salt (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.

out steam using a laboratory pellet mill (California Pellet Mill Co., San Francisco, California) with a 2.4-mm die. All diets were air-dried at room temperature for 48 hours and stored at approximately 20°C until used. The analyzed crude protein and crude fat levels were close to calculated values. Dietary trace mineral levels met or slightly exceed the requirement for rainbow trout (NRC 1993). Fish weight gain (WG), feed conversion ratio (FCR), survival, fish body composition, and apparent nutrient retention were determined. Apparent nutrient retention (%) was calculated as: [amount of nutrients in whole fish body (final) − amount of nutrients in whole fish body (initial)]/amount of nutrient intake from feed.

RESULTS AND DISCUSSION

Experiment 1

Chemical analyses of DDGS, a reference diet (diet 1) and five other diets containing DDGS and supplemented with 0, 300, 600, 900 and 1200 FTU phytase/kg diet (diets 2 to 6, respectively) are presented in Table 3. This batch of DDGS was diluted during production by mixing 25% molasses with DDG; therefore, the crude protein level was 19.28% instead of 27% (NRC 1993). Relatively high levels of crude protein and gross energy in DDGS are desirable in fish feeds. Phytate-phosphorus in DDGS was 0.28%, which was about 36% of total phosphorus. This level of phytate-phosphorus was low compared to phytate-phosphorus in other cereal grain and oilseed meals. This might due to the fermentation process used in producing ethanol, which could break the chemical bonds of the phytate.

Mineral levels in DDGS were slightly different from published values (NRC 1993). The crude protein level in the reference diet was 54%, higher than the other diets, which had about 44% crude protein because they were diluted at 70:30 ratio with DDGS. There were no significant differences in crude protein levels among the five test diets (diet 2 through diet 6). Although 54% crude protein in the reference diet seemed to be high, it was highly digestible in previous experiments conducted in our laboratory (Sugiura et al. 1998, 1999). Other nutrients including minerals in the five test diets were also not significantly different.

The amino acid composition of DDGS and experimental diets is presented in Table 4. The levels of arginine, methionine, and threonine were close to published values (NRC 1993) when considering the 25%

TABLE 3. Chemical composition of corn distiller's dried grain with solubles (DDGS) and experimental diets (as fed basis, n = 2 samples).

		Microbial phytase supplemental levels (FTU/kg diet)					
		0	0	300	600	900	1200
	Ingredient	Diets					
Items	DDGS	1	2	3	4	5	6
Moisture (%)	9.58	7.25	7.38	7.36	8.26	6.14	7.70
Ash (%)	6.39	4.63	4.23	4.49	4.64	4.51	4.46
Crude fat (%)	2.64	15.62	12.00	11.72	12.44	12.39	12.22
Crude protein (%)	19.28	54.12	44.22	44.18	43.84	44.45	43.90
Gross energy (mj/kg)	19.90	25.64	23.93	24.35	24.40	24.35	24.31
Calcium (%)	0.06	0.58	0.42	0.43	0.42	0.42	0.43
Potassium (%)	1.20	0.59	0.77	0.77	0.76	0.76	0.76
Magnesium (%)	0.32	0.15	0.20	0.20	0.20	0.20	0.20
Sodium (%)	0.65	0.18	0.32	0.33	0.32	0.32	0.32
Sulfur (%)	0.37	0.59	0.54	0.55	0.54	0.54	0.55
Phytate-phosphorus (%)	0.28	0.00	0.09	0.08	0.08	0.08	0.09
Total-phosphorus (%)	0.77	0.80	0.80	0.80	0.79	0.78	0.80
Copper (mg/kg)	5.10	7.50	5.65	5.70	5.80	5.65	5.65
Manganese (mg/kg)	18.50	5.00	9.50	9.00	9.00	9.00	10.00
Zinc (mg/kg)	57.50	90.50	100.00	110.00	110.00	110.00	110.00

molasses level in this batch of DDGS; cystine, histidine, lysine, and tryptophan levels were slightly higher, and those of isoleucine, leucine, phenylalanine, tyrosine, and valine were slightly lower than published values (NRC 1993). All essential and non-essential amino acid levels among the 5 test diets were not significantly different.

The ADCs of DDGS are presented in Table 5. The ADCs of dry matter, calcium, phytate-phosphorus, copper, manganese, and zinc in DDGS were low (< 50%), and manganese and zinc in DDGS were completely unavailable to rainbow trout. The ADCs of crude fat, magnesium, sulfur, and total-phosphorus were greater than 50%, and those of crude protein, potassium, and sodium were greater than 90% in DDGS. Phytase supplementation at 300 FTU/kg diet significantly improved ADCs of dry matter, calcium, magnesium, total-phosphorus, manganese and zinc ($P < 0.05$). At a supplemental level of 600 FTU/kg diet,

TABLE 4. Amino acid composition of corn distiller's dried grain with solubles (DDGS) and experimental diets (%, as fed basis, n = 2 samples).

		Microbial phytase supplemental levels (FTU/kg diet)					
		0	0	300	600	900	1200
	Ingredient	Diets					
Items	DDGS	1	2	3	4	5	6
Essential amino acids							
Arginine	0.82	2.87	2.24	2.28	2.23	2.24	2.22
Histidine	0.57	1.54	1.24	1.23	1.18	1.20	1.19
Isoleucine	0.67	2.32	1.84	1.88	1.77	1.78	1.78
Leucine	1.79	4.30	3.63	3.62	3.45	3.53	3.48
Lysine	0.59	4.17	3.17	3.18	3.04	3.08	3.08
Methionine	0.31	1.92	1.46	1.51	1.42	1.46	1.45
Phenylalanine	0.76	2.42	1.95	1.95	1.86	1.91	1.90
Threonine	0.73	1.99	1.68	1.68	1.60	1.65	1.66
Tryptophan	0.16	0.59	0.46	0.49	0.49	0.51	0.49
Valine	0.98	3.00	2.40	2.43	2.30	2.31	2.31
Non-essential amino acids							
Alanine	1.34	1.83	1.67	1.71	1.65	1.66	1.65
Aspartic acid	1.24	3.33	2.74	2.77	2.65	2.68	2.69
Cysteine	0.41	0.25	0.30	0.31	0.29	0.30	0.29
Glutamic acid	2.73	11.09	8.62	8.95	8.36	8.48	8.59
Glycine	0.84	2.98	2.29	2.38	2.35	2.31	2.30
Proline	1.57	5.33	4.22	4.36	4.10	4.16	4.19
Serine	0.73	1.98	1.78	1.85	1.72	1.77	1.84
Tyrosine	0.59	2.35	1.82	1.80	1.71	1.77	1.76

phytase also significantly improved the ADCs of crude fat, calcium, magnesium, phytate-phosphorus, total-phosphorus, manganese and zinc ($P < 0.05$). However, supplementation higher than 900 FTU/kg diet did not result in further improvement in terms of ADCs for the nutrients, except that ADCs of phytate-phosphorus and manganese were increased at the 1200 FTU/kg diet supplementation level. At this level, ADCs of phytate-phosphorus and manganese were 96.88 and 97.18%, respectively, essentially completely available to rainbow trout.

The ADCs of gross energy in DDGS supplemented with 0, 300, 600, 900, and 1200 FTU/kg diet were 57.7, 66.6, 51.7, 57.1, and 50.5%, respectively (Table 5). Digestible energy in DDGS supplemented with 0, 30, 600, 900, and 1200 FTU/kg diet were 13.8, 16.2, 12.6, 13.9, and 12.3 mj/kg, respectively. The average value of digestible energy in DDGS was 13.8 mj/kg for rainbow trout. No digestible energy value for DDGS was reported in NRC (1993) for rainbow trout.

Phytase supplementation did not improve ADCs of crude protein, potassium, sodium, and sulfur. This may be due to the fact that their ADCs in DDGS were already high (>88%). Although phytase supplementation did not significantly improve ADCs of crude protein, there was a trend that phytase supplementation increased ADCs of crude protein at 300 and 600 FTU/kg diet supplemental levels. This could be demonstrated by comparing the ADCs of their respective amino acids.

The ADCs of amino acids are presented in Table 6. The ADCs of essential amino acids in DDGS were high (>90%) except that of threonine (87.9%). ADCs of non-essential amino acids in DDGS were also high (>85%) except that of cystine (75.9%). Supplementing phytase at 300

TABLE 5. Apparent digestibility coefficients of nutrients (or dry matter and energy) in corn distiller's dried grain with solubles for rainbow trout (%, Mean± S.D., n = 2 tanks). Means in the same row that do not share a common letter differ significantly ($P < 0.05$).

	Microbial phytase supplemental levels (FTU/kg diet)				
Items	0	300	600	900	1200
Dry matter	49.1±2.5a	58.6±0.7b	46.7±2.7a	51.1±2.4a	47.6±0.3a
Crude fat	81.8±0.3ab	85.8±1.8bd	88.9±0.7cd	78.9±1.9a	83.5±0.6b
Crude protein	90.4±0.9a	91.9±0.3a	90.9±0.6a	88.0±0.3b	88.9±0.4b
Gross energy	57.7±3.3a	66.6±0.8a	51.7±3.7a	57.1±3.9a	50.5±3.1a
Calcium	34.0±0.8a	90.8±4.4b	83.0±4.7b	68.8±0.5b	84.6±13.6b
Potassium	99.6±0.1a	99.6±0.1a	99.6±0.1a	99.7±0.0a	99.5±0.1a
Magnesium	55.3±1.2a	76.3±0.1b	68.8±3.3b	74.3±4.1b	72.5±0.5b
Sodium	93.8±1.6a	95.1±0.8a	98.8±0.5a	97.0±2.8a	97.3±1.2a
Sulfur	88.1±0.2ac	89.7±0.3bc	88.9±0.7a	86.7±0.8a	86.9±1.1a
Phytate-phosphorus	22.2±6.7a	17.8±2.7a	64.1±7.9b	53.8±3.2b	96.9±4.4c
Total-phosphorus	80.1±0.0a	87.0±0.1b	89.1±0.7c	86.3±0.7b	87.5±0.1b
Copper	33.9±11.8a	53.7±1.3a	47.1±8.9a	48.6±9.7a	45.1±1.6a
Manganese	−7.3±21.8a	60.7±6.8b	70.8±17.2b	54.9±4.2b	97.2±21.5b
Zinc	−7.2±5.6a	59.1±6.5b	53.4±9.3b	54.4±1.7b	58.3±1.2b

TABLE 6. Apparent digestibility coefficients of amino acids in corn distiller's dried grain with solubles for rainbow trout (%, Mean±S.D., n = 2 tanks). Means in the same row that do not share a common letter differ significantly ($P < 0.05$).

Amino acids	Microbial phytase supplemental levels (FTU/kg diet)				
	0	300	600	900	1200
Essential amino acids					
Arginine	94.9±0.2a	95.4±0.2b	94.3±0.1c	92.9±0.2d	93.6±0.2e
Histidine	92.5±0.2a	93.4±0.2b	91.8±0.1ad	89.7±0.3c	91.2±0.2d
Isoleucine	91.4±0.1ac	93.1±0.1a	91.7±0.2ac	88.1±0.9b	89.9±0.8c
Leucine	90.4±0.2a	92.2±0.2b	90.2±0.2a	87.3±0.8c	88.7±0.4d
Lysine	95.2±0.2a	96.8±0.1b	95.1±0.1a	93.1±0.3c	94.4±0.1d
Methionine	95.5±0.2ac	96.2±0.3a	95.2±0.1ac	93.4±0.5b	94.8±0.1c
Phenylalanine	92.4±0.1a	93.7±0.1b	92.1±0.2a	89.3±0.7c	90.6±0.4d
Threonine	87.9±0.5a	90.4±0.5b	88.3±0.4a	86.5±0.7a	87.4±0.2a
Tryptophan	93.0±0.1a	94.6±0.4b	93.3±0.3a	92.5±0.1ac	91.8±0.3c
Valine	90.8±0.3a	92.4±0.3b	90.4±0.1a	87.1±0.5c	89.1±0.5d
Non-essential amino acids					
Alanine	86.4±0.1a	88.5±0.2c	86.8±0.2a	84.7±0.9b	85.9±0.5ab
Aspartic acid	89.9±0.3ab	91.4±0.4a	90.4±0.2a	86.9±0.7b	88.8±0.4b
Cysteine	75.9±0.9ab	79.7±2.1b	73.9±1.1a	73.9±0.9a	77.1±1.5ab
Glutamic acid	94.2±0.1a	95.6±0.3b	94.4±0.2c	91.0±0.3d	92.9±0.2e
Glycine	91.5±0.1ab	92.7±0.2c	91.9±0.3ab	90.8±0.0d	91.4±0.1b
Proline	93.3±0.0a	94.9±0.1b	93.5±0.3a	90.4±0.6c	92.1±0.4d
Serine	91.9±0.1a	93.7±0.9b	91.7±0.9a	89.8±0.2a	91.1±0.0a
Tyrosine	94.1±0.3a	95.2±0.1b	93.9±0.2a	91.7±0.4c	92.2±0.2c

FTU/kg diet significantly improved the ADCs of arginine, histidine, leucine, lysine, phenylalanine, threonine, tryptophan and valine ($P < 0.05$), although the absolute differences were small. Supplementing phytase at 300 FTU/kg diet also significantly improved the ADCs of alanine, glutamic acid, glycine, proline, serine and tyrosine ($P < 0.05$). However, at levels higher than 600 FTU/kg diet, phytase supplementation did not result in further improvement in ADCs of amino acids. The results of ADCs of amino acids were similar to their respective ADCs for crude protein.

Recently, we conducted another large scale digestibility trial, showing that supplementing microbial phytase at 600 FTU/kg diet improved ADCs of phytate-phosphorus from 14, 45, 12, and 16% to 100, 75, 93, and 81%, respectively, in barley, canola, wheat and wheat middlings,

for rainbow trout (Cheng and Hardy, In press). This indicated that the inclusion rate of phytase plays an important role in releasing phytate-phosphorus in plant protein-based fish diets and that the effectiveness of phytase varies with the plant ingredient source. Phytase supplementation in diets for rainbow trout and other fish species have previously been shown to be effective in releasing nutrients (Cain and Garling 1995; Rodehutscord and Pfeffer 1995; Storebakken et al. 1998; Vielma et al. 1998, 2000; Papatryphon et al. 1999).

Experiment 1 showed high ADCs of crude protein and amino acids in DDGS, and the effectiveness of phytase on the release of dietary phosphorus, especially phytate-phosphorus and certain minerals. Phytase supplementation is recommended in rainbow trout diets to release phosphorus and other minerals if DDGS is used as an alternative protein ingredient. With the use of phytase, the supplemental levels of trace minerals such as manganese and zinc may be reduced. This can be an advantage in fish production, especially at late stage of fish growth because most of the feed used in a production cycle is consumed at late growth stage. Feeding trials to determine the optimum inclusion levels of DDGS and optimum dosages of phytase in various feed formulations of fish diets are needed. As global fish meal production is not increasing, plant protein meals will be increasingly used in fish diets. The use of combinations of DDGS and phytase may be justified for its relatively high crude protein level and high ADCs of crude protein and amino acids, and the effectiveness of phytase in releasing phosphorus, calcium, magnesium, manganese and zinc, and thus reducing water pollution.

Experiment 2

Initial weight, final weight, WG, FCR, and survival of rainbow trout fed diets for 70 days are presented in Table 7. There were no significant differences in initial weight, final weight, WG, FCR, and survival among fish fed all diets ($P > 0.05$), indicating that trace mineral premix supplemental levels could be reduced with phytase supplementation at 500 FTU/kg diet in rainbow trout diets without significantly reducing fish WG, FCR, and survival.

Whole body composition of rainbow trout fed experimental diets for 70 days is presented in Table 8. There were no significant differences in fish body composition except that fish fed diet 6 had a significantly lower zinc level than fish fed diets 1 to 4, but not significant different from fish fed diet 5. Apparent nutrient retention of rainbow trout fed experimental diets for 70 days is presented in Table 9. There were no significant dif-

TABLE 7. Performance of rainbow trout fed diets for 70 days (Mean±S.D.). No significant differences were found in each item among fish fed different diets ($P > 0.05$).

	Diets					
	1	2	3	4	5	6
	Trace mineral supplemental levels (%)					
	0.1	0.08	0.06	0.04	0.02	0
	Microbial phytase supplemental levels (FTU/kg diet)					
Items	0	500	500	500	500	500
Initial weight (g)	20.0±0.3	19.9±0.6	19.9±0.2	20.0±0.8	20.1±0.7	19.9±1.0
Final weight (g)	78.5±5.2	79.6±0.7	71.6±14.5	82.4±3.8	68.4±12.0	74.2±11.6
Weight gain (g)	58.5±5.0	59.7±0.9	51.7±14.4	62.4±3.4	48.3±11.6	54.3±10.7
Feed conversion ratio (g diet/g gain)	1.08±0.06	1.07±0.06	1.16±0.08	1.05±0.04	1.16±0.13	1.13±0.11
Survival (%)	98.9±1.9	97.8±1.9	96.7±0.0	97.8±1.9	95.6±3.9	97.8±3.9

TABLE 8. Whole body composition of rainbow trout (Mean±S.D., as is basis). Means in the same row that do not share a common letter differ significantly ($P < 0.05$).

	Diets					
	1	2	3	4	5	6
	Trace mineral supplemental levels (%)					
	0.1	0.08	0.06	0.04	0.02	0
	Microbial phytase supplemental levels (FTU/kg diet)					
Items	0	500	500	500	500	500
Moisture (%)	68.1±0.8	68.5±0.3	68.2±0.8	68.1±0.4	67.7±0.5	67.7±0.5
Crude protein (%)	16.1±0.5	15.9±0.7	15.8±0.6	15.9±0.9	16.5±0.4	16.2±0.4
Crude fat (%)	14.2±0.5	14.1±0.3	13.9±0.9	14.3±0.2	14.1±0.5	14.5±0.3
Ash (%)	2.5±0.1	2.7±0.3	2.6±0.2	2.6±0.2	2.8±0.4	2.6±0.2
Calcium (%)	0.42±0.0	0.41±0.0	0.41±0.1	0.40±0.0	0.40±0.0	0.47±0.0
Phosphorus (%)	0.48±0.0	0.47±0.0	0.49±0.1	0.44±0.0	0.48±0.0	0.50±0.0
Potassium (%)	0.34±0.0	0.35±0.0	0.35±0.1	0.35±0.0	0.37±0.0	0.32±0.0
Magnesium (%)	0.03±0.0	0.03±0.0	0.03±0.0	0.03±0.0	0.03±0.0	0.03±0.0
Sodium (%)	0.07±0.0	0.06±0.0	0.07±0.0	0.07±0.0	0.08±0.0	0.07±0.0
Sulfur (%)	0.20±0.0	0.20±0.0	0.20±0.0	0.20±0.0	0.21±0.0	0.20±0.0
Copper (mg/kg)	0.99±0.0	0.98±0.0	1.07±0.0	1.00±0.0	1.02±0.2	1.05±0.1
Manganese (mg/kg)	1.09±0.0	0.79±0.1	1.00±0.4	1.05±0.0	1.29±0.1	1.49±0.1
Zinc (mg/kg)	25.1±2.0a	26.0±3.3a	23.5±3.6a	23.9±1.8a	19.9±1.1ab	14.7±2.1b

TABLE 9. Apparent nutrient retention for rainbow trout fed diets for 70 days (%, Mean±S.D.). Means in the same row that do not share a common letter differ significantly ($P < 0.05$).

	Diets					
	1	2	3	4	5	6
	Trace mineral supplemental levels (%)					
	0.1	0.08	0.06	0.04	0.02	0
	Microbial phytase supplemental levels (FTU/kg diet)					
Items	0	500	500	500	500	500
Crude protein	36.7±1.6	36.4±2.3	33.4±1.9	36.7±2.9	35.9±1.6	36.1±1.5
Calcium	35.5±0.7	37.7±1.1	33.8±13.4	38.6±2.5	36.4±3.7	42.5±0.7
Phosphorus	44.6±3.6	44.5±2.3	42.7±6.2	43.2±0.7	44.5±7.6	43.5±3.5
Potassium	38.3±3.5	40.2±1.3	38.3±4.7	42.0±1.4	42.1±3.6	34.4±0.3
Magnesium	18.4±0.4	18.7±0.9	18.0±4.1	18.8±1.2	17.6±1.7	18.3±1.3
Sodium	51.7±2.3	46.3±4.4	50.8±6.3	54.1±4.4	52.1±2.1	44.4±2.1
Sulfur	30.9±1.0	30.1±1.3	27.6±0.5	29.2±0.9	29.9±0.3	28.3±1.4
Copper	11.2±0.2	11.1±1.1	12.0±0.4	11.7±0.3	11.8±2.0	12.8±1.8
Manganese	1.7±0.0ab	1.2±0.1a	1.6±1.2ab	2.3±0.1ab	3.1±0.2b	4.5±0.0c
Zinc	19.4±2.2	24.6±2.8	22.3±3.3	30.9±1.3	29.3±1.7	24.8±5.8

ferences in apparent nutrient retention among fish fed different diets except that fish fed diet 6 had significantly higher manganese retention than fish fed all other diets. Results indicated that trace mineral premix supplemental levels in rainbow trout diets could be reduced with phytase supplementation at 500 FTU/kg diet without affecting body composition and apparent nutrient retention for rainbow trout. Further research is needed to confirm the results obtained in this experiment.

Results of experiment 2 show that trace mineral premix supplemental levels in rainbow trout diets can be reduced with phytase supplementation at 500 FTU/kg diet without affecting fish WG, FCR, survival, body composition and apparent nutrient retention. Future research should focus on the whole growing stages of fish to elucidate the advantages of supplementing microbial phytase in DDGS-based diets, and the economic analyses comparing feeding fish meal and DDGS-based diets for rainbow trout.

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