Beta Hatch

Title: Evaluation of meal worm protein digestibility and efficacy as a fish meal replacement for rainbow trout

Subcontract: University of Idaho, Aquaculture Research Institute

Sponsor:
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Estimate: $63,748

Statement of Work:

The overall aim of the proposed project is to evaluate Beta Hatch defatted mealworms (DMW) fed to rainbow trout for use in commercial aquaculture.

The specific objectives of this study are to:

- To evaluate the diet, nutrient and energy digestibility of defatted mealworms (DMW) fed to rainbow trout as part of a complete feed.
- To evaluate growth performance and health of rainbow trout fed five diets containing graded levels of DMW (0, 25, 50, 75, 100% fishmeal replacement).

Work Plan and Research Approach

The use of experimental animals will be according to the scientific research and animal care and use protocols of the University of Idaho, which comply with all relevant local and/or international animal welfare laws, guidelines and policies. Institutional Animal Care and Use protocol number and study approval memo will be provided upon approval and prior to start of the study.

Experimental procedures for fish feeding trial

Beta Hatch will supply the DMW for the digestibility trial and production of the five dietary treatments to be used in a feeding trial with rainbow trout. Preliminary proximate composition of DMW ins shown in Table 1. All fish work will be performed at the University of Idaho Hagerman Fish Culture Experiment Station, Hagerman, Idaho. A diagram of tanks and treatments will be provided upon assignment and in the final report. The project will consist of a digestibility trial and a growth trial using rainbow trout. The trials will be preceded by chemical analysis of feeds and protein ingredients, including proximate composition, energy content, and amino acid composition.

***Trout digestibility trial*:**

In vivo digestibility of DMW will be determined following feeding to rainbow trout. A reference diet (Table 2) containing practical ingredients and 0.1% indigestible inert marker (yttrium oxide) will be prepared, with which a test diet containing 30% test ingredient and 70% reference diet mash on dry-matter basis will prepared. Samples of DMW ingredient, diet and respective feces will be analyzed for proximate composition, energy and amino acid content. Digestibility of macro-nutrients, energy and amino acids will be calculated according to standard methods.

Rainbow trout sourced as eggs from a commercial supplier and reared to approximately 200 ± 20 g average weight will be used in this study. At stocking, groups of 30 fish will be placed into 8 145-L tanks supplied with constant temperature (15°C) spring water. The experimental diet and the control diet will be fed in replicate to four tanks of fish. Photoperiod will be maintained at a constant 14 h light: 10 h dark with timer-controlled fluorescent lights. Fish will be fed their respective diets twice daily to apparent satiation for one week. Apparent satiation will be achieved by offering small quantities of feed to the fish by hand until feeding activity stopped. During week two, fish in each tank will be lightly anaesthetized using tricaine methanesulfonate (MS-222, 100 mg L^{-1}, buffered to pH 7.0), removed from water for 20-30 seconds, and feces gently expelled using light pressure on the abdomen near the vent, a process called stripping. Feces will be collected in aluminum pans and pooled by tank. Fish will be stripped at intervals of 3-4 days until sufficient fecal samples are obtained. Feces will be frozen between stripping collections. Fecal samples will be analyzed for marker and nutrient composition. Apparent digestibility coefficients (ADC) will be calculated for dry matter, protein, lipid, energy, and amino acids.

Sample collection and analysis:

Feed and fecal samples will be finely ground by mortar and pestle. Proximate composition of ingredients, feed and fecal samples will be determined using AOAC (1990) procedures. Briefly, samples will be dried in a convection oven at 105°C for 12 h to determine moisture level. Samples will be analyzed for crude protein (total nitrogen × 6.25) using the combustion method (AOAC 990.03) with a nitrogen determinator (Elementar nitrogen analyzer, Ronkonkoma, NY). Crude lipid will be analyzed using an ANKOM XT15 extractor (AOCS Am 5-04; ANKOM Technology, Macedon, NY) with petroleum ether as the extracting solvent, and ash by incineration at 550 °C in a muffle furnace for 5 hr (AOAC 942.05). Energy content of samples will be determined using an isoperibol bomb calorimeter (Parr 6300, Parr Instrument Company Inc., Moline, IL). Analyses of amino acids in samples will be conducted using a BioChrom 30+ amino acid analyzer (AOAC 994.12). Analyses of minerals including yttrium will be subcontracted to the Department of Agricultural Chemistry, Louisiana State University Agricultural Center, Baton Rouge, LA, using inductively coupled plasma (ICP; AOAC 985.01). All analyses will be done in duplicate.

Analytical Labs	Analyses
University of Idaho Aquaculture Research Institute Nutritional Service Center 3059F National Fish Hatchery Road Hagerman, Idaho 83301	Proximate composition, Energy, Amino Acids
Agricultural Chemistry Laboratory Department of Agricultural Chemistry Louisiana State University Agricultural Center Baton Rouge, LA 70803	Minerals, including yttrium
Fish Biologist-Histology Dept. Bozeman Fish Health Center USFWS- Dept. of Interior 1805 South 22nd Avenue, Suite #1 Bozeman, MT 59718-7069	Histology

Calculations:
ADC of diets and ingredients, for dry matter, protein, lipid, amino acids and energy will be calculated using the following formula:

- ADC diet = 1 − [(F/D) × (Di/Fi)]
  - where D = % nutrient of diet, F = % nutrient of feces,
  - Di = % digestion indicator of diet, Fi = % digestion indicator of feces
  - ADC ingredient = ADCT + [((1 − s) DR)/s DI] × (ADCT − ADCR)
  - where ADCT = ADC of test diet, ADCR = ADC of reference diet, DR = % nutrient of reference diet,
  - DI = % nutrient of test ingredient, s = proportion of test ingredient in test diet (0.3)

Growth trial:
The trout growth trial will consist of the 5 dietary treatment groups (0, 25, 50, 75, 100% fishmeal replacement with DMW), each fed in quadruplicate to tanks of juvenile rainbow trout for 12 weeks. The diets (Table 3) will meet be isonitrogenous (47% crude protein), isoenergetic (5000 kcal/kg), and meet or exceed the nutrient requirements for rainbow trout (NRC, 2011). Final formulation will be based on the actual nutrient profile of the DMW ingredient. Experimental feeds will be produced by cooking extrusion at the Bozeman Fish Technology Center, Bozeman, MT. Feed samples will be analyzed for proximate composition and energy using the standard protocols described above. The personnel responsible for the day-to-day care and management of the animals and for making and recording observations will be blinded to the experimental treatments. The final study report will include information regarding the extent of blinding (for example, monitor, investigator, caretakers), blinding methods and procedures, and a list of personnel with access to treatment codes and the rationale for the access.

Rainbow trout fry hatched from eggs purchased from a commercial source and reared to approximately 10 ± 2 g will be used. Forty fish will be stocked into each of twenty 145-L tanks supplied with 8 L min^{-1} of constant temperature (15°C), gravity-fed spring water and acclimated for 1 week on a standard commercial trout feed (Classic Trout, Skretting USA, Tooele, UT). Each diet will be fed by hand to four replicate tanks of fish to apparent satiation. Apparent satiation will be achieved by offering small quantities of feed to the fish by hand until feeding activity stopped. A completely randomized design will be used to assign diets to account for any tank position effects. The fish will be fed three times per day, six days per week. Photoperiod will be held constant at 14 h light: 10 h dark with electric timers. Tanks will be cleaned daily; any mortality will be recorded, and dead fish removed. Feed intake will be recorded. Fish will be weighed and counted every 3 weeks for the duration of the study (12 weeks). At the end of the study (day 84), length and weight will be measured for all fish, and 4 fish per tank fish will be sacrificed for analysis of whole-body and fillet proximate composition, calculation of hepatosomatic index (HIS = 100 x liver weight/body weight), and collection of blood for blood chemistry using a VetScan i-STAT® 1 handheld analyzer (Abaxis products, Union City, CA) for hematocrit, hemoglobin, ionized chloride, glucose, sodium, potassium, pH, pCO_2, HCO_3, TCO_2, base excess, PO_2, and sO_2. Another 4 fish per tank will be necropsied, and distal intestine, liver, and trunk kidney will be sampled for histological analysis. Growth and feed utilization will be evaluated using conventional calculated indices (Hardy and Barrows, 2002), such as specific growth rate, thermal growth unit coefficient, feed conversion ratio, protein retention efficiency, and proximate composition.

Medication and/or vaccination during acclimation period:
Fish will not be medicated or vaccinated during the acclimation period.

Provisions for removal from study and necropsy:
Abnormal behavior, i.e., erratic swimming, loss of equilibrium or death, will be cause for removal from the study. Fish removed from the study or die during the treatment period will be necropsied an effort to determine cause of death. Dead fish weight, date of mortality, and necropsy observations will be recorded and reported.

Histological analysis: The Bozeman Fish Health Center (Bozeman, MT) will be subcontracted to fix distal intestine, liver, and trunk kidney samples following standard histological techniques used by the US Fish and Wildlife Service (Mumford, 2004) and histopathological evaluation following staining with hematoxylin and eosin.

Statistical analyses: Tank means will be used as units of observation for statistical analysis. Fish growth performance and body composition and nutrient digestibility will be tested for normality and homogeneity of variance prior to one-way ANOVA. If required, data will be transformed to achieve normal distribution. If significant differences are found, data will be subjected to Tukey's HSD test to separate the means at a significance level of $P<0.05$. Nonparametric procedures may be used rather than ANOVA if the data are not distributed normally with homogenous variance across control and test groups.

Final Report: Within (90) days after completion of the animal and laboratory work, the PI will submit to Beta Hatch a final written report containing all and any findings, analysis and conclusions, including the raw data as well as statistical software lists and logs for the statistical analyses conducted.

Table 1. Proximate composition of defatted mealworm (DMW) meal (MidWest Laboratories, Omaha, Nebraska), based on preliminary analysis prior to final ingredient processing.

Ingredient	**Inclusion level**
Moisture	4.2
Crude Protein	72.4
Crude Fat	5.3
Fiber (acid detergent)	11.4
Ash	6.6

Table 2. Ingredient composition of reference mash (%, as-fed basis)

Ingredient	**Inclusion level**
Fishmeal, sardine[a]	33.0
Soy protein concentrate[b]	13.9
Corn protein concentrate[c]	10.0
Wheat flour[a]	18.0
Wheat gluten meal[a]	7.10
Dicalcium phosphate[a]	1.20
Choline chloride (60%)[a]	0.60
Vitamin C (Stay C, 35%)[a]	0.20
Vitamin premix, ARS 702[d]	0.80
Trace mineral mix, Trouw Nutrition[e]	0.10
Fish oil, Alaska pollock[f]	15.0
Yttrium oxide[g]	0.10
Total	**100**

[a] Rangen Inc., Buhl, ID, USA
[b] Profine VF, The Solae Company, St. Louis, MO, USA
[c] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.
[d] US Fish and Wildlife Service Trace Mineral Premix #3. It supplied the following (mg/kg diet): Zn (as $ZnSO_4.7H_2O$), 75; Mn (as $MnSO_4$), 20; Cu (as $CuSO_4.5H_2O$), 1.54; I (as KIO_3), 10
[e] Vitamin premix supplied the following per kg diet: vitamin A, 2.4 mg; vitamin D, 0.15 mg; vitamin E, 267 mg; vitamin K as menadione sodium bisulfite, 20 µg; thiamin as thiamin mononitrate, 32 mg; riboflavin, 64 mg; pyridoxine as pyridoxine-HCl, 64 mg; pantothenic acid as Ca-d-pantothenate, 192 mg; niacin as nicotinic acid, 240 mg; biotin, 0.56 mg; folic acid, 12 mg; vitamin B_{12}, 50 µg; and inositol as meso-inositol, 400 mg.
[f] Skretting USA, Tooele, UT, USA.
[g] Sigma Aldrich, St. Louis MQ, USA.

Table 3. Approximate ingredient and nutrient composition of the experimental diets replacing fishmeal with mealworm protein meal (DMW) fed to rainbow trout juveniles over a 12-week growth trial (%, as-fed basis). Actual composition will be adjusted following analysis on ingredient nutrient content.

Ingredients	Diet 1 FM 40 / DMW 0	Diet 2 FM 30 / DMW 10	Diet 3 FM 20 / DMW 20	Diet 4 FM 10 / DMW 30	Diet 5 FM 0 / DMW 40
Fish meal, sardine[a]	40	30	20	10	0
Mealworm protein (DMW)	0	10	20	30	40
Soybean meal[a]	9.5	11.5	10	8	6
Wheat gluten meal[a]	7	7	7	7	7
Corn protein conc.[c]	10	8	7	6	5
L-lysine HCl[g]	0	0	0.53	1.00	1.65
DL-methionine[g]	0	0	0	0.12	0.23
Wheat flour[a]	16.5	15.4	16.5	17.9	19.2
Dicalcium phosphate[a]	0	1.42	2.52	3.62	4.72
Trace mineral mix, Trouw[d]	0.1	0.1	0.1	0.1	0.1
Vitamin Premix, ARS 702[e]	1	1	1	1	1
Choline chloride (60%)[a]	0.6	0.6	0.6	0.6	0.6
Stay C (35%) vitami [a]	0.2	0.2	0.2	0.2	0.2
Fish oil[f]	15.1	14.8	14.6	14.5	14.3
Nutrients (% as-fed basis)					
Dry Matter	94.0	94.1	94.4	94.7	95.0
Protein	47.5	47.6	47.6	47.5	47.6
Fat	20.0	19.8	19.8	19.8	19.8
Ash	7.89	7.71	7.13	6.53	5.93
Gross energy (kcal/kg)	5020	5002	5017	5034	5053

[a] Rangen Inc., Buhl, ID, USA
[b] Profine VF, The Solae Company, St. Louis, MO, USA
[c] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.
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[f] Skretting USA, Tooele, UT, USA.
[g] Sigma Aldrich, St. Louis MQ, USA.

References:

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, pp. 1298.

Hardy, R.W. and Barrows, F.T. 2002. Diet Formulation and Manufacturing, in: Halver, J.E. and Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Academic Press, San Diego, CA, USA, pp. 505–600.

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NRC (National Research Council). 2011. Nutrient requirements of fish and shrimp. National Academy Press, Washington D.C., pp. 376.