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Effects of supplementing methionine hydroxy analogue in soybean meal and distiller's dried grain-based diets on the performance and nutrient retention of rainbow trout [*Oncorhynchus mykiss* (Walbaum)]

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Abstract

Two experiments were conducted to evaluate the effects of supplementing methionine hydroxy analogue (MHA) on the performance, body composition, and nutrition retention of rainbow trout. In experiment 1, a 2×4 factorial arrangement with two MHA supplemental levels (with and without) and four fish meal replacement levels (25%, 50%, 75%, and 100%, replaced with soybean meal (SBM) and wheat gluten) was used. A fish meal diet was included as a control. Results showed that fish meal replacement levels had significant effects on fish weight gain (WG), feed conversion ratio (FCR), body moisture, crude protein, crude fat, and ash ($P < 0.05$). In experiment 2, a fish meal reference diet and seven other diets were made using SBM and distiller's dried grain with solubles supplemented with 0, 0, 0.55, 1.1, 1.65, 2.2, and 2.75 g MHA kg^{-1} diet to replace 50% of fish meal. WG, FCR, and apparent retention of crude protein and phosphorus were significantly improved in rainbow trout fed the diet containing 1.65 g MHA kg^{-1} diet compared with fish fed an equivalent diet without MHA.

Keywords: methionine hydroxy analogue, soybean meal, distiller's dried grain with solubles, rainbow trout *Oncorhynchus mykiss*

Introduction

The production of feeds for carnivorous fish such as rainbow trout *Oncorhynchus mykiss* (Walbaum) requires large amounts of fish meal. The amounts of fish meal used in trout diets range from 300 to 500 g kg^{-1} diet. Fish meal has traditionally been used in commercial fish feeds as the major source of dietary protein (Hardy 1999). However, fish meal production from capture fisheries has been more or less stable over the past decade, except in El Nino years when production decreases. When global fish meal production declines and fish meal prices increase, feed manufacturers turn to less expensive plant protein sources such as soybean meal (SBM) and distiller's dried grain with solubles (DDGS). Both products are abundant, inexpensive, and more sustainable compared with fish meal.

Aquaculture is one of the fastest growing food-producing activities in the world. However, it is subject to increasing environmental regulations. Nitrogen and phosphorus are two nutrients of concern in fish farm effluent water. Therefore, reducing nitrogen and phosphorus discharges is a critical strategy in reducing the environmental impacts of intensive aquaculture operations. SBM and DDGS are relatively high-protein ingredients, containing 440–480 and 270 g crude protein kg^{-1} respectively. SBM and DDGS also contain much less phosphorus than fish meal: 6.5, 6.6, and 17–42 g phosphorus kg^{-1} in SBM, DDGS, and fish meal respectively (NRC 1993). Therefore, substituting SBM and DDGS for fish meal in fish feeds reduces the total phosphorus level of the diet and lowers the level of phosphorus in hatchery discharge water. However, substitution levels of SBM and DDGS in fish feeds are limited by the levels of

lysine and methionine in these ingredients. Lysine contents are 29, 6.5, and 40–56 g kg^{-1}, and methionine contents are 6.5, 5.5, and 10–20 g kg^{-1} in SBM, DDGS, and fish meal respectively. Thus, supplementing methionine hydroxy analogue (MHA), and other amino acids, if necessary, into trout diets to increase methionine (and other amino acids) levels may improve the nutritional values of diets containing SBM and DDGS. There is little information on the use of MHA in diets for aquatic animal species. Robinson, Otis, Poe & Wilson (1978) determined that the efficacy of MHA was about 26% compared with L-methionine for catfish *Ictalurus punctatus*. Results with warm-water fish are not always predictive of the response of cold-water fish species such as rainbow trout to dietary nutrients. Therefore, the effects of dietary MHA supplementation on rainbow trout performance using practical feed ingredients need to be determined by experimentation. This was the objective of the study.

Materials and methods

Experiment 1: experimental design and objective

The objective of experiment 1 was to determine the effect of supplementing MHA in diets in which SBM partially replaced fish meal on weight gain (WG), feed conversion ratio (FCR), and whole-body composition of rainbow trout. This experiment involved nine experimental diets (Table 1). It was designed as a 2×4 factorial arrangement, with and without MHA supplementation, and four fish meal replacement evels (25%, 50%, 75%, and 100%). A fish meal-based diet was included as a control. The MHA inclusion level in experimental diets was based on the analysed methionine level in fish meal, SBM (hexane extracted), wheat gluten, whole wheat, and corn gluten meal (data not shown). The MHA-supplemented diets were formulated to contain the same amount of methionine as that in the fish meal-based diet.

Fish rearing and feed manufacturing

Rainbow trout (average weight 9.1 g) were selected from a large population, counted into groups of 30 fish per tank, and placed in 27 tanks (150 L), with three tanks randomly assigned to each diet. Fish tanks were supplied with 4 L min^{-1} of untreated, constant-temperature (14.5 °C) spring water at the Hagerman Fish Culture Experiment Station (University of Idaho, Hagerman, ID, USA). Fish were fed three times per day and 6 days per week to apparent satiation for a period of 56 days.

A total of nine diets were made by compression pelleting using a laboratory pellet mill (California Pellet Mill, San Francisco, CA, USA) without steam, air-dried for 48 h at about 20 °C, and stored at room temperature for 1 week until use. All diets were formulated to be isonitrogenous and isocaloric, and contained 450 g crude protein kg^{-1} diet and a calculated digestible energy of 15.1 mJ kg^{-1} diet (Table 1). The analysed crude protein values were close to expected values. The experiment was designed as completely randomized arrangements for statistical evaluation of data, and diets were assigned randomly to tanks within the fish-rearing laboratory.

Sampling and analyses

Fish in each tank were bulk-weighed and counted at the beginning and end of the experiment. Mortalities were removed and recorded daily. From the initial population of the fish, and from each tank at the end of the experiment, five fish were killed, processed into a puree using a Robot Coupe food processor (Robot Coupe R-2, Ridgeland, MS, USA), and subsampled for chemical analyses. The moisture of feeds was determined by oven drying at 105 °C for 2 h according to AOAC (1990), and the moisture in fish samples was dried overnight. Crude protein was analysed by nitrogen determination ($N \times 6.25$) using a LECO FP 428 nitrogen analyser (LECO Instruments, St Joseph, MI, USA), crude fat by extraction using LECO TFE 2000 (LECO Instruments) employing super-critical CO_2 as the extracting solvent, and ash by incineration at 550 °C in a muffle furnace. The experiment followed the guidelines approved by the Animal Care and Use Committee of the University of Idaho (Moscow, ID, USA).

Calculations and statistical methods

Fish WG was determined by fish final weight minus initial weight, and FCR was calculated as feed consumed $(WG)^{-1}$. The data were subjected to one-way analysis of variance (ANOVA) for fish fed all diets. In addition, two-way ANOVA was used to determine the effects of MHA supplemental levels and fish meal replacement levels on fish WG and FCR. Prism, version 3.0 (GraphPad, San Diego, CA, USA), was used

Table 1 Diet composition in experiment 1 (g kg^{-1} diet, as-fed basis)

	Control	Without MHA				With MHA supplementation			
Ingredients*	D1†	D25	D50	D75	D100	M25	M50	M75	M100
Herring meal	328	246	164	82	0	246	164	82	0
Soybean meal	0	82	164	246	328	82	164	246	328
Wheat gluten	155	155	185	215	245	155	182.9	212	239.3
Whole wheat	223	186	156	121	86	185.4	156.8	122	88.9
Fish oil	180	185	185	190	195	185	185	190	195
MHA‡	0	0	0	0	0	0.6	1.3	2	2.8
Corn gluten (white)	90	122	122	122	122	122	122	122	122
Vitamin C (Stay-C)	3	3	3	3	3	3	3	3	3
Choline chloride	5	5	5	5	5	5	5	5	5
Trace mineral salt§	1	1	1	1	1	1	1	1	1
Vitamin premix¶	15	15	15	15	15	15	15	15	15
Chemical analyses									
Moisture	66.1	65.8	63.3	66	68.9	62.2	62.8	61.8	63.4
Crude protein	441.2	440.4	457	454.5	479.8	447.9	452.4	455.8	462.3
Crude fat	208.3	210.7	205.8	206.1	179.3	214.7	214.3	202	212.1
Ash	58.4	51.6	43.8	36.2	30.5	48.9	42.4	36.1	30.7
Calculated analyses									
Lysine	22.7	20.8	19	17.1	15.2	20.8	19	17.1	15.2
Methionine	10	9.4	8.7	8	7.3	10	10	10	10

*Origin of ingredients: herring meal, wheat gluten, whole wheat, corn gluten, choline chloride, trace mineral salt, and vitamin premix were purchased from Nelson & Sons (Murray, UT, USA); soybean meal, fish oil, and vitamin C were obtained from Rangen (Buhl, ID, USA); MHA was obtained from Aventis Animal Nutrition (Alpharetta, GA, USA).

†D1 was a fish meal control diet; D25, D50, D75, and D100 represent diets that were formulated to replace 25%, 50%, 75%, and 100% of fish meal, respectively, using soybean meal; M25, M50, M75, and M100 represent diets formulated to replace 25%, 50%, 75%, and 100% of fish meal, respectively, using soybean meal supplemented with MHA.

‡Methionine hydroxy analogue, its supplementation was on an equimolar methionine basis.

§Composition of trace mineral salt (mg kg^{-1}): 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.

¶Composition of vitamin premix (g kg^{-1} of premix, unless otherwise listed): D calcium pantothenate, 26.84; pyridoxine HCl, 7.7; riboflavin, 13.2; niacinamide, 55; folic acid, 2.2; thiamin mononitrate, 8.8; biotin, 0.088; vitamin B_{12}, 0.0055; menadione sodium bisulphite complex, 0.00275; DL-α-tocopherol acetate, 88 000 IU; vitamin D_3 (stabilized), 110 000 IU; vitamin A palmitate (stabilized), 1 650 000 IU.

to perform statistical calculations, and $P < 0.05$ was considered to be significant.

Experiment 2

The objective of experiment 2 was to determine the optimum MHA supplemental level in SBM–DDGS-based practical diets for rainbow trout. Six diets were prepared using SBM supplemented with 4.8 g lysine kg^{-1} diet and 0, 0.55, 1.1, 1.65, 2.2, and 2.75 g MHA kg^{-1} diet (diets 3–8), respectively, to replace 50% of fish meal (Table 2). In addition, a fish meal-based reference diet (diet 1) and a low lysine diet (diet 2) were also used. All diets were formulated to be isonitrogenous and isocaloric. Diet supplemented with 2.2 g MHA kg^{-1} diet (diet 7) contained the same amount of methionine as that in diet 1. A total of 720 rainbow trout (initial weight 49.5 g) were randomly placed in 24 150-L fibreglass tanks with 30 fish per tank and three tanks per diet. Fish were fed three times per day and 6 days per week to apparent satiation. Five fish from each tank were killed and pooled for whole-body analyses at the end of the experiment. Diet preparation, fish rearing, and sample and data analyses were similar to those in experiment 1. In addition, phosphorus was analysed using the method of Taussky & Shorr (1953). The concentration of crude protein and phosphorus in fish body at the beginning and end of the experiment, and the amount of each nutrient fed during the experiment were used to calculate apparent nutrient retention (crude protein and phosphorus) during the 49-day experiment, expressed on a per-fish basis for each dietary treatment group. Nutrient retention was calculated as [nutrient in fish whole body (final) – nutrient in fish whole body (initial)] (nutrient intake)$^{-1}$ from feed. All the data in experiment 2 were analysed using one-way ANOVA.

Table 2 Diet composition for experiment 2 (g kg^{-1}, as-is basis)

	Diets*							
Ingredients†	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Herring meal	350	175	175	175	175	175	175	175
Soybean meal	0	175	175	175	175	175	175	175
DDGS‡	185	185	185	185	185	185	185	185
Fish oil	192	198	198	198	198	198	198	198
Whole wheat	149	81	83	83.55	84.1	84.65	86.4	86.4
Corn gluten (white)	100	162	155.2	154.1	153	151.9	149.6	149.05
Lysine	0	0	4.8	4.8	4.8	4.8	4.8	4.8
MHA	0	0	0	0.55	1.1	1.65	2.2	2.75
Vitamin C	3	3	3	3	3	3	3	3
Choline	5	5	5	5	5	5	5	5
TM salt	1	1	1	1	1	1	1	1
Vitamin premix	15	15	15	15	15	15	15	15
Chemical analyses								
Moisture	61	62.8	62.2	63.4	60	61.6	59.9	59
Crude protein	380.4	400.7	392	400.1	398.9	402.6	393.4	399.5
Crude fat	217.4	203.5	213.5	209.3	212.9	210.8	212.7	214.3
Ash	83.6	62.9	62.7	60.6	61.3	63.3	61.7	64.2
Phosphorus	9.7	8	8.2	8.2	8.2	8.2	8.2	8.2
Calculated analyses								
Lysine	22	16	22	22	22	22	22	22
Methionine	10.7	8.7	8.7	9.2	9.7	10.2	10.7	11.2

*Diet 1 is a fish meal-based reference diet; diets 2–8 represent diets using soybean meal to replace 50% of fish meal and supplemented with 0, 0, 0.55, 1.1, 1.65, 2.2, and 2.75 g MHA kg^{-1} diet, respectively, on an equimolar methionine basis (MHA: methionine hydroxy analogue).

†Origin of ingredients, methods of MHA supplementation, and compositions of trace mineral salt and vitamin premix were the same as those used in experiment 1.

‡Distiller's dried grain with solubles, obtained from Rangen.

Results

Experiment 1

The initial weight, final weight, WG, and FCR of rainbow trout fed experimental diets for 56 days are presented in Table 3. There were no significant differences in initial fish weight ($P = 0.99$); fish were selected based on no visual defect and sign of diseases. Fish fed SBM-based diets to replace 25% (D25) and 50% (D50) of fish meal did not grow significantly different from fish fed fish meal-based control diet (D1). However, fish fed 75% (D75) and 100% (D100) SBM-based diets grew slower than fish fed D1 ($P < 0.0001$). Fish fed SBM-based diets to replace 25%, 50%, and 75% fish meal had FCR values similar to those fed D1, but fish fed D100 had the highest FCR. Fish fed SBM-based diets supplemented with MHA had a pattern of WG and FCR similar to fish fed SBM-based diets without MHA supplementation. Two-way ANOVA (Table 3) showed that there were no significant interactions between MHA supplemental levels and fish meal replacement levels in WG ($P = 0.4463$) and FCR ($P = 0.83$). The MHA supplementation did not affect WG ($P = 0.8496$) and FCR ($P = 0.8405$). However, fish meal replacement levels had significant effects on WG and FCR ($P < 0.0001$).

The whole-body composition of rainbow trout fed experimental diets for 56 days is presented in Table 4. There were significant differences in fish body moisture, crude protein, and ash ($P < 0.05$), but not crude fat ($P = 0.5470$). Fish fed diet M100 had significantly higher body moisture than those fed D1 and M50; fish fed D1 had the highest crude protein levels; and fish fed D25 and M25 had higher ash levels than those fed D75, D100, and M75. Two-way ANOVA (Table 4) showed that there were no significant interactions between MHA supplemental levels and fish meal replacement levels in fish body moisture, crude protein, crude fat, and ash ($P > 0.05$). MHA supplemental levels did not affect fish body moisture, crude protein, crude fat, and ash ($P > 0.05$). However, fish meal replacement levels had significant effects on fish

Table 3 Initial weight, final weight, weight gain, and feed conversion ratio of rainbow trout fed experimental diets for 56 days (mean ± SD, $N = 3$ tanks)*

Item	Control	Without MHA				With MHA supplementation				*P* value
	D1	D25	D50	D75	D100	M25	M50	M75	M100	
Initial weight (g)	9.1 ± 0.1	9.2 ± 0.2	9.1 ± 0.2	9.1 ± 0.2	9.2 ± 0.2	9.2 ± 0.3	9.1 ± 0.2	9.1 ± 0.1	9.2 ± 0.2	0.9992
Final weight (g)	65.5 ± 1.2^{a}	62.9 ± 4.6^{a}	64.6 ± 3.5^{a}	54.4 ± 3.6^{b}	43.0 ± 1.2^{c}	67.0 ± 5.9^{a}	61.3 ± 4.5^{a}	52.9 ± 4.1^{b}	42.6 ± 3.5^{c}	< 0.0001
Weight gain (g)	56.4 ± 1.3^{a}	53.7 ± 4.7^{a}	55.5 ± 3.3^{a}	45.3 ± 3.4^{b}	33.9 ± 1.1^{c}	57.8 ± 5.8^{a}	52.2 ± 4.4^{a}	43.8 ± 4.0^{b}	33.4 ± 3.3^{c}	< 0.0001
Feed conversion ratio (feed/gain)	1.02 ± 0.0^{a}	0.93 ± 0.0^{a}	0.97 ± 0.0^{a}	1.15 ± 0.1^{ab}	1.40 ± 0.1^{b}	0.95 ± 0.0^{a}	1.02 ± 0.0^{a}	1.17 ± 0.1^{ab}	1.34 ± 0.2^{b}	0.0001

	MHA supplemental levels	Fish meal replacement levels	Interaction
Two-way ANOVA analyses (P value summary)			
Weight gain	0.8496	< 0.0001	0.4463
Feed conversion ratio	0.8405	< 0.0001	0.8300

Means in the same row that do not share a common superscript differ significantly ($P < 0.05$).
*D1 was a fish meal control diet; D25, D50, D75, and D100 represent diets that were formulated to replace 25%, 50%, 75%, and 100% of fish meal, respectively, using soybean meal; M25, M50, M75, and M100 represent diets formulated to replace 25%, 50%, 75%, and 100% of fish meal, respectively, using soybean meal supplemented with methionine hydroxy analogue (MHA).

Table 4 Whole-body composition of rainbow trout fed experimental diets for 56 days (%, mean ± SD, $N = 15$ pooled fish)*

Item	Control	Without MHA				With MHA supplementation				*P* value
	D1	D25	D50	D75	D100	M25	M50	M75	M100	
Moisture	69.2 ± 0.6^{a}	70.1 ± 1.0^{ab}	69.7 ± 0.3^{ab}	69.6 ± 1.2^{ab}	70.5 ± 0.4^{ab}	69.9 ± 0.2^{ab}	69.3 ± 0.6^{a}	70.1 ± 0.6^{ab}	71.5 ± 0.9^{b}	0.0316
Crude protein	14.6 ± 0.4^{a}	13.6 ± 0.3^{b}	13.2 ± 0.4^{b}	13.5 ± 0.2^{b}	12.8 ± 0.4^{b}	13.5 ± 0.2^{b}	13.7 ± 0.2^{b}	13.5 ± 0.4^{b}	12.8 ± 0.6^{b}	0.0005
Crude fat	14.7 ± 0.9	14.0 ± 1.2	14.7 ± 0.5	15.1 ± 1.4	15.2 ± 0.4	14.8 ± 0.5	15.2 ± 0.7	14.8 ± 0.5	14.0 ± 0.2	0.5470
Ash	2.7 ± 0.4^{ab}	3.1 ± 0.5^{a}	2.5 ± 0.3^{ab}	2.1 ± 0.3^{bc}	1.9 ± 0.3^{c}	2.8 ± 0.2^{a}	2.4 ± 0.1^{ab}	2.3 ± 0.3^{bc}	2.5 ± 0.5^{ab}	0.0156

	MHA supplemental levels	Fish meal replacement levels	Interaction
Two-way ANOVA analyses (P value summary)			
Moisture	0.5026	0.0148	0.3780
Crude protein	0.0065	0.4682	0.6249
Crude fat	0.8055	0.5774	0.1916
Ash	0.2835	0.0041	0.1509

Means in the same row that do not share a common superscript differ significantly ($P < 0.05$).
*D1 was a fish meal control diet; D25, D50, D75, and D100 represent diets that were formulated to replace 25%, 50%, 75%, and 100% of fish meal, respectively, using soybean meal; M25, M50, M75, and M100 represent diets formulated to replace 25%, 50%, 75%, and 100% of fish meal, respectively, using soybean meal supplemented with methionine hydroxy analogue (MHA).

body moisture, crude protein, and ash ($P < 0.05$), but not on crude fat ($P = 0.5774$).

Experiment 2

The initial weight, final weight, WG, FCR, and survival of rainbow trout fed experimental diets for 49 days are presented in Table 5. There were significant differences in fish final weight, WG, and FCR ($P < 0.05$), but not in survival ($P = 0.4663$). Fish fed a diet with MHA supplementation at 1.65 g kg^{-1} diet (diet 6) grew faster than fish fed a diet without lysine and MHA supplementation (diet 2) or without MHA supplementation (diet 3). Fish fed diet 6 also had a lower FCR than those fed a diet with the highest MHA supplemental level (diet 8). There were no significant differences in WG and FCR between fish fed diet 1 and those fed all other diets, indicating that SBM could be used in rainbow trout diets to replace

Table 5 Initial weight, final weight, weight gain, feed conversion ratio, and survival of rainbow trout fed experimental diets for 49 days (mean ± SD, $N = 3$ tanks)

	Diets*								
Items	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	***P* value**
Initial weight (g)	49.3±0.4	49.4±0.5	49.5±0.7	49.5±0.7	49.4±0.6	49.5±0.8	49.4±0.5	49.4±0.5	1.0000
Final weight (g)	114.6±4.4^{ab}	105.1±3.4^{b}	106.8±4.3^{b}	110.3±3.9^{ab}	113.7±1.2^{ab}	118.2±4.9^{a}	110.5±5.6^{ab}	107.7±5.0^{ab}	0.0267
Weight gain (g)	65.3±4.6^{ab}	55.8±3.0^{b}	56.7±4.2^{b}	60.8±3.9^{ab}	64.3±1.1^{ab}	68.8±4.3^{a}	61.2±5.9^{ab}	58.3±4.6^{ab}	0.0183
Feed conversion ratio (feed/gain)	1.16±0.1^{ab}	1.28±0.0^{ab}	1.29±0.1^{ab}	1.22±0.1^{ab}	1.16±0.0^{ab}	1.08±0.1^{a}	1.23±0.1^{ab}	1.30±0.1^{b}	0.0482
Survival (%)	100.0±0.0	100.0±0.0	98.9±1.9	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	0.4663

Means in the same row that do not share a common superscript differ significantly ($P < 0.05$).
*Diet 1 is a fish meal-based reference diet; diets 2–8 represent diets using soybean meal to replace 50% of fish meal and supplemented with 0, 0, 0.55, 1.1, 1.65, 2.2, and 2.75 g MHA kg^{-1} diet, respectively, on an equimolar methionine basis (MHA: methionine hydroxy analogue).

Table 6 Whole-body composition of rainbow trout fed experimental diets for 49 days (%, mean ± SD, $N = 15$ pooled fish)

	Diets*								
Items	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	***P* value**
Moisture	70.9±0.1	71.3±0.1	71.4±0.1	71.1±0.4	71.6±0.5	71.4±0.6	71.5±0.3	71.7±0.5	0.2386
Crude protein	15.8±0.3	15.1±0.2	16.0±0.2	14.6±2.2	16.0±0.3	15.8±0.7	16.3±0.4	15.7±0.3	0.2983
Crude fat	11.4±0.6	11.4±0.5	10.8±0.6	10.6±1.5	10.8±0.4	11.0±0.7	10.9±0.5	11.2±0.7	0.8633
Ash	3.0±0.4^{ab}	2.4±0.1^{a}	2.7±0.1^{ab}	3.0±0.6^{ab}	3.5±0.5^{b}	2.7±0.2^{ab}	2.6±0.1^{ab}	3.1±0.3^{ab}	0.0359
Phosphorus	0.29±0.0	0.29±0.0	0.28±0.0	0.28±0.0	0.27±0.01	0.28±0.0	0.28±0.01	0.27±0.0	0.4234

Means in the same row that do not share a common superscript differ significantly ($P < 0.05$).
*Diet 1 is a fish meal-based reference diet; diets 2–8 represent diets using soybean meal to replace 50% of fish meal and supplemented with 0, 0, 0.55, 1.1, 1.65, 2.2, and 2.75 g MHA kg^{-1} diet, respectively, on an equimolar methionine basis (MHA: methionine hydroxy analogue).

50% of fish meal. Fish fed diet 6 also had the highest WG and the lowest FCR.

The whole-body composition of rainbow trout fed experimental diets for 49 days is presented in Table 6. The MHA supplementation did not affect fish body moisture, crude protein, crude fat, and phosphorus ($P > 0.05$), but affected ash level ($P = 0.0359$). Fish fed a diet without lysine and MHA supplementation (diet 2) had a significantly lower ash level than fish fed a diet containing 1.1 g MHA kg^{-1} diet (diet 5), but had no significant differences compared with fish fed other diets.

The apparent retention of crude protein and phosphorus in rainbow trout fed experimental diets for 49 days is presented in Table 7. Significant differences existed in the apparent retention of crude protein and phosphorus ($P < 0.05$) for fish fed different diets. Fish fed diets 3, 5, 6, and 7 did not have significant differences in the apparent retention of crude protein compared with fish fed diet 1, but fish fed diets 2, 4, and 8 had a significantly lower apparent retention of crude protein compared with fish fed diet 1. The results showed that fish fed the MHA-supplemented diet at 1.65 g kg^{-1} diet had the greatest apparent crude protein retention. There were no significant differences in the apparent phosphorus retention between fish fed MHA-supplemented diets and fish fed diet 1, but fish fed diet 6 had a significantly higher apparent phosphorus retention than fish fed diet 8. The results indicated that fish fed a diet with 1.65 g MHA kg^{-1} diet supplementation had the highest apparent phosphorus retention.

Discussion

MHA has been used in poultry feeds successfully in some studies (Gutteridge & Lewis 1964; Katz & Baker 1975; Harter & Baker 1977; Harms, Eldred & Blakeslee 1977; Christensen, Anderson & Dobson 1980; Muramatsu, Yokota, Okumura & Tasaki 1984; Rostagno & Barbosa 1995). However, most of these studies were

Table 7 Apparent retention of crude protein and phosphorus of rainbow trout fed experimental diets for 49 days (%, mean ± SD, $N = 15$ pooled fish)

	Diets*								
Items	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	***P* value**
Crude protein	35.4 ± 2.4^{ab}	27.7 ± 0.7^{c}	31.6 ± 1.2^{bc}	27.5 ± 2.5^{c}	34.4 ± 1.2^{bd}	35.8 ± 2.4^{b}	34.1 ± 3.7^{bd}	29.6 ± 2.4^{cd}	0.0008
Phosphorus	25.9 ± 1.7^{ab}	28.4 ± 0.6^{ab}	25.9 ± 1.0^{ab}	27.2 ± 2.4^{ab}	26.9 ± 1.2^{ab}	30.7 ± 2.6^{a}	27.0 ± 3.2^{ab}	23.8 ± 2.0^{b}	0.0333

Means in the same row that do not share a common superscript differ significantly ($P < 0.05$).
*Diet 1 is a fish meal-based reference diet; diets 2–8 represent diets using soybean meal to replace 50% of fish meal and supplemented with 0, 0, 0.55, 1.1, 1.65, 2.2, and 2.75 g MHA kg^{-1} diet, respectively, on an equimolar methionine basis (MHA: methionine hydroxy analogue).

focused on comparing the efficacy of MHA and methionine, and the experimental treatments in most of these studies were not large enough to elucidate the optimum level of MHA supplementation in animal feeds to optimize animal growth performance.

The results from experiment 1 suggested that SBM could replace up to 50% of fish meal in rainbow trout feed formulations without significantly reducing fish WG and FCR, but MHA supplementation did not affect fish performance. The reason for this may be that the dietary methionine levels were already high (10 g kg^{-1} diet) and lysine levels were low in MHA-supplemented diets; the methionine and cystine requirement for rainbow trout is 10 g kg^{-1} diet (NRC 1993). The lysine to methionine ratios in fish meal-based control diet and diets without MHA supplementation were higher than those in diets with MHA supplementation. The dietary lysine level at 21 g kg^{-1} diet was reported to maximize rainbow trout growth performance (Ogino 1980; Cheng, Hardy & Usry 2003). In the present study, fish fed a diet containing 20.8 g lysine kg^{-1} diet (M25) had the highest numerical WG, suggesting that the lysine to methionine ratio played an important role in fish growth, and the optimum amino acid ratio in fish diets needs to be investigated.

Experiment 2 was based on the results of experiment 1. Experiment 1 showed that SBM could replace 50% of fish meal without significantly reducing fish WG and FCR; thus, SBM was used in diets to replace 50% of fish meal in experiment 2 (Table 2). DDGS was chosen based on its low methionine level and was included at 185 g kg^{-1} diet in all diets. The results of experiment 1 also showed that the lysine level in experimental diets may be low; thus, lysine was supplemented in diets 3–8. MHA supplementation increased fish WG and improved FCR (diets 3–6). Higher MHA supplemental levels did not improve fish performance further. The positive effects of supplemental amino acids, especially lysine and methionine, have also been demonstrated by Webster, Tidwell, Goodgame, Yancey & Mackey (1992), El-Dahhar & El-Shazly (1993), Bai & Gatlin (1994), Robinson & Li (1994), Reigh (1999), and Roubach & Lovell (2001).

Conclusions

Based on the results obtained from both experiments, we conclude that SBM can replace up to 50% of fish meal or can be used at a 17.5% inclusion level in rainbow trout feed formulations without significantly reducing fish WG and FCR, and MHA supplementation does not affect fish WG, FCR, and body composition. MHA supplementation at 1.65 g kg^{-1} diet in SBM–DDGS-based practical diets improves the WG, FCR, and apparent retention of crude protein and phosphorus for rainbow trout. Thus, MHA has a positive effect on fish performance when a diet deficient in methionine and cystine is used.

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