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Glyphosate Tolerant Canola Meal Is Equivalent to the Parental Line in Diets Fed to Rainbow Trout

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Two separate studies were conducted to evaluate the utility of glyphosate tolerant canola (GTC) as a feed ingredient in diets fed to rainbow trout. In the first study, two forms of GTC were compared to a parental line, Westar. In the second study, one line of GTC was reevaluated to Westar. In each study, processed canola meals were incorporated at 5, 10, 15, or 20% of the dry diet and a diet containing no canola was fed for comparison. All diets were fed to triplicate groups of fish in each study. In the first study, weight gain, feed efficiency (FE), protein efficiency ratio (PER), and protein retention (PR) were not significantly different in fish fed either Westar or GT200 at any level of substitution. Fish fed GT73 exhibited a gradual reduction in weight gain, FE, and PER as the level of GTC increased. However, the only significant reduction was in weight gain of fish fed 20% GT73 as compared to fish fed 5% GT73. Because of an error in preparing samples prior to the experiment, samples GT200 and GT73 were essentially equivalent in composition. The differences were explained by differences in processing temperatures that occurred after the sample mixing error occurred. In the second study, mean weight gain, PR, and survival were not significantly different among forms of canola. FE and PER values were significantly lower in fish fed 15% Westar as compared to fish fed 10% Westar; other FE and PER values were not significantly different. On the basis of these results, GTC processed into a toasted meal and incorporated into diets for rainbow trout is equivalent to a parental line of canola.

KEYWORDS: Glyphosate tolerant canola; canola; rainbow trout; nutrition; protein

INTRODUCTION

The development of biotechnology has heightened the awareness of how some crop plants are produced. Much of the early work was focused on incorporation of genes that express proteins that confer resistance to disease, pests, or chemical agents. One of the more successful early efforts was incorporation of genes that confer resistance to the broad spectrum herbicide Roundup (*1*). Incorporation of a modified 5-enolpyruvylshikimate-3-phosphate synthase gene (*cp4 epsps*) into soybeans did not significantly alter the nutritional composition as compared to a parent line (*2*). Furthermore, there were no adverse effects of feeding a processed meal from the modified soybeans to mice, rats, catfish, chickens, or dairy cows (*3*, *4*). Similarly, two genes were introduced into canola (*Brassica napus* L.), which resulted in commercial levels of tolerance to glyphosate, the herbicidal agent in Roundup herbicide. Unlike soybean, efficacious glyphosate tolerance in canola required the use of both the *cp4 epsps* protein and a protein that catalyzes the breakdown of glyphosate (*gox*) into a well-known metabolite, aminomethylphosphonic acid.

Canola or rapeseed is one of the major oilseeds grown, and interest in production and distribution is increasing because of its relatively low concentration of saturated fatty acids and high concentration of n-3 fatty acids. The meal resulting after extraction of oil is an ingredient in diets fed to some fish and has potential in additional markets. Processed canola meal was probably first evaluated in diets fed to salmonids, and it remains an important potential ingredient (*5*−*10*). The potential market has expanded to several other fish (*11*−*13*) and crustaceans (*14*−*16*). Maximum levels of incorporation into diets for salmonids is usually less than 20% of the dry matter. Increased availability and reasonable price of processed canola meal led to routine incorporation into diets for rainbow trout (*Oncorhynchus mykiss*) in North America. However, the genetically modified form of canola has not been evaluated as a feedstuff. The objective of these studies was to evaluate glyphosate tolerant canola (GTC) as an ingredient in diets fed to rainbow trout as compared to a traditional (nonmodified) canola.

MATERIALS AND METHODS

Diets. Two separate studies were conducted. In the first study, two lines of GTC, designated GT200 and GT73, and a parental line, Westar, were used. The two experimental canola lines express the same proteins

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Table 1. Chemical Analyses of Three Canola Lines[a]

canola	moisture	crude protein	fat	crude fiber	ash	nitrogen solubility
Westar	7.2	38.2	3.8	13.9	5.9	19.8
GT200	7.8	39.0	4.1	12.6	6.4	20.0
GT73	5.6	42.2	3.4	12.3	6.5	14.7
Westar[b]	10.8	36.8	4.0	12.9	6.5	28.3
GT73[b]	10.0	37.3	3.1	13.1	6.6	29.1

[a] Westar is the parental line, and GTC lines 200 and 73 are the test ingredients. Values are the means of three determinations. Crude protein, fat, crude fiber, and ash are expressed as a percentage of dry matter, and nitrogen solubility is expressed on an as-is basis. [b] Westar and GT73 used in the second experiment.

Table 2. Composition of Diets Fed to Rainbow Trout to Determine Utility of GTC Processed into a Toasted Meal

	first experiment	second experiment
fish meal[a]	37.8–47.0	33.9–40.2
canola meal[b]	0–20.0	0–20.0
soybean meal	20	0
wheat midds	8.7–19.4	26.0–40.0
fish oil[c]	15.0	11.3–11.6
mineral premix[d]	8.0	8.0
vitamin premix[d]	0.3	0.3
choline-Cl	0.7	0.7
ascorbic acid	0.1	0.1
carboxymethylcellulose	0.0	2.0

[a] Fish meal was SolAtlantique. [b] Canola meal was incorporated at either 0, 5, 10, 15, or 20%. Three forms of canola were evaluated in the first experiment: a parental line, Westar, GT200, and GT73. Two forms of canola were evaluated in the second experiment, Westar and GT73. [c] Menhaden fish oil. [d] Mineral and vitamin premixes were the same as reported in ref *21*.

that confer resistance to glyphosate. However, there is a three amino acid difference between the two lines, which impacts kinetic properties of the enzyme. In the second study, GT73 was reevaluated using the same parental line as a control. All lines of canola were grown in the same field test sites in Canada and processed into toasted meal at the Texas A&M University Engineering and Biosciences Research Center (College Station, Texas). Processed canola meal was then shipped to Purdue University. Two separate feeding studies were conducted with rainbow trout (*O. mykiss*). Good laboratory practices (GLP) (*17*) were used in the production of the canola lines, harvest, transportation, processing, and testing. Furthermore, all protocols used in acquisition, quarantine, and testing with fish were approved by the Purdue Animal Care and Use Committee.

In both studies, a basal dietary formulation was developed using nutrient and ingredient recommendations of Crampton (*18*) and Cho and Cowey (*19*) and chemical analyses of the test ingredients (**Table 1**). In the first study, the basal diet contained fish meal, soybean meal, and wheat midds as the main sources of crude protein (**Table 2**). Both GT200 and GT73 were incorporated at 5, 10, 15, or 20% of the dry matter for an equal amount of crude protein from fish meal, with minor adjustments in wheat midds to maintain similar essential amino acid concentrations. In the second study, the basal diet contained fish meal and wheat midds as the major sources of crude protein (**Table 2**), and substitution was as described above. The source of fish meal used in both studies was SolAtlantique (Sanofi Sante Animale, Cambridge, Ontario). Wheat midds (Wabash Valley Feeds, Lafayette, IN) and soybean meal (Cargill, Inc., Lafayette, IN) were obtained locally. Fish oil was supplied by Zapata Proteins, Inc. (Reedville, VA). Vitamin and mineral premixes used in both studies were nutritionally complete (*20, 21*) and manufactured in our laboratory. Vitamins and carboxymethylcellulose were obtained from U.S. Biochemical (Cleveland, OH), and minerals were of reagent grade and obtained from Sigma Chemical, Inc. (St. Louis, MO).

All diets were mixed and pelleted at Purdue University using methods described previously (*21*). Diets were dried in a forced-air convection oven at 60 °C and stored frozen (−20 °C) in sealed bags prior to feeding.

Fish and Experimental System. In both studies, juvenile rainbow trout, Shasta strain, were acquired from the Michigan Department of Natural Resources and transported to Purdue University. Fish were quarantined prior to initiation of either study and fed a commercial trout diet during that time (Nelson and Sons, Inc., Murray, UT). After quarantine, fish were randomly distributed among 36 glass aquaria in the first study and 27 in the second study. Eighteen fish with an average weight of 10 g were stocked into each aquarium in the first study. After an adjustment period of 9 days, numbers of fish were reduced to 15 per aquarium and the study was initiated. In the second study, numbers of fish and acclimation times were the same, but mean initial fish weight was 16.1 g. Treatments in both studies were randomly assigned to triplicate aquaria. All fish were fed a restricted rate twice per day based on fish weight and water temperature (*22*). An initial sample of fish was collected at the beginning of each study. Those fish were killed by hypothermia and placed in a freezer (−20 °C) prior to chemical analyses.

All aquaria were 120 L and each contained water, an air supply, and an external drain. All aquaria had black polyethylene wrapped around each side and the bottom of the tanks to prohibit interaction with other fish and to diminish effects of activity in the laboratory. Water from each aquarium drained into a common solids filter and then into a biological filter. Water chillers were used to maintain temperature at 15 °C.

Water quality variables were monitored daily in each study. Temperature and dissolved oxygen were measured with a dissolved oxygen meter (YSI, Inc., Yellow Springs, OH). Ammonia-N and nitrite-N were measure daily with a HACH DREL 1-C water quality test kit (HACH Chemical, Co., Loveland, CO). The temperature ranged from 15 to 17 °C during the course of the studies, and dissolved oxygen ranged 8.0−11.1 mg/L. Ammonia-N concentrations did not exceed 1.0 mg/L, and nitrite-N did not exceed 2 mg/L.

Chemical Analyses. At the end of each study, final numbers and weights of fish were recorded. Three average size fish were collected from each replicate and stored frozen (−20 °C) prior to proximate analysis. Fish collected at the beginning of the studies and at termination were chopped into sections approximately 0.25 cm wide and dried in a convection oven at 100 °C for determination of moisture concentration. Dried samples were ground in a mortar and redried prior to further analyses. Crude protein and ash were determined by AOAC methods (*23*), and lipid concentrations were determined by chloroform:methanol extraction (*24*).

Statistical Analyses. Weight gain, feed efficiency (FE), survival, protein efficiency ratio (PER), protein retention (PR), and whole body proximate composition data were analyzed as a 3×4 factorial in the first experiment and a 2×4 factorial in the second experiment (*25*) using the Statistical Analysis System. If analysis of variance indicated significant differences, Student Neuman Keuls was used to separate mean values. The accepted level of significance was 0.05.

RESULTS

The type of canola and level of inclusion significantly affected weight gain of rainbow trout (**Table 3**). Weight gain of fish fed GT73 was significantly lower than fish fed GT200. Weight gain of fish fed Westar was not significantly different from either GT200 or GT73. Weight gain of fish fed 5% canola was significantly higher than in fish fed any other level, and weight gain of fish fed 10 and 15% canola was significantly higher than in fish fed 20%. FE values were not significantly affected by the type of canola; values ranged from 0.88 to 0.93. The FE of fish fed 10% canola was significantly lower than fish fed 5%, but the FE of fish fed 15 or 20% canola was not significantly different than fish fed either 5 or 10%. Similarly, PER values were not significantly affected by the type of canola but were by level of inclusion. Fish fed 10% canola exhibited

Table 3. Mean Weight Gain,[a] FE,[b] PER,[c] and PR[d] of Rainbow Trout Fed Graded Levels of a Commercial Variety of Canola (Westar) or Graded Levels of Two GTC Lines (GT200 or GT73) in the First Experiment[e]

	canola			level of inclusion					ANOVA P value[g]		
	Westar	GT200	GT73	5	10	15	20	pooled SEM[f]	Can	Inc	Can × Inc
weight gain	521.1a,b	537.6a	495.2b	560.4x	504.7y	517.8y	489.7z	1.98	0.038	0.004	0.121
FE	0.88	0.93	0.91	0.97x	0.86y	0.90x,y	0.90x,y	0.01	0.368	0.040	0.129
PER	2.5	2.6	2.5	2.7x	2.4y	2.5x,y	2.5x,y	0.01	0.309	0.031	0.163
PR	28.4	27.7	28.9	29.2	28.5	28.0	27.6	0.16	0.700	0.710	0.002
					whole body analyses						
moisture	71.0	70.7	70.5	71.0 7	0.6	70.7	70.6	0.02	0.412	0.553	0.417
protein	49.1	45.2	48.2	45.9	49.8	47.4	46.9	0.21	0.053	0.197	0.002
fat	41.1	42.3	43.2	42.6	41.1	42.3	42.8	0.09	0.067	0.343	0.058
ash	7.4	7.1	7.2	7.3	7.1	7.2	7.3	0.01	0.533	0.789	0.519

[a] Weight gain was expressed as percentage increase from initial weights. [b] FE = wet weight gain/dry weight of feed offered. [c] PER = wet weight gain/protein intake. [d] PR = (final body protein − initial body protein/total protein fed) × 100. [e] All three canola samples were processed into toasted meal. Values in the same row under each main effect with the same letter designation were not significantly different as determined by Student Neuman Keuls. [f] Pooled standard error of the mean. [g] Probability of calculated *F* value exceeding tabular *F* statistic.

Table 4. Mean Weight Gain,[a] FE,[b] PER,[c] and PR[d] of Rainbow Trout Fed a Commercial Variety of Canola or a GTC (GTC73) at Various Levels of Incorporation in Diets[e]

	canola		level of inclusion					ANOVA P value[g]		
	Westar	GTC73	5	10	15	20	pooled SEM[f]	Can	Inc	Can × Inc
weight gain	145.5	144.2	156.9x	161.2x	129.3y	131.9y	0.73	0.857	0.007	0.584
FE	0.62	0.61	0.64x,y	0.67x	0.57y	0.58y	0.04	0.637	0.006	0.359
PER	1.7	1.7	1.8x,y	1.9x	1.6y	1.6y	0.01	0.647	0.006	0.348
PR	26.1b	29.0a	26.1	29.0	27.4	27.6	0.15	0.022	0.397	0.780
				whole body analyses						
moisture	67.5a	66.6b	67.7	66.9	66.5	67.1	0.04	0.021	0.166	
protein	52.5a	54.6b	51.6y	52.0y	55.1x	55.5x	0.10	0.026	0.004	0.643
fat	36.7a	33.0b	35.7	34.6	34.9	34.3	0.09	0.001	0.815	0.457
ash	7.4	7.4	7.3	7.7	7.4	7.3	0.09	0.972	0.618	0.929

[a] Weight gain was expressed as percentage increase from initial weights. [b] FE = wet weight gain/dry weight of feed offered. [c] PER = wet weight gain/protein intake. [d] PR = (final body protein − initial body protein/total protein fed) × 100. [e] All three canola samples were processed into toasted meal. Values in the same row under each main effect with the same letter designation were not significantly different as determined by Student Neuman Keuls. [f] Pooled standard error of the mean. [g] Probability of calculated *F* value exceeding tabular *F* statistic.

significantly lower PER than fish fed 5%, while PER values for fish fed either 15 or 20% canola did not differ significantly from fish fed 5 or 10%. PR was significantly affected by the interaction of canola and level of inclusion. None of the other whole animal responses were significantly impacted by the interaction of main effects. Whole body moisture, fat, and ash concentrations were not significantly affected by the type of canola, the level of inclusion, or the interaction (**Table 3**). The interaction of canola and level of inclusion did significantly affect whole body protein concentration. Five mortalities occurred in the first experiment, and they were in five different treatments.

Mean weight gain, FE, and PER values were not significantly affected by the type of canola used in the second experiment (**Table 4**). However, all three variables were significantly affected by the level of inclusion. Values for fish fed either 15 or 20% canola were not significantly different from each other but were significantly lower than in fish fed either 5 or 10% canola. Weight gain, FE, and PER values in fish fed 5 or 10% canola were not significantly different from one another. Mean PR values were significantly lower in trout fed Westar than in fish fed GTC. However, PR values were not significantly affected by the level of inclusion. There were no significant interactions of main effects in the whole animal responses. However, whole body moisture concentrations were significantly affected by the interaction of the type of canola and level of inclusion. Whole body protein concentrations were significantly lower in fish fed Westar than in fish fed GTC and significantly lower in fish fed 5 and 10% canola as compared to fish fed 15 or 20%. Whole body protein concentrations of fish fed 5 and 10% canola were not significantly different, and those fed 15 or 20% canola were not significantly different. Whole body fat concentrations were significantly higher in trout fed Westar than in fish fed GTC, but those values were not significantly affected by the level of inclusion. Whole body ash concentrations were not significantly affected by the type of canola, the level of inclusion, or the interaction of main effects. Twelve mortalities occurred in the second experiment, and those were in seven of the eight treatments.

DISCUSSION

On the basis of the results of the first experiment, a toasted meal from GTC is comparable in nutritional quality to a processed parent line when incorporated into diets for rainbow trout. There were no significant differences in any of the response variables measured in the first experiment between fish fed Westar and those fed sample GT200. However, because of a mixing error that occurred prior to the first study, samples of seed labeled GT200 and GT73 were essentially equivalent in composition. Examination of processing records indicated that the reduction in growth and FE in trout fed GT73 appears to be the result of improper processing temperatures. The temperature of the steam used to toast GT73 was 84 °C, while the temperatures used for Westar and GT200 were 94−99 °C, which is the desired range for toasting solvent-extracted canola meal.

The only apparent change resulting from the improper toasting temperature was nitrogen solubility (14.7 in GT73 vs 19.8 and 20.0 in Westar and GT200, respectively). A decreased response attributed to reduction in nitrogen solubility has been identified in other animals (*26*, *27*), and we suspect that decreased nitrogen solubility impaired amino acid absorption, thus weight gain, in our first experiment. All other aspects of the study were closely monitored in conjunction with GLP and maintained similarly across experimental and test systems.

On the basis of the results of the second experiment, it seems clear that a toasted meal made from GT73 is also equivalent to the parental line Westar. The only significant differences detected in the second experiment were PR and whole body moisture, protein, and fat concentrations. In each case, fish fed GT73 exhibited improvements in the three variables as compared to fish fed Westar. Increased retention of dietary protein is a positive attribute of a feed, and the higher protein, lower fat concentrations should also be considered a positive attribute of the fish produced.

Increasing dietary canola in both experiments generally resulted in decreasing response variables. Feeding up to 20% canola meal to trout in a previous study did not have an adverse impact (*8*). Differences in basal dietary formulations or strain of fish may have influenced this comparison (*28*).

We simplified our basal diet from the first to the second experiment, and that may have contributed to the reduced weight gain, FE, PER, and PR that we recorded in the second experiment. The initial size of fish was also larger in the second experiment, which may have led to a reduced response.

As pointed out by Hammond et al. (*3*), feeding studies with animals are typically not conducted with new genetic lines of crop plants; compositional analysis usually suffices; however, transgenic plants are under closer scrutiny than those developed from classical breeding experiments. In this evaluation, the transgenic meal was equivalent to the parental line in terms of nutritional efficacy and there were no apparent health problems identified in the test organism. Thus, transgenic canola meal appears to be a viable feedstuff for trout. Conducting animal feeding studies reassures the potential user of the processed meals but may provide little biological information beyond compositional analyses (*29*).

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