EFFECT OF POULTRY BY-PRODUCT MEAL SUPPLEMENTED WITH L-LYSINE, DL-METHIONINE AND L-HISTIDINE AS A REPLACEMENT FOR FISHMEAL ON THE PERFORMANCE OF RAINBOW TROUT
(Oncorhynchus mykiss)

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Executive Summary

Four experiments were conducted to evaluate the nutritional value of poultry by-product meal (PBM) and the effects of supplementing synthetic lysine, methionine, and histidine on growth, feed conversion ratio, and survival of rainbow trout.

In experiment #1, apparent digestibility coefficients (ADCs) of three types of PBMs (feed grade, prime and refined) and two fish meals (herring and menhaden) were measured using settlement method of fecal collection. Although there were significant differences in ADCs of nutrients among these ingredients, refined PBM (R-PBM) had similar ADCs to herring meal.

In experiment #2, Rainbow trout were fed R-PBM based diets supplemented with or without synthetic lysine (LYS) to replace 25, 50, 75, and 100% herring meal. Six-wk feeding results showed that R-PBM could replace up to 75% fishmeal without reducing WG, FCR, and survival; Furthermore, when LYS was supplemented at 0.29% in R-PBM based diets, it could replace 100% fishmeal without reducing WG, FCR, and survival.

Lysine supplementation significantly improved WG but not FCR; fishmeal replacement also had significant effects on WG and FCR for fish fed R-PBM based diets.

In experiment #3, R-PBM was supplemented with synthetic methionine (MET) and LYS to replace 83.3, 91.6, and 100% herring meal. Eight-wk data indicated that fish fed MET and LYS supplemented diets grew faster than those fed the fishmeal diet although the differences were not statistically significant (P > 0.05), indicating that R-PBM could replace 100% herring meal when MET (0.13%) and LYS (0.29%) were supplemented.

In experiment #4, R-PBM was supplemented with synthetic histidine (HIS), LYS and MET. Six-wk feeding trial demonstrated that when synthetic LYS (0.29%) and MET (0.13%) were supplemented, R-PBM could replace 100% of high quality herring meal without reducing fish WG, FCR, and survival. HIS level of 0.66% in R-PBM based diets was sufficient to promote fish growth. There was no necessary to supplement HIS to R-PBM based diets for rainbow trout.

Key words: Rainbow trout; lysine; methionine; histidine; fishmeal; apparent digestibility coefficients.

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GENERAL OBJECTIVE:

To determine the effect of supplementing L-lysine, DL-methionine and L-histidine in poultry by-product meal to replace fishmeal on the performance of rainbow trout.

SPECIFIC OBJECTIVES:

(1) To determine the *in vivo* apparent digestibility coefficients for protein, amino acids and phosphorus in feed grade, prime and refined poultry by-product meals for rainbow trout.

(2) To determine the optimum replacement level of poultry by-product meal for fishmeal in rainbow trout diets.

(3) To evaluate the nutritional value of poultry by-product meal supplemented with lysine, methionine and histidine for rainbow trout.

Introduction

Fish feed production requires large amounts of fishmeal. Fishmeal has been used in fish feeds as the major source of dietary protein (Hardy, 1999). Fishmeal accounts for about 30-50% of fish feeds for carnivorous species. Peru and Chile are two countries that produce about 1/3 of annual global fishmeal. However, fishmeal production from these countries can fluctuate periodically by over 80% in El Nino years. When this occurs, the fishmeal production can be decreased dramatically and the cost of fishmeal can double in value. If other fish meal-producing countries are experiencing declines in their fisheries during the El Nino years, the supply/demand situation can become even worse. Additional

pressures will result from use of industrial fish as human food sources, a practice that is increasing as world capture fisheries to produce seafood decline (Aiken and Sinclair, 1995).

When global fishmeal production declines and fishmeal prices become too expensive to include in fish feeds, feed manufacturers turn to alternative animal protein sources, such as rendered animal protein meals. There are also increasing global concerns about the ethics and long-term sustainability of using fishmeal in aquatic feeds. Furthermore, fishmeal often is more expensive and in shortage worldwide, and according to industry forecasts, production can be expected to decrease by 20% (down from 6.6 million tons to 5.1 to 5.2 million tons) as the result of El Nino (Barlow, 1989; 1997; Pike, 1998).

Fish feeds typically account for >50% of the operating cost of fish production, and the over-dependence of the salmonid industries on fish meal makes them vulnerable to shifts in supply and demand that cause the price of fish meal to fluctuate (Hardy and Kissil, 1996; Higgs et al., 1995). Therefore, it is important for feed manufacturers to identify and utilize less expensive and more sustainable ingredients such as poultry by-product meal (PBM) in fish feeds and yet make these feeds nutritious so that they are of equal or even better nutritional quality compared to feeds based mainly on fishmeal.

Feed grade, prime and refined PBM have similar nutrient profiles compared to fishmeal. In addition, PBM has a low ash content similar to anchovy fishmeal (NRC, 1993), which is desirable in fish feeds because ash contributes to phosphorus (P) levels in fish farm effluents and ponds. This stimulates algae and other aquatic plant growth in aquatic environment and is becoming subject to strict regulation associated with the Clean Water Act. Numerous studies conducted in our laboratory demonstrated that the potential of PBM as protein source in fish feeds was promising. Their nutritional values for fish can be further improved if supplemented with limiting AA (Cheng, 2000).

Fishmeal used for trout feeds is currently estimated to be 184,000 metric tons worldwide (Hardy, 1999) and, if 3/4 of the fishmeal can be replaced by PBM, trout producers can save about $25 million/year in ingredient costs alone. More importantly, increased usage of rendered animal protein products could have a positive impact on our environment.

Procedures of the experiments

This project consisted of four experiments. All experiments were related to evaluating and improving the nutritional value of PBM for rainbow trout and to compare them to high quality fishmeal. Experiment #1 was started on January 29, 2001. It was designed to evaluate the digestibility of test ingredients to provide digestibility data to formulate trout diets. Three types of poultry by-product meals and two types of fish meals were compared. Experiment #2 was started on July 17, 2001, Experiment #3 on August 17, 2001, and Experiment #4, September 18, 2001. All experiments were conducted at Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho, and all followed the guidelines approved by Animal Care and Use Committee of the University of Idaho. The detailed procedures and results for each experiment are presented below:

Experiment #1: Determination of Apparent Digestibility Coefficients of Nutrients in Poultry By-Product Meals

Abstract

Fish meal production is not growing worldwide; therefore, it is important to search for alternative protein sources. Three types of poultry by-product meals (feed grade, prime and refined) and two fish meals (herring and menhaden) were mixed into casein-gelatin purified reference diets at 30% to measure apparent digestibility coefficients of those ingredients for rainbow trout Oncorhynchus mykiss. A total of 90 trout (initial mean body weight 294.6 ± 10.7 g) were stocked into six 40-L fiberglass digestibility tanks at 15 fish per tank. A single tank was assigned randomly to each of the 5 diets made from these 5 ingredients and the reference diet. Fecal collection by settlement lasted for 2 wk. After first collection, fish were moved to different tanks, and fed their respective diets, and then fecal was collected again. Feces collected in each wk represented a replicate, and they were analyzed separately. The apparent digestibility coefficient of nutrients for herring meal, menhaden meal, feed grade poultry by-product meal, prime poultry by-product meal, and refined poultry by-product meal were: dry matter, 81.3, 70.9, 70.9, 71.5 and 74.5%, respectively ($P = 0.0168$); crude protein, 89.8, 85.8, 83.1, 84.8 and 87.1%, respectively ($P = 0.0032$); fat, 91.5, 90.7, 79.7, 82.7 and 79.9%, respectively ($P = 0.0004$); ash, 76.6, 66.2, 74.2, 77.4 and 79.6%, respectively ($P = 0.2880$); phosphorus, 58.2, 46.9, 49.4, 45.8 and 56.0%, respectively ($P = 0.0143$); gross energy, 88.2, 84.0, 81.9, 83.4 and 79.8%, respectively ($P = 0.2466$). Significant differences in apparent essential amino acid digestibility existed only for arginine and leucine when comparing refined poultry by-product meal with herring meal. Results showed that refined poultry by-product meal had similar nutrient digestibility values to herring meal. Thus, it has a similar nutritional value and can replace portions of herring meal in trout feeds. Furthermore, differences exist in digestibility values among grades of poultry by-product meals that should be taken into consideration when appropriate use levels in fish feeds are being selected.

Introduction

Feed grade PBM (F-PBM), prime PBM (P-PBM) and refined PBM (R-PBM) are three types of PBM that are currently used in the pet-food industry. Their applications in aquaculture remains to be explored. PBM has lower ash content compared to most fish meals (NRC, 1993), which is desirable in fish feeds because ash contributes to phosphorus (P) levels in fish farm effluents and ponds. Unassimulated P stimulates algae and other aquatic plant growth in aquatic environment and is subject to strict regulation associated with the Clean Water Act. However, the apparent nutrient digestibility, especially amino acid (AA) and P digestibility, for these three types of PBM has not been determined in rainbow trout. Previous studies used PBM without distinguishing among the different types of PBM available in the market place. Advanced rendering technologies have made different types of PBM available for the purpose of feeding different species of animals. The assumption is that their nutritional values are different, but this has not been confirmed in fish. Thus, the objectives of this study were to determine the in vivo apparent digestibility coefficients (ADCs) for crude protein (CP), AA, fat, P and gross energy (GE) in F-PBM, P-PBM and R-PBM for rainbow trout and to compare them to ADCs of fish meals.

Materials and methods

F-PBM, P-PBM and R-PBM (Georgia Feed Products Co., Cuthbert, GA), herring and menhaden meals (Silver Cup, Murray, Utah) were used in this experiment to measure ADCs for CP, AA, fat, P and GE. Each of these 5 ingredients was added at 30% into a purified reference diet (70%, Table 1) according to the methods used by Cho and Slinger (1979), and Hardy et al. (1984). The nutrients in this reference diet are highly digestible to rainbow trout and coho salmon (Sugiura et al., 1998). Yttrium (Y) at 100 ppm was used as an inert marker. Thirty percent of water and 10% of trace mineral solution were mixed with each diet before pelleting. The experimental diets were prepared as cold-extruded pellets using a noodle-making machine with a 4 mm die. Pellets were dried using forced air at room temperature overnight and stored at 0–5 °C until use.

This experiment was conducted at Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho. A total of 90 rainbow trout, Oncorhynchus mykiss (Walbaum), (initial mean body weight 294.6 ± 10.7 g), were stocked in six 40-L fiberglass digestibility tanks with 15 fish per tank. A single tank was assigned randomly to each of the 5 diets made from these 5 ingredients and the reference diet. Each tank was supplied continuously with spring water (15 °C) at 5 L/min.

The fish rearing laboratory was lighted from 0500 to 1900 h with fluorescent lighting controlled by timers. Fish were acclimated to experimental conditions for two weeks, during which all fish received a commercial trout feed (Silver Cup, Murray, Utah). Experimental diets were fed once daily at 1330 h to apparent satiation for one week before fecal collection. After feeding, tanks were completely cleaned and feces were collected at 1300 h next day by the settlement technique. The fecal collection lasted for two weeks. After fecal collection in the first week, fish were moved to different tanks and fed their respective diets for another week, and then fecal were collected again. Feces collected in each week were pooled and represented a replicate according to the methods used by Cho et al. (1982), Sugiura et al. (1998) and Rawles and Gatlin (2000), and they were analyzed separately. During the entire experiment, no fish mortality or disease signs occurred. The experiment protocol was approved by the Animal Care and Use Committee of the University of Idaho.

Feed samples were dried in a convection oven at 105 °C for 2 h to determine moisture content (AOAC, 1990) and feces were dried overnight. The dried samples were finely ground by mortar and pestle and were analyzed for total nitrogen using LECO FP-428 nitrogen determinator (LECO Instruments, St. Joseph, MI). Fat was analyzed by extraction using LECO TFE-2000 employing super-critical CO_2 as the extracting solvent, and ash by incineration at 550 C in a muffle furnace. GE was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL). P and Y were analyzed using an Optima 3200 radial inductively-coupled plasma atomic emission spectrometry (Perkin-Elmer Corp., Norwalk, CT). The levels of AA, arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), valine (Val), alanine (Ala), aspartic acid (Asp), cysteine (Cys), glutamic acid (Glu), glycine (Gly), proline (Pro), serine (Ser), tyrosine (Tyr) were analyzed by AAA Laboratory (Mercer Island, WA), using a Beckman System 6300 AA analyzer (Beckman Instruments, Inc., Fullerton, CA).

The ADCs of dry matter (DM), CP (N x 6.25), AA, fat, ash, GE, and P were calculated as a fractional net absorption of nutrients from diets based on Y (0.01%) as a non-absorbable indicator (Marcus and Lengemann 1962; Hardy 1989; Sugiura et al. 1998). Chromic oxide at 0.5% was also included as a cross reference check. The digestibility data were subjected to one way ANOVA to determine the differences among the 5 ingredients. ADCs for reference and test diets were calculated as: ADCs (%) =

100 x [1 – (% marker in diets/% marker in feces) x (% nutrient in feces/% nutrient in diets)] (Maynard and Loosli 1969; NRC 1993).

ADCs values for test ingredients were calculated using the formula below (Cho and Slinger 1979; Cho et al., 1982; Hardy et al. 1984): ADC (%) = [ADC_{test} – (1-i) x ADC_{ref}]/i, where ADC_{test} and ADC_{ref} were the apparent nutrient (or 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. Computer software, Prism, version 3.0 (GraphPad, Inc., San Diego, CA), was used to perform statistical calculations. Mean values of experimental variables for each ingredient and each diet were compared using all pair-wise multiple comparison procedures of the Student-Newman-Keuls method. $P < 0.05$ was considered statistically significant.

Results

The results of chemical analyses of test ingredients are presented in Table 2. Significant differences existed in moisture, CP, fat, ash, energy, and AA among all ingredients ($P < 0.05$). Moisture level was highest in menhaden meal, followed by herring meal and P-PBM; F-PBM and R-PBM had the lowest moisture levels. CP contents were highest in herring meal and R-PBM, followed by P-PBM; menhaden meal and F-PBM had the lowest CP contents. F-PBM had the highest fat level, followed by R-PBM, herring meal and menhaden meal; P-PBM had the lowest fat level. Menhaden meal had the highest ash content, followed by F-PBM and R-PBM; herring meal and R-PBM had the lowest ash contents. P level in each ingredient had pattern similar to ash level. GE was the highest in R-PBM, herring meal and F-PBM, followed by P-PBM. Menhaden meal had the lowest GE.

AA contents in these five ingredients showed a similar trend to respective CP contents. In general, F-PBM and menhaden meal had the lowest AA contents; AA level in P-PBM was in between; R-PBM had similar AA profile to herring meal except that R-PBM had significantly higher Arg, Ala, Cys, Gly, and Pro contents but lower His, Ile, Lys, Met, and Val contents than those of herring meal.

ADCs values for all ingredients are presented in Table 3. ADCs of DM was the highest in herring meal, followed by R-PBM, P-PBM, menhaden meal and F-PBM. ADCs of CP were the highest in herring meal and R-PBM, followed by menhaden meal, P-PBM and F-PBM. ADCs of fat were the highest in herring and menhaden meals, followed by P-PBM, R-PBM and F-PBM, indicating that trout digest polyunsaturated lipid better than saturated fat. There were no significant ADCs differences in ash and GE ($P > 0.05$) among all ingredients. Based on ADCs and GE levels, the digestible energy (DE) was calculated as follows: DE (kcal/g) = GE (kcal/g) x ADCs (%). DE values were 4.43, 3.27, 4.09, 3.89, and 4.16 Kcal/g, for herring meal, menhaden meal, F-PBM, P-PBM, and R-PBM, respectively. ADCs of P was the highest in herring meal and R-PBM, followed by F-PBM, menhaden meal and P-PBM.

ADCs values of AA for all ingredients are also presented in Table 3. In general, ADCs of AA for F-PBM was the lowest among ingredients tested. There were no significant differences ($P > 0.05$) in ADCs of essential AA between P-PBM and R-PBM. There were almost no significant differences in ADCs of non-essential AA between P-PBM and R-PBM except that of Cys and Gly which were higher in R-PBM than in P-PBM. ADCs of AA for two fish meals were high; there were no significant differences ($P > 0.05$) in ADCs of essential AA between these two ingredients. There were almost no significant differences in ADCs of non-essential AA between the two fish meals except that of Gly which was higher in herring meal than that in menhaden meal. Most ADCs of essential AA between R-

PBM and herring meal were not significantly different (P > 0.05), except that ADCs of Arg and Leu was lower in R-PBM than that of herring meal, but ADCs of Trp was significantly higher in R-PBM than that of herring meal.

Discussion

Rendered animal protein meals have been used in animal feeds since the middle of 19th century. However, only recently have researchers shown that animal protein meals such as PBM can successfully be used as protein source for growing fish. Sugiura et al. (1998) reported that there were no significant ADCs differences in CP among herring meal (94.6%), menhaden meal (89.8%) and PBM (95.9%) for rainbow trout, but the type of PBM used in their study was not specified. Aksnes and Opstvedt (1998) reported that ADCs of CP ranged from 76.7 to 90.7% in various types of fish meals. ADCs of CP were found to be 87-91% and 82%, respectively, by Bureau et al. (1999) and Pfeffer et al. (1995) in PBM which were similar to our results (83.1-87.1%). However, Cho and Slinger (1979) reported that ADCs for CP was low (68%), Dong et al. (1993) also reported low values (64.4-77.7%) for PBM. Given the wide range in ADCs of CP for various PBM samples reported by various investigators, fish feed formulators would be wise to utilize specific sources rather than simply purchase PBM from any source.

In the present study, ADCs of fat were higher in fish meal than in PBM. Austreng et al. (1979) also found that polyunsaturated fatty acids were highly digestible by trout. Ellis and Smith (1984) reported that ADC of lipids in herring meal was 89.8%, was similar to the value obtained in this study (91.5%). Bureau et al. (1999) also found lower lipid ADCs for one type of PBM (78%). DE values for herring meal and three types of PBM were slightly higher than their values estimated earlier, 4.34 and 3.46 kcal/g, for herring meal and PBM, respectively (NRC, 1993), type of PBM was not specified in NRC reports. ADCs of P in fish meals and PBM were similar to those of Sugiura et al. (1998) who reported that ADCs of P were 44.4, 36.5, and 63.5%, respectively, for herring meal, menhaden meal, and PBM. These values were not high, possibly due to high P levels in these ingredients. The dietary P requirement for rainbow trout is only 0.6% (NRC, 1993) and, excess P in trout diets is partially excreted in feces, thus causing lower ADCs for P. Ketola and Harland (1993) reported that P retention for salmon was around 20%, indicating that most dietary P was discharged into aquatic environment. However, this retention value depends upon the total and available P level of the diet (Sugiura, 2000).

Few studies in the past measured ADCs of AA for rainbow trout. Our results showed that ADCs of AA were higher in fish meals than those found in PBM. The differences between fish meals and PBM were significant for most AA. However, when comparing R-PBM with herring meal, only ADCs of Arg and Leu were lower in R-PBM than those found in herring meal, whereas the ADC of Trp was higher in R-PBM than that of herring meal. These results showed that R-PBM has the potential to replace significant portions of high quality herring meal in trout feeds, especially given the similarity in protein content and ADCs of proteins between the two ingredients.

In trout studies, Alexis et al. (1985) used PBM to replace fish meal for rainbow trout in a 197-day growth trial. Their results showed that fish meal as sole protein source gave results inferior to most of the other experimental diets, a higher fish growth performance could be obtained when the fish meal was partially substituted by PBM. Viola and Zohar (1984) reported that up to 50% of fish meal could be successfully replaced by PBM in tilapia feeds. Fowler (1991) indicated that up to 20% of PBM could be included in fall chinook salmon diets without adversely affecting growth performance.

Nengas et al. (1999) found that gilthead seabream fed PBM to replace 75 and 100% fish meal had a slight reduction in growth, but this reduction was not statistically significant (p > 0.05) from those fed control diet containing white fishmeal for 12 weeks. El-Sayed (1994) reported that silver seabream fingerlings fed diets containing 15% PBM and 43% fish meal performed as well as those fed 54% fishmeal.

Numerous studies conducted in our laboratory have shown that PBM is a promising rendered animal protein ingredient for trout and salmon. Sugiura et al. (1998) reported that PBM had higher digestibility than the average of four types of fish meal for DM, CP and P in trout and salmon diets. Hardy (University of Idaho, unpublished data) found that PBM had higher nutritional value than meat and bone meal for trout. The three types of PBM used in this study were all processed in the same rendering facility using a continuous cooker. The basic difference among them is in segregation of raw material to obtain the variables in total CP and ash levels (G. Pearl, Fats and Protein Research Foundation, Inc., personal communication). With this separation, CP and ash contents were different, thus causing the differences measured in their respective ADCs.

Rainbow trout require a relatively high portion of CP in their diet, approximately 38% (NRC, 1993). Comparing CP levels between fish meals and PBM, herring meal and R-PBM had the highest CP and there were no significant differences in CP between herring meal and R-PBM, suggesting that R-PBM and herring meal may have similar nutritional values for trout. This can be further demonstrated by comparing their AA profiles as well as their ADCs for CP and AA. Feeding trials using specific types of PBM to replace varying portions of fish meal are needed to determine the optimum level of inclusion of PBM as a major protein source in trout diets.

Experiment #2. Effect of Synthetic L-lysine Supplementation In Refined PBM To Replace Fishmeal On Trout Performance

Abstract

A 2 x 4 factorial experiment, in which refined poultry by-product meal (R-PBM) supplemented with or without synthetic lysine to replace 25, 50, 75, and 100% fishmeal (herring meal), was designed to evaluate the nutritional value of R-PBM and the effect of lysine supplementation in PBM based diets on the performance of rainbow trout. In addition, a fishmeal diet was used as a control. Eight hundred and ten fish, initial body weight of 14 g, were randomly arranged into 9 test groups. The amount of lysine supplementation was based on the results of Experiment #1 so that lysine supplemented diets had lysine levels equal to the control diet. Three tanks per diet and 30 fish per tank was arranged in a completely randomized design. All diets were isonitrogenous and isocaloric. After a 6-wk growing period, significant (P < 0.05) differences in fish weight gain (WG) and feed conversion ratio (FCR), but not survival, existed among fish fed different diets. Two-way ANOVA showed that lysine supplementation significantly improved WG (P = 0.0056), but not FCR (P > 0.05); fishmeal replacement also had significant effects on WG (P = 0.0014) and FCR (P = 0.0134). Survival was greater than 98% for fish fed all diets, and neither lysine supplementation nor fishmeal replacement had effects on survival. Results demonstrated that R-PBM could replace up to 75% of high quality fishmeal without reducing WG, FCR, and survival; Furthermore, when lysine was supplemented at 0.29% in R-PBM based diets, it could replace 100% fishmeal without reducing WG, FCR, and survival.

Key words: Lysine, poultry by-product meal, fishmeal, rainbow trout.

Introduction, materials, and methods

R-PBM and herring meal were selected based on the results of ADCs for CP, AA and P in Experiment #1. These two selected meals had similar ADCs for CP, AA, and P (Table 3). R-PBM was used to replace fishmeal at 25, 50, 75, and 100% with or without synthetic lysine (Heartland Lysine, Inc., Chicago, IL) supplementation in a 2 x 4 factorial design (Table 4). The amount of R-PBM used to replace fishmeal was on w/w, isonitrogenous and isocaloric basis. A fishmeal diet was also used as a control. The amount of lysine supplementation was based on the results of Experiment #1 such that lysine supplemented diets had lysine levels equal to the control diet. Eight hundred and ten fish, initial body weight of 14 g, were randomly arranged into 9 test groups with three tanks per diet and 30 fish per tank in a completely randomized design. All diets were isonitrogenous and isocaloric. Fish were fed for 6 weeks and, at the end of the experiment, five fish from each tank were sacrificed for body composition analyses. Fish WG, FCR, and survival were calculated and compared among treatment groups.

Fish diets were pelleted using a laboratory pellet mill (California Pellet Mill Co., San Francisco, California, USA) with a 2.4 mm die, and air-dried for 48 hours. Samples of each diet were taken for chemical analyses. Methods used for chemical analyses of feed and fish body were the same as those described in experiment #1. Resulting data for fish fed all diets were subjected to one-way ANOVA using Student-Newman-Keuls method to determine the significance. Data were also analyzed using two-way ANOVA to determine the effects of lysine supplementation and fishmeal replacement levels on fish performance. Computer software, Prism, version 3.0 (GraphPad, Inc., San Diego, California, USA) was used to perform statistical calculations, and $P < 0.05$ was considered to be statistically significant. The objectives of this experiment were to minimize fishmeal and maximize PBM in trout diets and to improve nutritional value of PBM by supplementing the most limiting AA, lysine.

Results

Results of chemical analyses of all diets were presented in Table 4. Diets were formulated to contain 2.07, 1.99, 1.92, 1.85, 1.77, 2.07, 2.07, 2.07, and 2.07% dietary lysine for diet 1 to 9, respectively, and all diets had 42% CP and a calculated digestible energy value of 3.6 Kcal/g diet. Analyzed proximate values were close to those expected values. Fish performance data were presented in Table 5. There were no significant differences in fish initial weight ($P = 1.0000$), these fish were selected based on a large population, and on the basis of no visible body defects or disease signs. After a 6-wk growing period, significant ($P < 0.05$) differences in fish final weight, WG, and FCR existed among fish fed different diets. However, survival was not significant different ($P = 0.5548$) among fish fed different diets. Fish fed diets containing R-PBM to replace 25% (diet 2) and 50% (diet 3) fishmeal grew faster than those fed fishmeal control diet, although the differences were not statistically significant. In addition, when lysine was supplemented in R-PBM to replace 25% (diet 6) and 50% (diet 7), fish grew faster than those fed respective R-PBM diets without lysine supplementation, and those fed fishmeal control diet.

Two-way ANOVA showed that there were no significant interactions between lysine supplementation and fishmeal replacement. Lysine supplementation significantly improved WG ($P = 0.0056$), but not FCR ($P > 0.05$); fishmeal replacement also had significant effects on WG ($P = 0.0014$) and FCR ($P = 0.0134$), meaning that as R-PBM inclusion rate increased in the diets, fish growth were reduced and FCR were poorer. Survival was greater than 98% for fish fed all diets, and neither lysine

supplementation nor fishmeal replacement had effects on survival. Results demonstrated that R-PBM could replace up to 75% of high quality fishmeal without reducing WG, FCR, and survival; Furthermore, when lysine was supplemented at 0.29% in R-PBM based diets, it could replace 100% fishmeal without reducing WG, FCR, and survival.

Table 6 presented analyses of whole trout body composition. Significant (P < 0.05) differences in moisture, CP, fat, and ash existed among fish fed different diets. Although differences were significant, the absolute values were very small. e.g., 73.7% moisture for fish fed diet 4 vs. 72.2% moisture for fish fed diet 2. Two-way ANOVA showed that lysine supplementation had no significant effects on moisture (P = 0.6178) and CP (P = 0.5658) levels in fish body, but fishmeal replacement had significant effects on moisture (P = 0.0040) and CP (P = 0.0004), meaning that as R-PBM inclusion rate increased in the diets, there was a trend that fish body moisture would increase and CP decrease. There were significant interactions for fat and ash (P < 0.05), therefore, the effects of lysine supplementation and fishmeal replacement on fish fat and ash could not be compared.

Discussion

AA nutrition studies on fish and shrimp have received significant attention in the past. Andrews et al. (1977) supplemented a semi-purified casein diet with free AA (arginine, cystine, methionine or tryptophan) and gelatin and fed these diets to catfish. Their results showed that the addition of synthetic arginine, cystine, methionine or tryptophan to casein had little effect on fish growth and feed efficiency. The authors concluded that catfish were unable to utilize dietary free AA. However, lysine was not supplemented in that study. Data from several studies conducted in 1990's with fish and shrimp showed that supplementing synthetic AA, especially lysine, into fish or shrimp diets usually improved aquatic animal performances (Kim et al., 1992; Lim, 1993; El-Dahhar and El-Shazly, 1993; Bai and Gatlin, 1994; Fernandez and Sukumaran, 1995; Fox et al., 1995). Recently, Cheng (2000) completed a study evaluating the effects of supplementing synthetic L-lysine and DL-methionine into feather meal based diets on the performance of juvenile Pacific white shrimp (*L. vannamei*). Results demonstrated that feather meal could replace only 1/3 fishmeal, but with AA supplementation, feather meal could replace 2/3 fishmeal. Furthermore, when AA were supplemented into feather meal to replace 1/3 fishmeal, shrimp grew faster than those fed the fishmeal control diet (P < 0.05). Viola et al. (1992) evaluated the reduction of feed protein levels by lysine supplementation in intensive carp culture. Their results showed that 30% protein carp feeds could be decreased to 25% by replacing soybean meal with grains and supplementing lysine (0.5% lysine-HCl) and methionine (0.3%) without impairing fish growth performance. In their earlier study, Viola and Lahav (1991) showed that carp fed diet containing 25% protein supplemented with 0.5% lysine had equal growth and FCR as compared with fish fed 30% protein diet.

Research on dietary lysine requirement is of great importance. Our present study indicated that dietary lysine level at 2.07% (diet 6) could maximize WG and FCR for rainbow trout. As dietary lysine level decreased from 1.99% in diet 2 to 1.77% in diet 5, WG and FCR of fish were linearly decreased. This 2.07% dietary lysine level was higher than estimated requirement value of 1.8% (NRC, 1993), 1.9% (Walton et al., 1984), or 1.3% (Kim et al., 1992) in rainbow trout diets, but lower than the estimated 2.9% by Ketola (1983), and close to 2.1% estimated by Ogino (1980). Rodehutscord et al. (2000 a) reported that the growth of rainbow trout fed diets containing 0.9, 1.3, and 1.7% dietary lysine did not reach a plateau when diets contained either 35 or 55% CP, indicating that the requirement of lysine in

rainbow trout was higher than 1.7%. In their second study, Rodehutscord et al. (2000 b) further confirmed that the growth of rainbow trout was not maximum when fed diets containing 0.9, 1.3, and 1.7% dietary lysine (either in L-lysine.HCL or L-lysine sulphate) and 55% CP.

Our results showed that lysine supplementation in R-PBM based diets did not have significant effects on increasing CP and lysine levels, and reducing fat content in whole body of rainbow trout. This was in disagreement with results of Rodehutscord et al. (2000 a). In their study, retention of lysine and CP in trout body increased linearly with increased dietary lysine supplementation disregarding dietary CP levels, and retention of fat decreased linearly with increased lysine supplementation. The reasons for this disagreement were not clear.

Future research in investigating the effect of lysine supplementation on the performance of larger fish or for a longer period is needed because most feeds are consumed in later growth stages. Research on evaluating the effect of supplementing other essential AA, i.e., methionine and histidine, on the performance of fish is also needed since PBM is also deficient in these AA compared to fishmeal.

Concluding remarks

Rainbow trout fed R-PBM based diets could replace up to 75% fishmeal without reducing WG, FCR, and survival. Furthermore, when lysine was supplemented at 0.29% in R-PBM based diet, it could replace 100% fishmeal without reducing WG, FCR, and survival at least in a short-term study. Lysine supplementation significantly improved WG but not FCR; fishmeal replacement also had significant effects on WG and FCR for fish fed R-PBM based diets. However, lysine supplementation in R-PBM based diets did not increase CP or reduce fat contents in whole body of rainbow trout.

Experiment #3. Effect Of Supplementing Graded Levels Of Methionine In Refined PBM Based Diets On Trout Performance

Abstract

Poultry by-product meal (PBM) is deficient in lysine (LYS) and methionine (MET) compared to fishmeal. Therefore, supplementing LYS and MET in PBM diets may improve animal performance. Nine diets were made using refined PBM (R-PBM) supplemented with synthetic LYS and graded levels of MET to contain total dietary Met levels of 0.73% (D-2 to D-4), 0.83% (D-5 to D-7), and 0.93% (D-8 to D-10), and to replace 83.3, 91.6, and 100% fishmeal (herring meal) for rainbow trout. In addition, a fishmeal diet (D-1) containing 0.73% MET and 35% herring meal was used as a control. All diets had 2.07% LYS, 45% CP, and a digestible energy of 3.6 Kcal/g diet. A total of 900 rainbow trout (initial mean body weight 13.9 ± 0.4 g) were stocked into thirty 150-L fiberglass tanks with 30 trout per tank, and three tanks per diet. Fish were fed to apparent satiation 3 times/day and 7 days/week. After an 8-week period, average weight gain (WG) of fish fed D-1 to D-10 was: 83.3, 85.6, 88.9, 83.5, 80.9, 86.2, 82.3, 87.2, 80.8, and 83.0 g, respectively. Average FCR of fish fed D-1 to D-10 was: 1.01, 1.02, 1.07, 1.09, 1.12, 1.07, 1.06, 1.05, 1.11, and 1.15, respectively. Survival was > 97% for fish fed all diets. Results showed that fish fed diets supplemented with LYS and MET to replace 83.3, 91.6, and 100% fishmeal were not significantly different compared to fish fed the fishmeal diet in terms of WG, FCR, and survival (P > 0.05); indicating that R-PBM could replace 100% herring meal when LYS and MET were supplemented.

Key words: Methionine, lysine, poultry by-product meal, fishmeal, rainbow trout.

Introduction, materials, and methods

Rainbow trout fed R-PBM based diets could replace up to 100% fishmeal without reducing WG and survival, but FCR was significantly poorer when R-PBM was used to replace 100% fishmeal. Furthermore, when LYS was supplemented at 0.29% in fish diets, R-PBM could replace 100% fishmeal without reducing WG, FCR, and survival (Table 5). From experiment #1 (Table 2), it could be seen that MET levels in herring meal and R-PBM were 1.84 and 1.48%, respectively, indicating that R-PBM was also deficient in MET. Therefore, supplementing MET in R-PBM based diets may further improve fish performance.

In this experiment, a 3 x 3 factorial arrangement was used to evaluate the nutritional value of R-PBM and the effect of LYS and MET supplementation in PBM based diets on the performance of rainbow trout. R-PBM was supplemented with three levels of synthetic MET (0.11, 0.21, and 0.31%) to replace 83.3, 91.6, and 100% fishmeal. Due to the differences in MET levels between R-PBM and herring meal, MET supplementation levels were slightly higher than 0.11, 0.12, and 0.13% when R-PBM was used to replace 91.6 and 100% fishmeal. In addition, a fishmeal diet was used as a control. Nine hundred fish, initial body weight of 14 g, were randomly arranged into 10 test groups, the amount of LYS supplementation was based on the results of Experiment #1 such that LYS supplemented diets had equal amount of LYS level to control diet. The amount of MET supplementation was also based on the results of Experiment #1 such that MET supplemented diets, D-2, D-3, and D-4, had equal amount of MET levels to control diet. D-5, D-6, and D-7 had 0.83% MET; D-8, D-9, and D-10 had 0.93% MET. Three tanks per diet and 30 fish per tank were arranged in a completely randomized design. All diets were formulated to be isonitrogenous and isocaloric. Fish were fed for 8 weeks and, at the end of the experiment, five fish from each tank were sacrificed for body composition analyses. Fish WG, FCR, and survival were evaluated.

Fish diets were pelleted using a laboratory pellet mill (California Pellet Mill Co., San Francisco, California, USA) with a 2.4 mm die, and air-dried for 48 hours. Samples of each diet were taken for chemical analyses. Methods used for chemical analyses of feed and fish body were the same as those described in experiment #1. Resulting data for fish fed all diets were subjected to one-way ANOVA using Student-Newman-Keuls method to determine the significance. Data were also analyzed using two-way ANOVA to determine the effects of MET supplementation and fishmeal replacement levels on fish performance. Computer software, Prism, version 3.0 (GraphPad, Inc., San Diego, California, USA) was used to perform statistical calculations, and $P < 0.05$ was considered to be statistically significant. The objectives of this experiment were to further evaluate the nutritional value of R-PBM in trout diets and to improve nutritional value of R-PBM by supplementing synthetic LYS and MET.

Results

Results of chemical analyses of all diets were presented in Table 7. Diets were formulated to contain 45% CP, a calculated digestible energy value of 3.6 Kcal/g diet, 2.07% dietary LYS, and 0.73% MET (D-2 to D-4), 0.83% MET (D-5 to D-7), and 0.93% MET (D-8 to D-10). Analyzed proximate values were close to those expected values. Fish performance data were presented in Table 8. There were no

significant differences in fish initial weight (P = 1.0000). These fish were selected based on a large population, and on the basis of no visible body defects or disease signs.

After an 8-wk growing period, no significant (P > 0.05) differences existed among fish fed different diets in terms of final weight, WG, FCR and survival. However, fish fed D-2, D-3, D-4, D-6, D-8, and D-9 grew faster than those fish fed D-1 despite lacking of significant differences. Fish fed 0.11% MET supplemented diets (D-2 to D-4) had better growth performance compared to fish fed fishmeal based control diet, indicating that MET supplementation at 0.1% was sufficient for fish growth, further MET supplementation did not promote fish growth. FCR was the best for fish fed D-1. Two-way ANOVA showed that neither MET supplementation nor fishmeal replacement had effects on WG, FCR, and survival (P > 0.05). Results demonstrated that R-PBM could replace 100% of high quality fishmeal without reducing WG, FCR, and survival.

Results of whole trout body composition analyses are presented in Table 9. No significant (P > 0.05) differences in body moisture, fat, and ash existed among fish fed different diets. However, there were significant (P < 0.05) differences in body CP among fish fed different diets. Although differences were significant, the absolute values were very small. e.g., 16.2% CP for fish fed diet-1 vs. 15.1% CP for fish fed some of the MET supplemented diets. Two-way ANOVA showed that neither MET supplementation nor fishmeal replacement had significant effects on fish body moisture, CP, fat, and ash (P > 0.05).

Discussion

Results of this experiment showed that fish fed diets supplemented with 0.11, 0.12, and 0.13% MET to replace 83.3, 91.6, and 100% (D-2, D-3, and D-4) fishmeal, respectively, had better WG than those fed fishmeal control diet (D-1) although the differences were not statistically significant. Total MET level in these diets were 0.73%. However, in experiment #2, fish fed diets supplemented with LYS to replace 100% fishmeal (Diet 9) grew slightly slower than those fed the fishmeal control diet (Diet 1, Table 5) although the differences were not statistically significant. Total MET level in diet 9 (Experiment #2) was 0.6%. Results indicated that supplementing 0.13% MET into R-PBM based rainbow trout diet improved fish growth performance. Further MET supplementation did not support higher fish growth performance, suggesting that 0.73% MET in rainbow trout diet was sufficient for optimum trout growth. Recently, Cheng and Hardy (Unpublished data) completed a study using MHA as a MET source in soybean meal and dried distiller's grain based diets for rainbow trout. Seven week growth responses showed that a dietary MET level of 0.7% promoted best fish growth.

Kim et al. (1992) reported that the growth, feed efficiency, and nitrogen retention of rainbow trout were maximized when fed diets containing 0.6-0.8% dietary MET although these parameters were not significantly different from fish fed a 0.5% dietary MET diet. Walton et al. (1982) found that the requirement of MET for rainbow trout was 0.5-1.0% in the absence of cystine, and 0.5% dietary MET was adequate when cystine was 2% in the diet. Rodehutscord et al. (1994) reported that dietary MET level of 0.8-0.9% could maximize protein deposition in 50-150 g rainbow trout, and 0.35% dietary MET was used most efficiently. NRC (1993) estimated 1.0% total dietary MET and cystine for rainbow trout. Our result of 0.73% dietary MET in R-PBM based diet to maximize growth performance of rainbow trout was close to 0.72% dietary MET requirement estimated by Ogino (1980).

Experiment #4: Effect Of Supplementing Graded Levels Of Histidine In PBM Based Diets On Trout Performance

Abstract

Compared to herring meal, histidine (HIS) is relatively low in poultry by-product meal (PBM) which is also deficient in lysine (LYS) and methionine (MET). Therefore, supplementing HIS in PBM diets may affect animal performance. Six diets (D 2 to D 7) were made using refined PBM supplemented with LYS and MET, and had 0.66, 0.785, 0.91, 1.035, 1.16, and 1.285% dietary HIS, respectively. These diets were used to replace 100% herring meal in rainbow trout diets. In addition, a fishmeal diet (D 1) containing 0.91% HIS and 35% herring meal was used as a control. All diets had 2.07% LYS, 0.73% MET, 45% CP and a calculated digestible energy value of 3.6 Kcal /g diet.

A total of 630 rainbow trout (initial mean body weight 19.1 ± 0.5 g) were stocked into twenty-one 150-L fiberglass tanks with 30 trout per tank, and three tanks per diet. Fish were fed to apparent satiation 3 times per day, 7 days per week. After a 6-week period, there were no significant differences in average WG, FCR, and survival among fish fed different dietary treatments.

Results suggested that R-PBM could replace 100% herring meal when LYS (0.29%) and MET (0.13%) were supplemented. Supplementation of HIS in R-PBM diets did not yield improvements in the performance of the rainbow trout, indicating that the level of HIS in R-PBM is sufficient for rainbow trout.

Key words: Histidine, poultry by-product meal, fishmeal, rainbow trout

Introduction, materials, and methods

Rainbow trout fed R-PBM based diets supplemented with 0.29% LYS and 0.13% MET could replace 100% fishmeal without reducing WG, FCR, and survival. However, Table 2 showed that HIS levels in herring meal and R-PBM were 2.24 and 1.54%, respectively, indicating that R-PBM was also deficient in HIS compared to herring meal. Therefore, supplementing HIS in R-PBM based diets may further affect fish performance.

R-PBM used in Experiments #2 and #3 was used here. Five graded levels of HIS supplementation at 0.125% increment were used for this experiment (D 3 to D 7, Table 10) in a completely randomized design. A control diet (D 2) contained 0.66% HIS and a fishmeal reference diet (D 1) was also used. Diet formula is presented in Table 10. Three tanks per diet and 30 trout per tank was used. All diets were isonitrogenous and isocaloric. Fish were fed for 6 weeks, at the end of the experiment, five fish from each tank were sacrificed for body composition analyses. Fish WG, FCR, and survival were measured. The objectives of this experiment were to completely replacing fishmeal with PBM in rainbow trout diets and to improve nutritional value of PBM by supplementing essential AA, HIS, along with LYS and MET.

Results and Discussion

Results of chemical analyses of all diets are presented in Table 10. Diets were formulated to contain 45% CP, a calculated digestible energy value of 3.6 Kcal/g diet, 2.07% dietary LYS, and 0.73% MET.

Analyzed proximate values were close to expected values. Fish performance data are presented in Table 11. There were no significant differences in fish initial weight (P = 0.9849). These fish were selected based on a large population, and on the basis of no visible body defects or disease signs.

After a 6-wk growing period, no significant (P > 0.05) differences existed among fish fed different diets in terms of final weight, WG, FCR and survival. Results of whole trout body composition analyses are presented in Table 12. No significant (P > 0.05) differences in body moisture, fat, and ash existed among fish fed different diets. However, there were significant (P < 0.05) differences in body CP among fish fed different diets. Although differences were significant, the absolute values were very small. e.g., 15.6% CP for fish fed D 1 vs. 14.3% CP for fish fed D 5 and D 7.

Results demonstrated that when synthetic LYS (0.29%) and MET (0.13%) were supplemented, R-PBM could replace 100% of herring meal without reducing fish WG, FCR, and survival at least in a short-term study. HIS level of 0.66% in R-PBM based diets was sufficient to promote fish growth. There was no necessary to supplement HIS in R-PBM based diets for rainbow trout. The dietary level of HIS at 0.66% is close to the level of 0.7% estimated by NRC (1993).

Conclusions and Recommendations

Based on the results of four experiments conducted at our Station, we conclude that the nutritional value of R-PBM is similar to herring meal for rainbow trout. Furthermore, when synthetic LYS (0.29%) and MET (0.13%) are supplemented, R-PBM can replace 100% of herring meal without reducing fish WG, FCR, and survival. There is no necessary to supplement HIS into R-PBM based diets for rainbow trout. Future research should focus on production scale testing using large fish, e.g., greater than 100 g, to confirm the results of this project; and to further evaluate the effects of synthetic LYS and MET supplementation on the performance of rainbow trout using extruded feed, and the quality of the fillets of rainbow trout fed extruded feeds. We, at the University of Idaho, are more than happy to conduct more studies for Fats and Protein Research Foundation in the near future.

Table 1. Composition of the reference diet used in experiment #1.

Ingredients	%
Casein[1]	44.0
Gelatin[2]	10.0
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
Finstim[7]	1.39
Yttrium oxide[2]	0.01
Chromic oxide[2]	0.5
Herring oil[8]	17.0
Trace mineral solution[9]	(10)
Water	(30)

[1] Purchased from ICN Biomedicals, Inc., Cleveland, OH.

[2] Purchased from Sigma Chemical Co., St. Louis, MO.

[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; $MnSO_4H_2O$, 20 mg; $ZnSO_47H_2O$, 75 mg; Na_2SeO_3, 2 mg; $CoCl_36H_2O$, 1.0 mg; $CuSO_45H_2O$, 3 mg; $FeSO_47H_2O$, 50 mg.

Table 2. Chemical analysis of test ingredients (%, Mean ± SD, as is basis)[1].

	Herring meal	Menhaden meal	Feed grade PBM	Prime PBM	Refined PBM
Moisture	6.66±0.23a	8.83±0.05b	3.08±0.13c	3.92±0.02d	3.27±0.03c
CP	71.02±2.10a	61.22±0.17b	62.28±0.26b	66.22±1.18c	70.09±0.30a
Fat	9.18±0.13a	9.14±0.09a	11.00±0.28b	8.07±0.03c	9.95±0.01d
Ash	12.22±0.24a	21.69±0.59b	15.51±0.22c	15.21±0.97c	11.26±0.23a
P	2.40±0.00ab	3.05±0.35b	2.60±0.14b	2.80±0.14b	2.00±0.00a
GE (kcal/g)	5.01±0.01ad	3.90±0.12b	4.99±0.09d	4.67±0.06c	5.21±0.04d
Essential AA					
Arg	4.50±0.13ad	3.80±0.11a	4.43±0.45ac	4.94±0.16bcd	5.42±0.03b
His	2.24±0.19a	1.19±0.06b	1.40±0.01b	1.22±0.12b	1.54±0.03b
Ile	3.25±0.07a	2.37±0.15b	2.51±0.09b	2.51±0.12b	2.92±0.02c
Leu	5.25±0.21a	3.95±0.23b	4.36±0.15b	4.36±0.21b	5.12±0.04a
Lys	5.40±0.38a	4.18±0.18b	3.66±0.30b	3.71±0.23b	4.58±0.04b
Met	1.84±0.12a	1.53±0.04c	1.16±0.01b	1.26±0.11b	1.48±0.07c
Phe	2.84±0.17a	2.18±0.08b	2.30±0.11b	2.35±0.10b	2.76±0.01a
Thr	3.10±0.16ac	2.38±0.11b	2.46±0.06b	2.44±0.28b	2.95±0.04bc
Trp	0.33±0.00a	0.19±0.01a	0.48±0.02a	0.63±0.14a	0.62±0.03a
Val	3.63±0.11a	2.66±0.15b	2.84±0.09b	2.86±0.10b	3.30±0.04c
Non-essential AA					
Ala	4.03±0.13a	3.63±0.13b	3.93±0.16a	4.15±0.01a	4.52±0.00c
Asp	6.33±0.26ac	5.05±0.23b	4.96 ±0.20b	4.94±0.47b	5.89±0.05bc
Cys	0.61±0.01a	0.45±0.03b	0.66±0.08ad	0.78±0.06cd	0.82±0.04cd
Glu	9.00±0.38ac	7.68±0.35a	8.15±0.49a	8.11±0.59a	9.69±0.01bc
Gly	3.73±0.26a	4.41±0.09b	5.16±0.17c	6.25±0.25d	6.23±0.24d
Pro	3.22±0.26a	3.23±0.06a	4.06±0.00b	4.65±0.13c	4.83±0.13c
Ser	2.85±0.22acd	2.19±0.08b	2.40±0.02bc	2.49±0.21bd	2.91±0.08cd
Tyr	2.42±0.13a	1.78±0.13b	1.93±0.02b	2.04±0.11b	2.40±0.06a

[1]Values in the same row that do not share a common letter differ significantly (P < 0.05).
Abbreviations: PBM, poultry by-product meal; CP, crude protein; P, phosphorus; GE, gross energy; AA, amino acid; Arg, Arginine; His, Histidine; Ile, Isoleucine; Leu, leucine; Lys, lysine; Met, Methionine; Phe, Phenylalanine; Thr, Threonine; Trp, Tryptophan; Val, Valine; Ala, Alanine; Asp, Aspartic acid; Cys, Cysteine; Glu, Glutamic acid; Gly, Glycine; Pro, Proline; Ser, Serine; Tyr, Tyrosine.

Table 3. Apparent nutrient digestibility coefficients of test ingredients (%, Mean ± SD)[1].

	Herring meal	Menhaden meal	Feed grade PBM	Prime PBM	Refined PBM
DM	81.26±1.23a	70.94±3.42b	70.93±2.58b	71.50±1.34b	74.48±0.15b
CP	89.78±1.11a	85.83±1.04bd	83.10±0.48cd	84.75±0.89bc	87.14±0.04ab
Fat	91.52±0.05a	90.74±0.65a	79.69±2.64b	82.66±0.19b	79.88±0.40b
Ash	76.60±4.34a	66.19±4.98a	74.17±9.91a	77.44±2.45a	79.58±3.42a
P	58.24±0.62ac	46.88±2.73b	49.43±0.40bc	45.82±5.88b	56.03±1.70bc
GE	88.24±6.27a	84.01±1.77a	81.93±1.64a	83.35±1.65a	79.84±1.58a
Essential AA					
Arg	93.45±0.14a	93.56±0.17a	85.66±1.92b	86.71±0.99b	88.99±1.33b
His	91.67±0.95abc	93.27±2.29abc	85.23±1.35d	89.41±0.97bd	88.57±0.28cd
Ile	90.16±1.18ab	91.01±1.22ab	80.14±4.25c	83.64±0.45bc	83.23±2.46bc
Leu	92.18±0.66a	94.23±0.89a	83.80±2.69b	86.54±0.31b	86.38±1.49b
Lys	94.73±0.09a	94.80±0.59a	88.54±1.80b	91.63±0.04a	92.61±0.64a
Met	94.69±0.08ab	95.42±0.95a	92.19±1.29b	94.75±0.13ab	94.24±0.39ab
Phe	88.16±2.53a	91.78±5.07a	85.10±2.16a	85.38±0.85a	85.74±0.66a
Thr	90.29±0.09ac	91.86±1.23a	82.07±3.67b	84.62±0.46bc	84.60±1.25bc
Trp	74.51±5.59a	93.11±8.62ab	84.68±5.12ac	97.02±5.14bcd	96.13±3.69bcd
Val	91.17±1.26abc	92.03±0.94abc	81.03±4.63d	84.07±0.84bd	83.49±2.38cd
Non-essential AA					
Ala	89.38±1.70a	88.41±0.72a	77.60±4.77b	81.04±1.44ab	84.17±1.44ab
Asp	88.77±0.15a	88.80±1.42a	80.03±4.68a	82.62±0.85a	83.88±1.86a
Cys	82.35±0.26a	82.63±4.22a	46.52±6.51b	34.59±0.55c	56.40±4.65b
Glu	93.11±0.53ac	92.13±1.24ac	86.14±2.68b	89.23±0.15ab	90.11±1.25bc
Gly	89.38±1.83ab	84.03±1.18cd	82.53±1.96cd	81.06±0.84d	86.94±0.00bc
Pro	95.16±0.36a	93.58±0.92a	89.01±1.43b	89.46±0.21b	91.03±0.60b
Ser	86.31±0.13a	85.58±2.22a	76.83±5.04a	82.43±1.26a	83.84±1.17a
Tyr	95.25±0.29a	96.00±0.64a	90.48±0.44b	90.24±0.51b	90.63±1.52b

[1]Values in the same row that do not share a common letter differ significantly (P < 0.05). Abbreviations: PBM, poultry by-product meal; DM, dry matter; CP, crude protein; P, phosphorus; GE, gross energy; AA, amino acid; Arg, Arginine; His, Histidine; Ile, Isoleucine; Leu, leucine; Lys, lysine; Met, Methionine; Phe, Phenylalanine; Thr, Threonine; Trp, Tryptophan; Val, Valine; Ala, Alanine; Asp, Aspartic acid; Cys, Cysteine; Glu, Glutamic acid; Gly, Glycine; Pro, Proline; Ser, Serine; Tyr, Tyrosine.

Table 4. Composition of the test diets for experiment #2 (%, as fed basis) [1]

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Herring meal	35.0	26.3	17.5	8.7	0.0	26.3	17.5	8.7	0.0
Refined-PBM	0.0	8.7	17.5	26.3	35.0	8.7	17.5	26.3	35.0
Whole wheat	27.9	27.7	27.6	27.4	27.2	27.82	27.65	27.58	27.41
Corn gluten	18.0	18.2	18.3	18.5	18.7	18.0	18.1	18.1	18.2
Fish oil	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7
Vitamin C	0.3	0.3	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	0.5	0.5
TM salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-lysine	0.0	0.0	0.0	0.0	0.0	0.08	0.15	0.22	0.29
Calculated analyses (%, as fed basis)									
Crude protein	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0
DE (kcal/g diet)	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Lysine	2.07	1.99	1.92	1.85	1.77	2.07	2.07	2.07	2.07
Chemical analyses (%, as fed basis, n = 2)									
Moisture	7.9	7.6	7.7	7.6	7.8	7.5	7.9	7.6	7.4
CP	43.3	43.2	42.1	41.7	40.5	42.8	42.1	41.7	40.8
Fat	20.3	21.0	21.0	21.8	22.5	21.4	21.2	21.5	21.9
Ash	3.9	3.9	4.2	4.3	4.4	4.0	4.0	4.2	4.3

[1] Origin of ingredients is as follows: Herring meal, whole wheat, corn gluten, choline chloride, trace mineral salt, and vitamin premix were purchased from Nelson & Sons, Inc., Murray, Utah; Refined-PBM was from Georgia Feed Products Co., Cuthbert, GA; Fish oil and vitamin C were from Rangen, Inc., Buhl, Idaho; L-lysine was from Heartland Lysine Inc., Chicago, IL.

Table 5. Initial weight, final weight, weight gain, feed conversion ratio, and survival of rainbow trout fed for six weeks (n = 3)*

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	
	Fishmeal replacement level (%)									
	0	25	50	75	100	25	50	75	100	
	Dietary lysine level (%)									
Item	2.07	1.99	1.92	1.85	1.77	2.07	2.07	2.07	2.07	P value
Initial (g)	13.9±0.2a	14.0±0.1a	13.9±0.1a	13.9±0.3a	13.9±0.2a	13.9±0.1a	13.9±0.3a	14.0±0.2a	13.9±0.2a	1.0000
Final (g)	51.5±1.7abc	53.0±1.8bc	51.9±1.6ab	47.2±0.8ac	45.0±5.9ac	57.5±2.8b	52.3±2.9bc	51.0±1.8ab	50.8±1.2ab	0.0023
WG (g)	37.5±1.7abc	39.0±1.9bc	37.9±1.7ab	33.3±1.1ac	31.1±5.9ac	43.6±2.9b	38.4±2.6bc	37.0±1.7ab	36.8±0.8ab	0.0019
FCR (g/g)	0.97±0.05a	0.99±0.06a	1.06±0.02a	1.21±0.05a	1.25±0.22b	0.96±0.08a	1.08±0.07a	1.09±0.04a	1.08±0.04a	0.0105
Survival(%)	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	98.9±1.9a	98.9±1.9a	100.0±0.0a	0.5548

Two-way ANOVA (P value summary)

	Lysine supplementation	Fishmeal replacement	Interaction
Weight gain	0.0056	0.0014	0.4297
FCR	0.0611	0.0134	0.3085
Survival	0.1765	0.5847	0.5848

*Means in the same row that do not share a common letter differ significantly (P < 0.05).

Table 6. Analyses of whole trout body composition (%, Mean ± S.D., n = 3)*

Item	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	P value
					Fishmeal replacement level (%)					
	0	25	50	75	100	25	50	75	100	
					Dietary lysine level (%)					
	2.07	1.99	1.92	1.85	1.77	2.07	2.07	2.07	2.07	
Moisture	72.6±0.1ab	72.2±0.2a	72.8±0.3ab	73.7±1.3b	73.4±0.6b	73.0±0.5ab	72.9±0.1ab	73.5±0.6b	73.0±0.7ab	0.0025
CP	15.1±0.5a	14.7±0.2b	14.9±0.2ab	14.3±0.5b	14.1±0.2b	14.7±0.4b	14.7±0.3b	14.5±0.4b	14.4±0.2b	0.0002
Fat	9.8±0.5a	10.2±0.2a	9.2±0.3ab	9.4±1.1ab	9.0±0.6b	10.3±0.6a	10.8±0.4a	10.2±0.5a	10.6±0.8a	<0.0001
Ash	2.4±0.3a	2.6±0.3a	3.0±0.3b	2.3±0.1a	2.4±0.2a	3.1±0.3b	2.5±0.3a	2.2±0.2a	2.3±0.2a	<0.0001

Two-way ANOVA (P value summary)

	Lysine supplementation	Fishmeal replacement	Interaction
Moisture	0.6178	0.0040	0.1200
CP	0.5658	0.0004	0.1590
Fat	0.0001	0.2119	0.0204
Ash	0.4817	0.0001	0.0001

*Means in the same row that do not share a common letter differ significantly (P < 0.05).

Table 7. Composition of the test diets for experiment #3 (%, as fed basis)[1]

Ingredients	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10
Herring meal	35.0	5.85	2.93	0.0	5.85	2.93	0.0	5.85	2.93	0.0
Refined-PBM	0.0	29.15	32.07	35.0	29.15	32.07	35.0	29.15	32.07	35.0
Whole wheat	27.9	27.54	27.51	27.48	27.44	27.41	27.38	27.34	27.31	27.28
Corn gluten	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0
Fish oil	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7
Vitamin C	0.3	0.3	0.3	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	0.5	0.5	0.5
TM salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-lysine	0.0	0.25	0.27	0.29	0.25	0.27	0.29	0.25	0.27	0.29
DL-methionine	0.0	0.11	0.12	0.13	0.21	0.22	0.23	0.31	0.32	0.33

Calculated analyses (%, as fed basis)

	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10
Crude protein	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0
DE (kcal/g diet)	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Lysine	2.07	2.07	2.07	2.07	2.07	2.07	2.07	2.07	2.07	2.07
Methionine	0.73	0.73	0.73	0.73	0.83	0.83	0.83	0.93	0.93	0.93

Chemical analyses (%, as fed basis, n = 2)

	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10
Moisture	9.1	8.9	8.9	8.8	9.3	9.0	9.2	9.3	9.0	9.3
CP	44.9	44.9	45.4	45.5	45.3	45.9	44.8	46.1	45.5	45.2
Fat	16.3	17.4	17.2	17.3	17.2	17.2	17.1	17.0	16.9	16.8
Ash	4.1	4.4	4.5	4.4	4.5	4.6	4.6	4.5	4.5	4.7

[1]Origin of ingredients is as follows: Herring meal, whole wheat, corn gluten, choline chloride, trace mineral salt, and vitamin premix were from Nelson & Sons, Inc., Murray, Utah; Refined-PBM was from Georgia Feed Products Co., Cuthbert, GA; Fish oil and vitamin C were from Rangen, Inc., Buhl, Idaho; L-lysine was from Heartland Lysine Inc., Chicago, Illinois; DL-methionine was from Sigma Chemical Co., St. Louis, MO.

Table 8. Initial weight, final weight, weight gain, feed conversion ratio, and survival of rainbow trout for eight weeks (n = 3)[1]

	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10	P value
Fishmeal replacement level (%)	0	83.3	91.6	100	83.3	91.6	100	83.3	91.6	100	
Dietary methionine level (%)	0.73	0.73	0.73	0.73	0.83	0.83	0.83	0.93	0.93	0.93	
Item											
Initial (g)	13.9±0.4	13.8±0.4	13.9±0.3	14.0±0.2	13.9±0.5	13.9±0.5	13.9±0.5	13.9±0.3	13.9±0.6	14.0±0.9	1.0000
Final (g)	97.1±5.2	99.4±4.0	102.7±6.2	97.5±7.3	94.8±7.2	100.1±1.1	103.5±6.9	98.6±12.9	96.2±13.5	96.4±13.9	0.9677
WG (g)	83.3±4.9	85.6±3.8	88.9±6.2	83.5±7.4	81.0±7.3	86.2±0.6	82.3±13.0	87.2±12.1	83.8±7.8	83.0±9.3	0.9533
FCR (g/g)	1.01±0.06	1.02±0.01	1.07±0.01	1.09±0.04	1.12±0.07	1.07±0.02	1.06±0.07	1.05±0.12	1.11±0.16	1.15±0.12	0.6036
Survival (%)	100.0±0.0	99.0±0.0	98.0±0.0	99.0±0.0	97.7±0.1	96.7±0.0	97.7±0.1	98.0±0.0	100.0±0.0	96.7±0.1	0.7599

Two-way ANOVA (P value summary)

	Methionine supplementation	Fishmeal replacement	Interaction
Weight gain	0.7466	0.8277	0.7733
FCR	0.6352	0.6689	0.5290
Survival	0.1765	0.5847	0.5848

[1]No significant differences were found among fish fed different diets in terms of initial weight, final weight, weight gain, FCR, and survival (P > 0.05).

Table 9. Analyses of whole trout body composition (%, Mean ± S.D., n = 3)[1]

Item	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10	P value
Fishmeal replacement level (%)	0	83.3	91.6	100	83.3	91.6	100	83.3	91.6	100	
Dietary methionine level (%)	0.73	0.73	0.73	0.73	0.83	0.83	0.83	0.93	0.93	0.93	
Moisture	70.6±0.4	71.1±0.5	70.1±0.4	69.8±0.9	70.3±1.4	69.9±0.4	70.8±0.6	70.6±1.4	69.9±0.8	70.4±0.9	0.6670
CP	16.2±0.3a	15.4±0.3b	15.4±0.3b	15.1±0.1b	15.2±0.5b	15.2±0.1b	15.1±0.1b	15.1±0.3b	15.4±0.3b	15.1±0.3b	0.0015
Fat	10.5±0.5	10.5±0.8	11.4±0.6	11.7±1.1	11.3±1.6	12.9±0.4	11.3±0.8	11.3±1.5	11.9±0.9	11.6±0.6	0.1813
Ash	2.7±0.2	2.4±0.1	2.4±0.1	2.8±0.3	2.8±0.4	2.3±0.1	2.4±0.1	2.6±0.2	2.8±0.3	2.5±0.2	0.0602

Two-way ANOVA (P value summary)

	Methionine supplementation	Fishmeal replacement	Interaction
Moisture	0.9920	0.2807	0.5567
CP	0.5902	0.2082	0.7049
Fat	0.4122	0.0998	0.5466
Ash	0.5450	0.5452	0.0154

[1]Means in the same row that do not share a common letter differ significantly ($P < 0.05$).

Table 10. Composition of the test diets for Experiment #4 (%, as is basis)[1]

Ingredients	D 1	D 2	D 3	D 4	D 5	D 6	D 7
Herring meal	35.0	0.0	0.0	0.0	0.0	0.0	0.0
Refined-PBM	0.0	35.0	35.0	35.0	35.0	35.0	35.0
Whole wheat	27.9	27.48	27.85	27.83	28.0	27.98	28.15
Corn gluten	18.0	18.0	17.7	17.6	17.3	17.2	16.9
Fish oil	16.7	16.7	16.5	16.5	16.5	16.5	16.5
Vitamin 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
TM salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-lysine	0.0	0.29	0.29	0.29	0.29	0.29	0.29
DL-methionine	0.0	0.13	0.13	0.13	0.13	0.13	0.13
L-histidine	0.0	0.0	0.13	0.25	0.38	0.50	0.63
Calculated analyses (%, as fed basis)							
Crude protein	45.0	45.0	45.0	45.0	45.0	45.0	45.0
DE (kcal/g diet)	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Lysine	2.07	2.07	2.07	2.07	2.07	2.07	2.07
Methionine	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Histidine	0.91	0.66	0.785	0.91	1.035	1.16	1.285
Chemical analyses (%, as fed basis, n = 2)							
Moisture	9.3	9.0	8.9	8.7	8.6	8.3	7.9
CP	46.2	45.5	45.7	46.0	45.2	45.8	45.7
Fat	17.1	17.5	17.6	17.6	17.5	17.7	17.8
Ash	5.3	5.3	4.7	4.8	4.6	4.6	4.7

[1]Origin of ingredients is as follows: Herring meal, whole wheat, corn gluten, choline chloride, trace mineral salt, and vitamin premix were from Nelson & Sons, Inc., Murray, Utah; Refined-PBM was from Georgia Feed Products Co., Cuthbert, GA; Fish oil and vitamin C were from Rangen, Inc., Buhl, Idaho; L-lysine was from Heartland Lysine Inc., Chicago, Illinois; DL-methionine and L-histidine were from Sigma Chemical Co., St. Louis, MO.

Table 11. Initial weight, final weight, weight gain, feed conversion ratio, and survival of rainbow trout for six weeks (n = 3)[1]

| Item | Dietary histidine level (%) | | | | | | | P value |
	D 1	D 2	D 3	D 4	D 5	D 6	D 7	
	0.91	0.66	0.785	0.91	1.035	1.16	1.285	
Initial weight (g)	19.2±0.7	19.2±0.7	18.9±0.9	19.0±0.2	19.2±0.2	19.2±0.5	19.2±0.5	0.9849
Final weight (g)	79.6±4.6	78.2±2.3	73.1±1.9	75.4±4.1	74.5±2.8	76.0±0.5	76.7±1.1	0.1691
Weight gain (g)	60.5±4.0	59.1±1.6	54.2±1.3	56.5±4.0	55.4±3.0	56.8±0.3	57.5±1.4	0.1358
FCR (g/g)	0.98±0.06	1.04±0.03	1.12±0.05	1.10±0.09	1.09±0.01	1.03±0.02	1.05±0.02	0.0529
Survival (%)	100.0±0.0	98.9±1.9	97.8±1.9	96.7±5.8	98.8±1.9	94.4±1.9	100.0±0.0	0.5548

[1]No significant differences were found among fish fed different diets in terms of initial weight, final weight, weight gain, FCR, and survival (P > 0.05).

Table 12. Analyses of whole trout body composition (n = 3)[1]

| Item | Dietary histidine level (%) | | | | | | | P value |
	D 1	D 2	D 3	D 4	D 5	D 6	D 7	
	0.91	0.66	0.785	0.91	1.035	1.16	1.285	
Moisture	71.4±0.1a	70.9±0.1a	71.1±0.4a	71.7±0.6a	72.3±1.1a	71.7±0.5a	71.8±0.5a	0.1318
CP	15.6±0.5a	14.6±0.4b	14.4±0.0b	14.6±0.1b	14.3±0.2b	14.4±0.4b	14.3±0.2b	0.0014
Fat	11.8±0.0a	12.9±0.3a	12.7±0.5a	12.1±0.8a	11.5±0.9a	12.0±0.4a	12.1±0.5a	0.0810
Ash	2.3±0.2a	2.3±0.1a	2.5±0.2a	2.7±0.1a	2.3±0.2a	2.3±0.1a	2.5±0.2a	0.0573

[1]Means in the same row that do not share a common letter differ significantly (P < 0.05).

Literature Cited

Aksnes, A. and J. Opstvedt. 1998. Content of digestible energy in fish feed ingredients determined by the ingredient-substitution method. Aquaculture 161:45-53.

Alexis, M.N., E. Papaparaskeva-Papoutsoglou, and V. Theochari. 1985. Formulation of practical diets for rainbow trout (Salmo gairdneri) made by partial or complete substitution of fishmeal by poultry by products and certain plant by products. Aquaculture 50:61-73.

Andrews, J.W., Page, J.W., Murray, M.W., 1977. Supplementation of a semipurified casein diet for catfish with free amino acids and gelatin. J. Nutr. 107(7), 1153-1156.

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis, 15th edition. Association of Official Analytical Chemists, Washington, DC.

Austreng, E., A. Skrede, and A. Eldegard. 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agriculture Scandinavica 12:119-126.

Bai, S.C, Gatlin, D.M., 1994. Effects of L-Lysine supplementation of diets with different protein levels and sources on channel catfish, Ictalurus punctatus (Rafinesque). Aquaculture and Fisheries Management 25, 465-474.

Barlow, S. 1989. Fishmeal - World outlook to the year 2000. Fish Farmer October:40-43.

Barlow, S. 1997. Fishmeal - Supply limits demand. Fish Technology I:34-37.

Bureau, D.P., A.M. Harris, and C.Y. Cho. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). Aquaculture 180:345-358.

Cheng, Z.J., 2000. Effects of rendered poultry by-product and feather meals based diets on biological performance of the Pacific white shrimp (Litopenaeus vannamei). Ph.D. dissertation. Kansas State University, Manhattan, Kansas, USA.

Cho, C.Y. and S.J. Slinger. 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. Pages 239-247 In J.E. Halver and K. Tiews. Editors. Finfish nutrition and fish feed technology, Volume 2. Heinemann, Berlin, Germany.

Cho, C.Y., S.J. Slinger, and H.S. Bayley. 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. Vol. 73B, No. 1. pp. 25-41.

Dong, F.M., R.W. Hardy, N.F. Haard, F.T. Barrows, B.A. Rasco, W.T. Fairgrieve, and I.P. Forster. 1993. Chemical composition and protein digestibility of poultry by-product meals for salmonid diets. Aquaculture 116:149-158.

El-Dahhar, A.A, El-Shazly, K., 1993. Effect of essential amino acids (methionine and lysine) and treated oil in fish diet on growth performance and feed utilization of Nile tilapia, Tilapia nilotica (L.). Aquaculture and Fisheries Management 24, 731-739.

Ellis, R.W. and R.R. Smith. 1984. Determining fat digestibility in trout using a metabolic chamber. Progressive Fish-Culturist 46:116-119.

El-Sayed, A.M. 1994. Evaluation of soybean meal, spirulina meal and chicken offal meal as protein sources for silver seabream (Rhabdosargus sarba) fingerlings. Aquaculture 127:169-176.

Fernandez, C.H, Sukumaran, N.S., 1995. Effect of crystalline amino acids on the growth performance of Indian white shrimp, Penaeus indicus (Milne edwards). J. Aqua. Trop. 10, 245-260.

Fowler, L.G. 1991. Poultry by product meal as a dietary protein source during parr-smolt transformation in fall chinook salmon diets. Aquaculture 99:309-321.

Fox, J.M., Lawrence, A.L., Chan, E.L., 1995. Dietary requirement for lysine by juvenile Penaeus vannamei using intact and free amino acid sources. Aquaculture 131, 279-290.

Hardy, R.W., K.D. Shearer, and J. Spinelli. 1984. The nutritional properties of co-dried fish silage in rainbow trout (Salmo gairdneri) dry diets. Aquaculture 38:35-44.

Hardy, R.W. 1989. Diet preparation. Pages 473-546. In J.E. Halver, editor, Fish Nutrition, Second Edition. Academic Press, New York, NY, USA.

Hardy, R.W. and G. W. Kissil. 1996. Trends in world aquaculture production: issues limiting increased production. Pages 107-120. In Lyons, T.P. and K.A. Jacques, editors. Proceedings of the 12th annual symposium on biotechnology in the feed industry. Nottingham University Press, Nottingham, U.K.

Hardy, R.W. 1999. Aquaculture's rapid growth requirements for alternate protein sources. Feed Management 50: 25-28.

Higgs, D. A., B.S. Dosanjh, A.F. Prendergast, R.M. Beames, R.W. Hardy, W. Riley, and G. Deacon. 1995. Use of rapeseed/canola protein products in finfish diets. Pages 130-156. In Lim, C.E. and D. J. Sessa, editors. Nutrition and Utilization Technology in Aquaculture. AOCS Press, Champaign, Illinois, USA.

Ketola, H.G., 1983. Requirement for dietary lysine and arginine by fry of rainbow trout. J. Anim. Sci. 56, 101-107.

Ketola, H.G. and B.F. Harland. 1993. Influence of phosphorus in rainbow trout diets on phosphorus discharges in effluent water. Transactions of the American Fisheries Society 122:1120-1126.

Kim, K.I., Kayes, T.B., Amundson, C.H., 1992. Requirements for sulfur amino acids and utilization of D-methionine by rainbow trout (Oncorhynchus mykiss). Aquaculture 101, 95-103.

Kim, K.I., Kayes, T.B., Amundson, C.H., 1992. Requirements for lysine and arginine by rainbow trout (Oncorhynchus mykiss). Aquaculture 106, 333-344.

Lim, C. 1993. Effect of dietary pH on amino acid utilization by shrimp (Penaeus vannamei). Aquaculture 114, 293-303.

Marcus, C.S. and F.W. Lengemann. 1962. Use of radioyttrium to study food movement in the small intestine of the rat. Journal of Nutrition 76:179-182.

Maynard, L.A. and J.K. Loosli. 1969. Animal Nutrition, McGraw-Hill, New York, USA.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press, Washington, D.C., USA.

Nengas, I., M.N. Alexis, and S.J. Davis. 1999. High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream Sparus aurata L. Aquaculture 179:13-23.

Ogino, C. 1980. Requirements of carp and rainbow trout for essential amino acids. Bull. Jpn. Soc. Sci. Fish., 46, 171-174.

Pfeffer, E., S. Kinsinger, and M. Rodehutscord. 1995. Influence of the proportion of poultry slaughter by-product and of untreated or hydrothermally treated legume seeds in diets for rainbow trout, Oncorhynchus mykiss (Walbaum), on apparent digestibilities of their energy and organic compounds. Aquaculture Nutrition 1:111-117.

Pike, I. 1998. Fishmeal outlook. International Aqua Feed, January/February: 5-8.

Rawles, S.D. and D.M. Gatlin III. 2000. Nutrient digestibility of common feedstuffs in extruded diets for sunshine bass Morone chrysops x M. saxatilis. Journal of the World Aquaculture Society 31:570-579.

Rodehutscord, M., Jacobs, S., Pack, M., and Pfeffer, E., 1994. Response of rainbow trout (Oncorhynchus mykiss) growing from 50 to 150 g to supplements of DL-Methionine in a semipurified diet containing low or high levels of cystine. J. Nutr. 125:964-969.

Rodehutscord, M., Borchert, F., Gregus, Z., Pfeffer, E., 2000 a. Availability and utilisation of free lysine in rainbow trout (Oncorhynchus mykiss). 1. Effect of dietary crude protein level. Aquaculture 187, 163-176.

Rodehutscord, M., Borchert, F., Gregus, Z., Pack, M., Pfeffer, E., 2000 b. Availability and utilisation of free lysine in rainbow trout (Oncorhynchus mykiss). 2. Comparison of L-lysine.HCL and L-lysine sulphate. Aquaculture 187, 177-183.

Sugiura, S.H., F.M. Dong, C.K. Rathbone, and R.W. Hardy. 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159:177-202.

Sugiura, S.H., F.M. Dong, and R.W. Hardy. 2000. A new approach to estimating the minimum dietary requirement of phosphorus for large rainbow trout based on nonfecal excretions of phosphorus and nitrogen. Journal of Nutrition 130:865-872.

Tan, R.K.H., Dominy, W.G., 1997. Commercial pelleting of crustacean feeds. pp. 520-549. In: D'Abramo, L.R., D.E. Conklin & D.M. Akiyama (Eds), Crustacean Nutrition. Advances in World Aquaculture, 6: World Aquaculture Society, Baton Rouge, USA.

Viola, S. and G. Zohar. 1984. Nutrition studies with market size hybrids of tilapia (Oreochromis) in intensive culture. Bamidgeh 36:3-15.

Viola, S., Lahav, E., 1991. Effects of lysine supplementation in practical carp feeds on total protein sparing and reduction of pollution. The Israeli journal of Aquaculture-Bamidgeh 43(3), 112-118.

Viola, S., Lahav, E., Angeoni, H., 1992. Reduction of feed protein levels and of nitrogenous N-excretions by lysine supplementation in intensive carp culture. Aquat. Living Resour., 5, 277-285.

Walton, M.J., Cowey, C.B., Adron, J.W., 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J. Nutr., 112, 1525-1535.

Walton, M.J., Cowey, C.B., Adron, J.W., 1984. The effect of dietary lysine levels on growth and metabolism of rainbow trout (Salmo gairdneri). Br. J. Nutr., 52, 115-122.