Director's Digest
FATS AND PROTEINS RESEARCH FOUNDATION, INC.

DR. GARY G. PEARL D.V.M.
Director Technical Services
16551 Old Colonial Road
Bloomington, Illinois 61704
Telephone: 309-829-7744 FAX: 309-829-5147
<www.fprf.org>

#301

COMBINATIONS OF RENDERED ANIMAL PROTEIN INGREDIENTS AS PROTEIN SOURCES FOR SALMONID DIETS (97B-3)

Dominique P. Bureau
Fish Nutrition Research Laboratory, Department of Animal and Poultry Science
University of Guelph, Guelph, Ontario, N1G 2W1 Canada

SCIENTIFIC ABSTRACT

Two growth trials were conducted using spray-dried blood meal (BM), feather meal (FEM), meat and bone meal (MBM), poultry by-products meal (PBM) as major protein sources in the diet of rainbow trout. In the first trial, increasing levels of BM (6, 12%) or PBM (0, 10, 20, 30%) replaced fish meal and corn gluten meal in the diet. PBM and BM provided up to 40% of the total digestible protein of the diet. For the second trial, eight diets were formulated to contain following combinations: FEM+MBM, FEM+PBM or MBM+PBM replacing half the fish meal and the totality of the soybean meal (control containing 40% fish meal and 13% soybean meal, experimental diets containing 20% fish meal and no soybean meal). Rendered animal protein ingredients provided about 2/3 of the total digestible protein of the diet in this trial. Some of the experimental diets were supplemented with either L-lysine or DL-methionine. The fish were fed the experimental diets for 16 weeks in the first trial or 12 weeks in the second trial. Faecal samples were collected for the experimental diets of the first trial to measure apparent digestibility.

High growth rates and feed efficiencies were achieved for all diets in the two trials. Apparent digestibility coefficients (ADC) of protein and energy of the diets containing high levels of PBM and BM were high and confirm the high ADC measured for PBM and BM in previous studies in our laboratory. There were no significant differences in the growth rate, feed efficiency, nitrogen and energy retention efficiencies (N or E gain/digestible N or E intake) of the fish fed the eight diets in the first trial. This suggests that both BM and PBM have high nutritive values and that they can be used at fairly high levels in rainbow trout diets. In the second trial, growth rate of the fish fed the diet containing the FEM+PBM combination was not statistically different from the

growth rate of fish fed the control diet. Growth rates of the fish fed diets containing FEM+MBM or MBM+PBM combinations were significantly lower than that of the fish fed the control diet. Digestible nitrogen retention efficiencies of all the experimental diets were significantly lower than that of the control diet. Supplementation of diets with either L-lysine or DL-methionine had no effect on the performance of the fish. This suggests that diets containing high levels of combination of feather meals, poultry by-products meal, and meat and bone meals can support high growth performances and feed efficiencies. However, better definition of the nutritive value of these ingredients is required to be able to formulate diets that support performance levels matching those obtained with high fish meal diets.

INTRODUCTION

Feeds for salmonid fish species generally contain high levels of fish meal. Since fish meal is costly and of limited supply, it needs to be used more sparingly to improve the economic sustainability of salmonid aquaculture. The production of successful fish feed formulae which rely less on fish meal requires accurate information on the nutritive value of more economical protein sources.

Rendered animal protein ingredients are ingredients that have been used in fish feeds for decades but their use has been limited, or even avoided, for various reasons, such as poor digestibility and quality variability. Better manufacturing practices appear to be currently in use and recent studies have shown that blood meal, feather meal, meat and bone meal and poultry by-products produced in Canada are all relatively highly digestible for rainbow trout (Hajen et al., 1993; Sugiura et al., 1998; Bureau et al., 1999). Numerous studies have shown that these ingredients can be valuable protein sources when used individually in fish feeds (Tacon et al., 1985; Davies et al., 1989; Fowler, 1990; Pfeffer et al., 1994, 1995; Robaina et al., 1997; Bureau et al., 2000). Rendered animal protein ingredients often have complementary amino acid profiles (e.g. poultry by-product meal and feather meal). Combinations of these ingredients, along with supplementation of synthetic amino acids, may allow higher levels of incorporation of these ingredients in diet than possible when these are used individually.

The objectives of this research project were to: 1) evaluate the potential of rendered animal protein ingredients, individually or in combination, to be used as major protein sources in the diet of rainbow trout, and 2) evaluate the effect of crystalline amino acid supplementation on the nutritive value of diets with high levels of rendered animal protein ingredients.

EXPERIMENTAL PROCEDURES

2.1 Experimental diets

Two growth trials were conducted using spray-dried blood meal (BM), feather meal (FEM), meat and bone meal (MBM), poultry by-products meal (PBM) as major protein sources in the diet of rainbow trout. The rendered animal protein ingredients, with the exception of blood meal, were obtained from a local rendering plant (Rothsay Inc., Dundas, Ontario, Canada) long-time supporter of the Fats and Proteins Research Foundations (FPRF). The spray-dried whole blood meal (California Spray Dry Co., Stockton, CA, USA) and other ingredients were obtained from a local feed mills (Martin Mills, Elmira, Ontario, Canada). The chemical compositions of the ingredients used in the two trials (Table 1) were representative of that of similar ingredients on the market (Dale, 1995; Bureau et al., 1999).

All the experimental diets were formulated to contain all nutrients in excess of the levels recommended by NRC (1993), based on analysed or tabulated composition of these ingredients (NRC, 1993, Dale, 1995). The diets were mixed using a Hobart mixer (Hobart Ltd, Don Mills, Ontario, Canada) and pelleted using a laboratory steam pellet mill (California Pellet Mill Co., San Francisco, CA, USA). The feed pellets were subsequently dried in a current of air at room temperature for 24 h, sieved and stored at 4°C until used.

2.1.1 Trial #1

In the first trial, a series of diets was formulated to examine the nutritive value of spray-dried BM and PBM. Increasing levels of BM (6, 12%) or PBM (0, 10, 20, 30%) replaced fish meal and corn gluten meal in the diet of rainbow trout (Table 2). PBM and BM provided up to 40% of the total digestible protein of the diet. A few diets were also formulated to compare the nutritive value of whey powder to that of oat flour. This part of the study was to determine if this modification to the carbohydrate component of the experimental diets would affect their nutritive value.

2.1.2 Trial #2

For the second trial, eight experimental diets were formulated to be isoproteic and isoenergetic on a digestible basis (Table 3). These diets contained the following combinations: FEM+MBM, FEM+PBM or MBM+PBM replacing half the fish meal and the totality of the soybean meal (Table 3). Rendered animal protein ingredients provided about 2/3 of the total digestible protein of the diet in this trial. The diets containing FEM+MBM and FEM+PBM were supplemented with either L-lysine or DL-methionine. These two amino acids were predicted to potentially be the most limiting in these diets based on theoretical amino acid composition and estimates of apparent digestibility of crude protein of the ingredients, and the amino acid requirements of rainbow trout (NRC, 1993). A ninth diet (high-corn gluten meal diet), previously shown to be deficient in lysine, was also fed to groups of fish and served as a negative control.

2.2. Fish, Experimental Conditions and Feeding

Juvenile rainbow trout, *Oncorhynchus mykiss,* were held under artificial lighting with a photoperiod regime of 12 h light and 12 h dark. The fish were treated in accordance with the guidelines of the Canadian Council on Animal care (CCAC, 1984) and the University of Guelph Animal Care Committee.

The fish were reared in fibreglass tanks (60 l) supplied with a mixture of well water and city water at a rate of about 3 l min^{-1}. The tanks were individually aerated, and water temperature was controlled thermostatically at 15°C. Each experimental diet was allocated to three tanks and fed to the fish for a period of 16 weeks (trial #1) or 12 weeks (trial #2). The fish were hand-fed three times daily a predetermined ration calculated according to the method of Cho and Bureau (1998). All the diets were readily consumed by the fish and the predetermined amounts of feed appeared close to the maximum voluntary feed intake of the fish. Mortality and morbidity were checked daily. All fish were weighed every four weeks. Live weight gain, TGC, feed efficiency and percent mortality were calculated. After the first trial, three faecal samples were collected for each experimental diet to calculate apparent digestibility coefficients (ADC) for dry matter, crude protein, ash, and energy.

2.3 Sampling and chemical analyses

At the beginning of each trial, pooled sample of 25 fish (trial #1) and 10 fish (trial #2) were collected to serve as an initial carcass sample. At the end of each of the two trials, three fish were sampled at random from each tank and anaesthetised with t-amyl alcohol and killed with a cephalic blow. The five fish were pooled, autoclaved, ground into a homogeneous slurry, freeze-dried, reground and stored at -20°C until analysed. Diet, ingredients and carcass samples were analysed for dry matter (DM) and ash according to AOAC (1995), crude protein (%N x 6.25) by Kjedahl method using a Kjeltech autoanalyzer (Model 1030, Tecator, Höganäs, Sweden), total lipid according to the method of Bligh and Dyer (1959) and gross energy content of carcass samples was measured using an automated bomb calorimeter (Model 1272, Parr Instruments Inc., Moline, IL).

2.4 Statistical Analysis

Data were analysed as a complete random block design using the general linear model (GLM) of the SAS/STAT software (SAS, 1988). Means of dependent variables were compared using Tukey's honestly significant difference (HSD) test, with an $\alpha=0.05$ (Steel and Torrie, 1980). Pooled standard error of means (SEM) and minimum significant difference (HSD) according to Tukey's HSD are provided for each dependent parameter.

RESULTS AND DISCUSSION

Trial #1

Apparent digestibility coefficients (ADC) of protein and energy of the diets containing high levels of PBM and BM were high (Table 4). These results confirm the high ADC of protein and energy measured for PBM and BM in previous studies in our laboratory (Bureau et al., 1999).

High growth rates and feed efficiencies were achieved for all diets in the first trial (Table 5), lending much credibility to the results. There were no significant differences in the final weight, growth rate (expressed as thermal-unit growth coefficient, TGC), feed efficiency, nitrogen and energy retention efficiencies (N or E gain/digestible N or E intake) of the fish fed the eight diets in the first trial. (Table 5). These results indicate that BM and PBM are two ingredients with high nutritive value for rainbow trout. Significant levels of these ingredients can be used in the diet without effects on the performance of the fish.

Replacing oat flour (rich in digestible starch) or cellulose (indigestible carbohydrate) for whey powder had no effect on growth, feed efficiency and nitrogen and energy utilisation by the fish. This indicates that digestible carbohydrate (lactose or starch) contribute very little in terms of net energy when the diet is high in lipids and has a low digestible protein (DP) to digestible energy (DE) ratio (e.g. 20 g DP/MJ DE).

Trial #2

High growth rates and feed efficiencies were also achieved in the second trial. Final weight and growth rate of the fish fed the diet containing the FEM+PBM combination were not statistically different from the growth rate of fish fed the control diet (Table 6). Growth rates of the fish fed diets containing the FEM+MBM or MBM+PBM combinations were significantly lower than that of the fish fed the control diet (Table 6). Digestible nitrogen retention efficiencies of all the experimental diets were significantly lower than that of the control diet (Table 6). Supplementation of diets with either L-lysine or DL-methionine had no effect on the performance of the fish. Feeding the high corn gluten meal diet (diet 9) results in lower final weight, feed efficiency, and digestible nitrogen retention efficiencies than all the experimental diets (Table 6).

CONCLUSION

The results from this study show that BM, FEM, MBM and PBM can be very valuable protein sources for rainbow trout diets. Very significant levels of these ingredients, individually or in combination, can be used in fish feeds while maintaining high growth performance. Only minor fine-tuning of the diet formulation may all that is required to obtain levels of performance matching those of high fish meal diets. However, this may require better definition of the nutritive value of these ingredients (digestible amino acid). More attention should also be devoted to a better understanding of amino acid nutrition of fish.

ACKNOWLEDGMENTS

Sincere thanks to Greg Ardnt, Paula Azevedo and Ursula Wehkamp to their assistance, the Alma Aquaculture Research Station for donating the fish used in this study, and to Rothsay Inc. (Dundas, Ontario) and Martin Mills (Elmira, Ontario) for donating the ingredients used in this study. Special thanks to the Fats and Proteins Research Foundation (Bloomington, IL), the Ontario Ministry of Agriculture, Food and Rural Affair (OMAFRA) and the Ontario Ministry of Natural Resources (OMNR) for providing the financial support necessary to carry out this project.

REFERENCES

AOAC, 1995. Official Methods of Analysis of AOAC International. Vol. I. Agriculture Chemicals; Contaminants, Drugs. 16th ed. AOAC International, Arlington, VA.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

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

Bureau, D.P., Harris, A.M., Bevan, D.J., Simmons, L.A., Azevedo, P.A., and Cho, C.Y. 2000. Use of feather meals and meat and bone meals from different origins as protein sources for rainbow trout (*Oncorhynchus mykiss*) diets. Aquaculture 181, 281-291.

Canadian Council on Animal Care (CCAC). 1984. Guide to the Care and Use of Experimental Animals, Volume 2. CCAC, Ottawa, Ontario, Canada.

Cho, C.Y., Bureau, D.P., 1998. Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. Aquat. Living Resour. 11, 199-210.

Dale, N., 1995. Feedstuffs ingredient analysis table: 1995 edition. Feedstuffs, 1995 Reference Issue, July 19, 1995.

Davies, S.J., Williamson, J., Robinson, M., Bateson, R.I., 1989. Practical inclusion levels of common animal by-products in complete diets for tilapia (*Oreochromis mossambicus*, Peters). In: Takeda, M., Watanabe, T. (Eds.). The Current Status of Fish Nutrition in Aquaculture. Proc. Third Int. Symp. on Feeding and Nutr. in Fish, Toba, Japan, Aug 28 - Sept. 1, Japan, 1989, pp.325-332.

Fowler, L.G., 1990. Feather meal as a dietary protein source during parr-smolt transformation in fall chinook salmon. Aquaculture 89, 301-314.

Hajen, W.E., Higgs, D.A., Beames, R.M., Dosanjh, B.S., 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (Oncorhynchus tshawytscha) in sea water. 2. Measurement of digestibility. Aquaculture 112, 333-348.

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

Pfeffer, E., Kinsinger, S., Rodehutscord, M., 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. Aquacult. Nut.1, 111-117.

Pfeffer, E., Wiesmann, D. and Henrichfreise, B., 1994. Hydrolyzed feather meal as feed component in diets for rainbow trout (Oncorhynchus mykiss) and effects of dietary protein/energy ratio on the efficiency of digestible energy utilization of digestible energy and protein. Arch. Anim. 46, 111-119.

Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M., Montero, D., 1997. Corn gluten meal and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): Nutritional and histological implications. Aquaculture 157, 347-359.

SAS Institute, 1988. SAS/STAT User's Guide, Release 6.03 ed., SAS Institute, Cary, North Carolina.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed., McGraw-Hill Publishing Co., New York.

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

Tacon, A.G.J., Jackson, A.J., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. In: Cowey, C.B., Mackie, A.M., Bell J.G. (Eds.). Nutrition and Feeding of Fish. Academic Press, London, U.K. pp. 119-145.

Note: A scientific manuscript of this project is in preparation. It will be submitted for publication in the scientific journal *Aquaculture* in the coming weeks.

Table 1. Composition of the ingredients used in trial #1.

Composition (as is basis)	Herring meal 1	Herring meal 2	Corn gluten meal	Blood meal	Poultry by-products meal	Meat and bone meal	Feather meal	Oat Flour
Dry matter, %	91.9	94.9	90.2	91.9	95.6	95.3	91.7	90.4
Crude protein, %	68.2	69.9	61.7	80.5	64.2	46.1	70.9	12.4
Ash, %	14.9	12.4	1.6	6.5	12.1	29.6	2.1	0.9
Biogenic amines[1]								
Tyramine, mg/kg	-	27	-	-	145	<69	<69	-
Histamine, mg/kg	-	N.D.	-	-	N.D.	N.D.	N.D.	-
Putrescine, mg/kg	-	13	-	-	273	<43	<43	-
Cadaverine, mg/kg	-	142	-	-	413	64	54	-
Spermidine, mg/kg	-	21	-	-	53	<36	<36	-
Spermine. mg/kg	-	11	-	-	<104	<104	<104	-

Results courtesy of Dr. T.K. Smith, Dept. of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada. Methodological details and complete results are presented in a manuscript due for publication in the coming weeks.

Table 2. Formulation of the experimental diets used in the first trial.

Ingredients	Diet							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Fish meal, herring (1)	28	24.5	28	28	28	28	24	20
Corn gluten meal	28	24.5	28	28	31	28	24	20
Whey	10	10	-	7.5	-	11	9	7
Blood meal, spray-dried	6	12	6	6	6	-	-	-
Oat flour	-	-	15	7.5	-	-	-	-
Poultry by-products meal	-	-	-	-	-	10	20	30
Wheat middlings	5	5	-	-	-	-	-	-
Cellulose	-	-	-	-	12	-	-	-
Vitamins & minerals	4	4	4	4	4	4	4	4
L-Lysine	1	0.8	1.0	1.0	1.0	1.0	1.0	1.0
L-Arginine	-	0.2	-	-	-	-	-	-
Fish oil, herring	18	19	18	18	18	18	18	18
Total	100	100	100	100	100	100	100	100
Digestible Composition (measured)								
Digestible protein (DP), %	43.3	43.7	43.1	43.2	43.1	44.0	44.5	44.6
Digestible energy (DE), MJ/kg	21.3	21.3	21.0	21.5	20.0	21.6	21.5	21.6
DP:DE, g/MJ	20.3	20.5	20.6	20.1	21.6	20.3	20.7	20.6

Table 3. Formulation of the diet used in the second trial.

Ingredients	Diet							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Fish meal, herring (2)	40	20	20	20	20	20	20	20
Corn gluten meal	11	11	11	11	11	11	11	11
Blood meal, spray-dried	4.5	5	4.5	4.5	5	4.5	4.5	5.5
Soybean meal	13	-	-	-	-	-	-	-
Feather meal	-	17	17	17	17	17	17	-
Meat and bone meal	-	25	25	25	-	-	-	25
Poultry by-product meal	-	-	-	-	16	16	16	16
Wheat middlings	5	-	-	-	8	8	8	-
Whey	9.5	6	6	6	6	6	6	6.5
Vitamin & mineral premix	3	3	3	3	3	3	3	3
DL-Methionine	-	-	0.5	-	-	0.5	-	-
L-Lysine	-	-	-	0.5	-	-	0.5	-
Fish oil, herring	14	13	13	13	14	14	14	13
Total	100	100	100	100	100	100	100	100
Calculated Composition								
Digestible protein (DP), %	42	42	42	42	42	42	42	42
Lipid, %	19	23	23	23	22	22	22	22
Digestible energy (DE), MJ/kg	19	19	19	19	19	19	19	19
DP:DE, g/MJ	22	22	22	22	22	22	22	22

Table 4. Apparent digestibility coefficients of the experimental diets in the first trial (n=3 fecal samples per diet).

Apparent Digestibility Coefficients	**Diet**							
%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dry matter	88	88	84	88	78	89	88	87
Crude protein	96	95	95	96	95	95	94	93
Ash	57	59	49	56	42	57	53	54
Gross energy	92	91	90	92	85	93	92	92

Table 5. Growth performance of rainbow trout fed the diets for 16 weeks in the first trial.

Parameters	**Diet**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**SEM**	**HSD**
Initial live body weight, g/fish	17	17	17	17	17	17	16	18	0.3	1.3
Final live body weight, g/fish	209	215	215	207	211	201	202	209	7	34
Feed efficiency, wet gain: dry feed	1.18	1.26	1.22	1.25	1.18	1.17	1.19	1.18	0.03	0.16
TGC[1]	0.200	0.205	0.205	0.200	0.202	0.195	0.199	0.199	0.004	0.018
N gain, g/fish	4.9	5.0	5.0	4.8	4.8	4.6	4.6	4.8	0.2	1.1
N gain, % digestible N intake	41	43	42	43	41	39	40	40	1.5	7
Recovered energy, kJ/fish	1658	1802	1788	1728	1688	1690	1644	1613	67	334
Recovered energy, % DE intake	45	51	50	50	49	47	46	44	1.5	8

[1]Thermal-unit growth coefficient (TGC) = (Final body weight $)^{1/3}$ - (Initial body weight$)^{1/3}$ / Σ (Temperature °C x Days)
No statistically significant differences according to Tukey's Honestly Significant Difference Test.

Table 6 Growth performance of rainbow trout fed the diets for 12 weeks in the second trial.

Parameters	Diet										
	1	2	3	4	5	6	7	8	9	SEM	HSD
Initial live body weight, g/fish	35.4	35.3	35.5	35.3	35.5	35.5	35.6	35.5	35.2	0.1	0.6
Final live body weight, g/fish	278_{a}	247_{bc}	248_{bc}	242_{c}	264_{ab}	251_{bc}	261_{ab}	245_{bc}	202_{d}	4	19
Feed efficiency, gain as is: dry feed	1.26_{a}	1.11_{bc}	1.12_{bc}	1.08_{cd}	1.20_{ab}	1.12_{bc}	1.17_{abc}	1.09_{cd}	1.00_{d}	0.02	0.10
TGC[1]	0.261_{a}	0.241_{bc}	0.241_{bc}	0.238_{c}	0.252_{ab}	0.243_{bc}	0.250_{abc}	0.239_{bc}	0.209_{d}	0.003	0.013
N gain, g/fish	6.4_{a}	5.4_{b}	5.2_{b}	5.3_{b}	5.4_{b}	5.0_{b}	5.4_{b}	5.0_{b}	3.3_{c}	0.1	0.5
N gain, % digestible N intake	45_{a}	38_{b}	38_{b}	36_{b}	38_{b}	36_{b}	39_{b}	36_{b}	26_{c}	0.8	4
Recovered energy, kJ/fish	2108_{a}	1952_{abc}	1911_{abc}	1898_{bc}	1970_{ab}	1801_{bcd}	1901_{bc}	1759_{cd}	1655_{d}	39	197
Recovered energy, % DE intake	54_{a}	51_{ab}	51_{ab}	54_{ab}	51_{abc}	46_{c}	49_{abc}	47_{bc}	47_{bc}	1.0	5

[1]Thermal-unit growth coefficient (TGC) = (Final body weight $)^{1/3}$ - (Initial body weight$)^{1/3}$ / Σ (Temperature °C x Days).
Data in a same row sharing a common subscript are not statistically different according to Tukey's Honestly Significant Difference Test.