

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

U·M·I

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313 761-4700 800 521-0600

Order Number 9239445

**Effects of dietary histamine on fish meal protein quality to
rainbow trout (*Oncorhynchus mykiss*)**

Fairgrieve, William Thomas, Jr., Ph.D.

University of Washington, 1992

Copyright ©1992 by Fairgrieve, William Thomas, Jr. All rights reserved.

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Effects of Dietary Histamine on Fish Meal Protein Quality to Rainbow Trout (*Oncorhynchus mykiss*).

by

William Thomas Fairgrieve, Jr.

A dissertation submitted in partial fulfillment
of the requirements for the degree of

Doctor of Philosophy

University of Washington

1992

Approved by James M. Day
(Chairperson of the Supervisory Committee)
Ronald W. Hardy
William K. Hershberger
Robert K. Johnson
Paul R. Young

Program Authorized
to Offer Degree School of Fisheries
Date July 23, 1992

© Copyright 1992

William Thomas Fairgrieve, Jr.

Doctoral Dissertation

In presenting this dissertation in partial fulfillment of the requirements for the Doctoral degree at the University of Washington, I agree that the Library shall make its copies freely available for inspection. I further agree that the extensive copying of this dissertation is allowable only for scholarly purposes, consistent the "fair use" as prescribed in the U.S. Copyright Law. Requests for copying or reproduction of this dissertation may be referred to University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom the author has granted "the right to reproduce and sell (a) copies of the manuscript in microform and or (b) printed copies of the manuscript from microform."

Signature

William T. Sullivan

Date

7/23/92

University of Washington

Abstract

Effects of Dietary Histamine on Fish Meal Protein Quality to Rainbow Trout (*Oncorhynchus mykiss*).

by William Thomas Fairgrieve, Jr.

Chairperson of the Supervisory Committee: Faye M. Dong
Associate Professor
School of Fisheries

Dietary histamine, and its derivative, gizzerosine, cause gizzard erosion and black vomit disease in chickens. Rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) fed fish meal that evoked black vomit disease in chickens reportedly developed gastric lesions, but the role of thermal abuse and histamine content in the formation of ulcerogenic compounds had not been fully elucidated. The effects of dietary histamine and heat treatment on feed palatability, protein quality, and gastric abnormalities were evaluated in feeding trials with juvenile rainbow trout.

No correlation was found between feeding behavior and feed intake by rainbow trout and the results of toxicity bioassays with chickens. Palatability of nontoxic fish meal and casein was improved by histamine supplementation (0 vs. 2,000 mg/kg dry diet) and heat treatment. Free histamine in unheated fish meal was slightly repellent. Histamine and heat treatment of casein or of two other sources of fish meal had minimal effect on feed intake.

No correlation was found between the nutritional quality of fish meal protein to juvenile rainbow trout and the results of toxicity bioassays with chickens. Protein retention and growth was similar among groups of fish pair-fed diets containing fish meal either acutely toxic or nontoxic to chickens. Protein retention and fish growth was not interactively affected by histamine supplementation (0, 500, 1,000, 1,500, or 2,000 mg/kg dry diet) and heat treatment of nontoxic fish meal or casein.

Fish fed toxic Chilean fish meal for 111 d had distended stomachs, but no lesions or cellular abnormalities were observed. Similar effects were obtained by histamine supplementation (2,000 mg/kg dry diet) and heat treatment of nontoxic fish meal and casein. Histamine toxicity was not affected by addition of two suspected potentiators, putrescine and cadaverine.

These studies showed that the results of chicken bioassays cannot be used to predict fish meal palatability and nutritional quality to rainbow trout. Fish meals acutely toxic to chickens are not acutely toxic to rainbow trout, and chronic dietary exposure results in stomach distention without reductions in feed intake or fish growth rates.

TABLE OF CONTENTS

	Page
List of Figures.....	v
List of Tables.....	vii
Chapter 1: Problem statement.....	1
Chapter 2: Literature review.....	3
Chapter 3: Preliminary Studies on the Palatability, Nutritional Value, and Toxicity of Chilean Fish Meals to Rainbow Trout.....	15
3.1 Introduction.....	15
3.2 Methods and Materials.....	17
3.3 Results.....	25
3.4 Summary and Conclusions	28
Chapter 4: Effects of Histamine on the Development of Digestive Tract Abnormalities in Rainbow Trout.....	37
4.1 Introduction.....	37
4.2 Methods and Materials.....	43
4.3 Results.....	52
4.4 Summary and Conclusions	56
Chapter 5: Comparison of Toxic and Nontoxic Fish Meals as Protein Sources in the Diets of Rainbow Trout.....	72
5.1 Introduction.....	72

5.2	Methods and Materials.....	73
5.4	Results.....	79
5.5	Summary and Conclusions.....	83
Chapter 6: Effects of Feeding Behavior by Interstrain Hybrids of Rainbow Trout on the Measurement of Feedstuff Palatability.....		
6.1	Introduction.....	97
6.2	Methods and Materials.....	99
6.3	Results.....	101
6.4	Summary and Conclusions.....	103
Chapter 7: Effects of Protein Intake Level on Nutrient Partitioning.....		
7.1	Introduction.....	112
7.2	Methods and Materials.....	114
7.4	Results.....	118
7.5	Summary and Conclusions.....	121
Chapter 8: Effects of Dietary Histamine Level and Heating on the Nutritive Value of Fish Meal and Casein to Rainbow Trout.....		
8.1	Introduction.....	130
8.2	Methods and Materials.....	131
8.3	Results.....	136
8.4	Summary and Conclusions.....	140

Chapter 9: General Discussion, Contributions of Research, and Suggestions for Future Research.....	156
9.1 Introduction.....	156
9.2 Discussion.....	159
9.3 Conclusions.....	187
9.4 Suggestions for future research.....	189
Literature Cited.....	190
APPENDIX 1.....	203
APPENDIX 2.....	204
APPENDIX 3.....	208

LIST OF FIGURES

	Page
Figure 2.1. Formation of histamine by the decarboxylation of L-histidine.....	12
Figure 2.2. Schematic representation of the flow of raw material through a typical plant processing raw fish into whole fish meal.....	13
Figure 2.3. Chemical structure of gizzerosine.....	14
Figure 3.1. Chemical structures of putrescine and cadaverine.	31
Figure 4.1. Pathways of histamine metabolism <i>in vivo</i>	60
Figure 4.2. Feeding response scores for rainbow trout fed the experimental diets.	61
Figure 4.3. Gross external and internal morphology of rainbow trout fed the casein-based experimental diets for 111 d.....	62
Figure 4.4. Gross external and internal morphology of rainbow trout fed the LT fish meal-based diets for 111 d.....	63
Figure 4.5. Gross external and internal morphology of rainbow trout fed the Chilean fish meal-based diets for 111 d.....	64
Figure 4.6. Cross sections of stomach tissues from rainbow trout fed either the unsupplemented CG diet for 42 d or the CG diet supplemented with histamine, putrescine, and cadaverine for 98 d.	65

Figure 4.7. Cross sections of stomach tissues from rainbow trout fed either the unsupplemented LT-1-N diet for 42 d or the LT-1-N diet supplemented with histamine, putrescine, and cadaverine for 98 d.66

Figure 4.8. Cross sections of stomach tissues from rainbow trout fed either the unsupplemented C-4-T diet for 42 d or the C-5-T diet supplemented with histamine, putrescine, and cadaverine for 98 d.67

Figure 5.1. Feeding response scores for rainbow trout fed the experimental diets.86

Figure 6.1. Feeding response scores for rainbow trout fed the experimental diets.105

Figure 8.1. Feeding response scores for rainbow trout fed the experimental diets.142

LIST OF TABLES

	Page
Table 3.1. Proximate composition (% dry weight) and histamine content (mg/kg wet weight) of the fish meals.	32
Table 3.2. Formulation of the experimental diets (% dry ingredients).	33
Table 3.3. Proximate compositions (% dry weight) of the experimental diets.	34
Table 3.4. Mean growth of rainbow trout fed the experimental diets for 6 weeks.	35
Table 3.5. Feed intake, weight gain, and efficiency of diet and protein utilization by rainbow trout fed the experimental diets for 6 weeks.	36
Table 4.1. Proximate composition and selected biogenic amine content of vitamin-free casein and fish meals included in the experimental diets.	68
Table 4.2. Formulation of experimental diets.	69
Table 4.3. Proximate compositions of experimental diets.	70
Table 4.4. Growth, feed consumption, and efficiency of feed utilization by rainbow trout fed the experimental diets.	71
Table 5.1. Proximate composition of casein and fish meals used in the experimental diets.	87
Table 5.2. Formulation of the experimental diets.	88

Table 5.3. Proximate composition of the experimental diets.....	89
Table 5.4. Protein intake and feeding rates used in the nutritive value trial.....	90
Table 5.5. Growth of rainbow trout fed the experimental diets to satiation for 6 weeks in the palatability trial.....	91
Table 5.6. Growth of rainbow trout fed equal quantities of protein from the experimental diets for 6 weeks in the nutritive value trial.....	92
Table 5.7. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout fed the experimental diets for 6 weeks in the palatability trial.....	93
Table 5.8. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout fed equal quantities of protein from the experimental diets for 6 weeks in the nutritive value trial.....	94
Table 5.9. Whole body composition of rainbow trout fed the experimental diets for 6 weeks in the palatability trial.....	95
Table 5.10. Table 5.10. Whole body composition (% wet weight) of rainbow trout fed equal quantities of protein from the experimental diets for 6 weeks in the nutritive value trial.....	96
Table 6.1. Proximate composition of the fish meal used in the experimental diets.....	106
Table 6.2. Formulation of the experimental diet.....	107
Table 6.3. Proximate composition of the experimental diet.....	108

Table 6.4. Mean growth of two strains of rainbow trout fed the experimental diets to satiation for 6 weeks.	109
Table 6.5. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by two strains of rainbow trout fed the experimental diets to satiation for 6 weeks.	110
Table 6.6. Whole body composition (% wet weight) of two strains of rainbow trout fed the experimental diets to satiation for 6 weeks.	111
Table 7.1. Proximate composition (% wet dry weight) of fish meal used in the experimental diets.	122
Table 7.2. Formulation of the experimental diets (% dry ingredients).	123
Table 7.3. Proximate composition (% dry weight) of diets.	124
Table 7.4. Protein consumption (g/fish/week) by rainbow trout red diets containing either herring meal or herring meal heat treated by autoclaving.	125
Table 7.5. Mean growth of rainbow trout pair-fed the experimental diets at various rates of protein intake for 4 weeks.	126
Table 7.6. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout pair-fed the experimental diets for 4 weeks.	127
Table 7.7. Efficiency of protein utilization by rainbow trout pair-fed at various rates of protein intake for 4 weeks.	128

Table 7.8. Whole body composition (% wet weight) of rainbow trout pair-fed at various rates of protein intake for 4 weeks.	129
Table 8.1. Proximate composition (% dry weight) and histamine content (mg/kg wet weight) of protein sources used in the experimental diets.	143
Table 8.2. Formulation of experimental diets (% dry ingredients).	144
Table 8.3. Proximate composition (% dry weight) of diets used to evaluate the effects of histamine level and heat treatment on the nutritive value of fish meal and casein to rainbow trout.	145
Table 8.4. Histamine concentration (mg/kg dry weight) of selected protein sources and diets used to evaluate the effects of histamine level and heat treatment on the nutritive value of fish meal and casein to rainbow trout.	146
Table 8.5. Results of analysis of variance on growth, dry feed intake, and efficiency of protein utilization and retention by rainbow trout fed fish meal-based diets to satiation for 4 weeks.	147
Table 8.6. Mean growth of rainbow trout fed the fish meal based diets to satiation for 4 weeks in the palatability trial.	148
Table 8.7. Results of analysis of variance on growth and efficiency of protein utilization and retention by rainbow trout pair-fed equal quantities of protein from the experimental diets for 4 weeks in the nutritive value trial.	149

Table 8.8. Effects of dietary histamine supplementation level on growth, protein efficiency ratio, and protein productive value by rainbow trout pair-fed equal quantities of protein for 4 weeks in the nutritive value trial.	150
Table 8.9. Mean growth of rainbow trout pair-fed casein or fish meal based diets for 4 weeks in the nutritive value trial.	151
Table 8.10. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout fed fish meal based diets to satiation for 4 weeks in the palatability trial.	152
Table 8.11. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout pair-fed equal quantities of protein from casein or fish meal based diets for 4 weeks in the nutritive value trial.	153
Table 8.12. Whole body composition (% wet weight) of rainbow trout pair-fed equal quantities of protein from casein or fish meal based diets for 4 weeks in the nutritive value trial.	154
Table 8.13. Whole body composition (% wet weight) of rainbow trout fed fish meal based diets to satiation for 4 weeks in the palatability study.	155

ACKNOWLEDGEMENTS

The timely and successful completion of the research described in this dissertation was made possible by the efforts and contributions of many individuals and organizations. It is with pleasure that I acknowledge their contributions.

I would like to express my gratitude to Dr. Faye M. Dong, chair of the supervisory committee, Dr. R.W. Hardy, Dr. R.R. Stickney, Dr. W.K. Hershberger, and Dr. W. Yamanaka for their suggestions and assistance in preparing the original project proposal, execution of the research, and writing of the dissertation.

Dr. M. Rutman (INUAL, Santiago, Chile) and Mr. E. Castro Campos (Fundacion Chile, Santiago, Chile) provided numerous samples and conducted the chicken toxicity bioassays of fish meal used in the research. Technical assistance was given by Ms. C. Lopez (INUAL, Santiago, Chile).

Dr. J. Hungerford and Ms. S. Lee (U.S. Food and Drug Administration, Bothell, Washington) analyzed many protein source and diet samples for histamine content. Mr. M. Meyers (N.M.F.S., Seattle, Washington) conducted histopathological evaluation of innumerable fish samples, and prepared the photographs used in this dissertation. Mr. K. Masee managed the laboratory experiments and assisted in data collection.

This research was funded by support from the Cooperative State Research Service, U.S. Department of Agriculture, to the Western Regional Aquaculture Consortium, under Agreement No. 87-CSRS-2-3219.

Dedication

To the first, and best, of my teachers
William and Muriel

Chapter 1

Problem Statement

Traditionally, diets formulated for feeding trout and salmon have relied to a large extent on fish meal as the major source of dietary protein. While it has long been recognized that the nutritional quality of fish meal protein varies widely depending on the species of fish used as raw material and method of processing, researchers employing standard chemical analysis and *in vitro* and *in vivo* assays of protein digestibility have been generally unsuccessful in developing measures of protein quality useful in the formulation of practical diets for fish. Although there are relatively few published studies regarding the effects of raw material freshness on the nutritive value of fish meal protein, a growing body of evidence suggests that this factor may be a pivotal determinant, irrespective of source of raw material or processing method used (Pike et al., 1990; Pike, 1991).

While the need for proper post-harvest handling and storage of fish intended for human consumption is obvious, fish destined for reduction into fish meal are often stored at near-ambient temperatures for periods of time ranging from hours to days prior to processing. Under these conditions, bacterial spoilage proceeds rapidly with the concomitant production of several putrifactive amines, including histamine (from histidine), putrescine (from arginine), and cadaverine (from lysine). The role of these biogenic amines in scombroid poisoning of humans has been extensively studied but little is known concerning their effects on most other animals, except poultry, where feeding of fish meals high in histamine has been associated with gizzard erosion, and in severe cases, gizzard ulceration and mortality. Two recent reports (Watanabe et al., 1987; Romero and Castro, 1989) suggested that

these and perhaps other biogenic amines present in fish meal toxic to chickens were also detrimental to at least two species of farm-reared salmonids. These data were insufficient to definitively establish a cause-and-effect relationship between biogenic amine content and fish meal quality. However, much of the fish meal manufactured in Chile for use in fish feeds has been tested for histamine and certified as nontoxic using a standard chicken bioassay. Because these fish meals command premium prices on the world market, their use substantially increases the cost of fish feeds and, in the final analysis, directly affects the profitability of aquaculture. Given the direct economic importance of these practices, further research into the validity of these measures of fish meal quality was warranted.

The objectives of this research were to examine: (1) the relationship between results of toxicity bioassays with broiler chickens and growth bioassays with rainbow trout fed a variety of fish meals; (2) the effects of thermal abuse and histamine content on the palatability and nutritive value of casein and fish meal fed to rainbow trout; and (3) the effects of these processing factors on the digestive tract morphology of rainbow trout.

Chapter 2

Literature Review

Histamine, 4-(2-aminoethyl)imidazol, is a primary amine arising from the decarboxylation of the amino acid, L-histidine (Figure 2.1). In many animals, endogenous histamine has important physiologic functions, including stimulation of vasodilation, anaphylaxis, and secretion of mucous and gastric acid (Ganong, 1989). The toxic effects of histamine in the food supply of humans has received considerable attention, and its presence has been positively associated with scombroid-fish poisoning (Morrow et al., 1991). Scombroid-fish poisoning, also referred to as scombroticism or histamine poisoning, is a clinical syndrome that results from the ingestion of spoiled fish, usually of the families Scombridae and Scomberesocidae, although non-scombroid fish of the families Pomatomidae, Coryphaenidae, Carangidae, Clupeidae, and Engraulidae have also been implicated (Taylor, 1986).

Decomposition of many marine fish species of the families listed above is characterized by the formation of high levels of histamine by bacteria that possess the enzyme histidine decarboxylase (Taylor et al., 1978). Histamine production can be controlled by low storage temperatures, conditions which reduce enzymatic and most microbial activities. Barankowski et al. (1990) examined the effect of temperature on decomposition and histamine formation in dolphin (*Coryphaena hippurus*). In their study, filets incubated in seawater at 0°C decomposed slowly, and after 3 weeks incubation the histamine level was about 120 mg/kg. At higher temperatures, rates of decomposition and histamine formation were rapid. For example, filets incubated in seawater at 21°C decomposed rapidly, and after 2 d the histamine level was 1,540 mg/kg. At 32°C, the fish showed definite signs of deterioration after 12 h

incubation, but only 20 mg histamine/kg were present. However, the histamine content increased rapidly to a level of 2,500 mg/kg in 24 h. It is noteworthy that many of the marine species prone to histamine formation are captured in tropical regions where water temperatures may approach 30°C. If onboard refrigeration is inadequate, much of the fish captured could undergo significant deterioration prior to processing for human consumption or into fish meal.

During 1990, nearly 3.7 million metric tons of pelagic fish were processed into fish meal by the Chilean industry. Of the total, about 91% were species known to degrade rapidly and to produce high levels of histamine during transport from the fishing grounds and during storage prior to processing into fish meal. During that year, about 19% of the fish were sardines (*Sardinops sagax*), 18% were anchovies (*Engraulis ringus*), and 54% were Jack mackerel (*Trachurus murphyi*) (M. Rutman, INUAL, Santiago, Chile; personal communication). While reliable figures regarding the freshness of the fish prior to reduction into fish meal were not available, historically, a significant portion of the catch has been delivered to the plants in a partially decomposed state and has been found high in endogenous histamine (E. Castro, Fundacion Chile, Santiago, Chile; personal communication). Further degradation has been known to occur after the fish have been unloaded, since the raw material is stored in unrefrigerated concrete pits prior to processing.

Histamine content can be used as an index of raw material freshness but the histamine content of the fish meal cannot, since much of the histamine and other water soluble amines are removed with the liquid phase during processing (Toyama et al., 1981). The liquid phase, or press liquor, is centrifuged to remove the oil and the remaining aqueous portion of the press liquor, known as stickwater, is concentrated by evaporation. The concentrate is then added to the insoluble component to produce whole meal (Figure 2.2). It is

important to note that the concentrate added to a particular batch of fish meal may not be derived from the same batch of raw material (Pike et al., 1990). Therefore, fish meal produced from otherwise good quality raw material may contain significant quantities of histamine and other biogenic amines from decomposed concentrate. Conversely, the histamine content of fish meal prepared from degraded fish may be lower or higher than expected depending on the quality and quantity of concentrate used in the production of whole meal.

The effects of dietary histamine on growth, proventricular enlargement, and gizzard erosion in broiler chickens are well known. Shifrine et al. (1959) fed chickens diets containing various quantities of histamine from spoiled fish. In their study, levels of dietary histamine up to 400 mg/kg were associated with increased weight gain. However, weight gain of chickens fed diets containing 1,200 to 4,000 mg histamine/kg was found inversely proportional to histamine level. Harry et al. (1975) associated histamine with gizzard erosion and proventricular enlargement in feeding trials using fish meal in the diets, and reported a high incidence of gizzard erosion in chickens fed diets supplemented with histamine hydrochloride at levels ranging from 1,000 to 10,000 mg/kg. Histamine has also been implicated as a factor in malabsorption syndrome of broiler chickens (Stuart et al. 1986).

Few data presently exist in the scientific literature regarding the direct effects of histamine on the nutritive value of fish meal protein. In response to concerns expressed by the Food and Agriculture Organization of the United Nations that persons in the Indo-Pacific region may be deficient in dietary protein due to the consumption of marginally spoiled fish, Barankowski and Brust (1984) determined the net protein utilization (NPU) of fresh (0 mg histamine/kg), slightly spoiled (200 mg histamine/kg), and spoiled (400 mg histamine/kg) dolphin in male Wistar weanling rats. They

found that slightly spoiled dolphin had a higher nutritional value than either the fresh or moderately spoiled product, which were statistically identical. They suggested that very slight protein hydrolysis due to microbial decomposition may improve the digestibility of the protein, but that the effects may be offset by elevated histamine levels.

Several lines of experimental evidence indicate that excessive heating during the drying of fish high in histamine results in the formation of histamine derivatives acutely toxic to chickens. Early work by Umemura et al. (1981) showed that heating whole fish meal made from mackerel or a blend of histidine or histamine and casein to 135°C for 3 h produced unidentified compounds causative of gizzard erosion in chickens. The fact that the compounds appeared in the protein fraction strongly suggested that the agent was not free histidine or histamine, but perhaps some protein or amino acid derivative. Toyama et al. (1982) observed decreases in the histamine content of spoiled sardines and mackerel during each stage of the fish meal manufacturing process in which the material was heated, namely, cooking of the raw fish, drying of the presscake, and concentration of the solubles. They suggested that the apparent loss of histamine was attributable to chemical changes, rather than microbial decomposition or physical loss, and that the resultant compounds may be causative of gizzard erosion in chickens. Experiments conducted by Wessels and Post (1989) showed that excessive heating of raw fish to which various sources of stickwater had been added increased its toxicity to chickens.

Watanabe et al. (1987) examined the interactive effects of dietary histamine, histidine, and heat treatment of fish meal on stomach morphology of rainbow trout (*Oncorhynchus mykiss*). In one study, fish fed diets containing white fish meal as the sole source of protein and supplemented with either 5,000 or 10,000 mg free histamine/kg for 10 weeks exhibited severe erosion of the gastric

mucosa. In a second study, sardine meal alone or sardine meal supplemented with approximately 3,000 mg/kg histidine or histamine was heated to approximately 130°C for 3 h before incorporation into the experimental diets. After 8 weeks feeding, 33% of the fish fed diets containing heat-treated sardine meal or sardine meal to which histidine had been added prior to heating and 40% of the fish fed diets containing sardine meal to which histamine had been added before heat treatment exhibited mild to severe gastric erosion. However, fish fed diets containing unheated, unsupplemented sardine meal showed no signs of gastric erosion. Similar results were obtained when the sardine meals used in the second study cited above were fed to broiler chickens. The pathology and stomach abnormalities reportedly differed between the two species of animals, but a detailed description was not given. Based on the results of these studies, the authors concluded that the compounds causing gastric erosion in fish were probably the same as those responsible for induction of gizzard erosion in chickens.

Watanabe et al. (1987) examined the direct effects of supplemental histamine in diets containing white fish meal as the sole source of protein on the growth and efficiency of protein utilization by rainbow trout. In their studies, the addition of histamine to the diets in levels of 1,000, 5,000, or 10,000 mg/kg appeared to have no effect on growth, although in general, NPU appeared to increase with increasing histamine level. Protein efficiency ratio (PER) appeared unaffected by dietary histamine level. The interactive effects of histamine level and heat were not determined. The studies were conducted without replication and the data were not statistically analyzed.

Although the results of the laboratory study conducted by Watanabe et al. (1987) showed that histamine could induce changes in the gastric morphology of rainbow trout that they presumed to be detrimental, published results of field trials using diets made with

commercially produced fish meal high in histamine and shown to induce gizzard erosion in chickens were almost nonexistent. Romero and Castro (1989) reported that coho salmon (*Oncorhynchus kisutch*) reared in marine net-pens and fed a practical diet containing fish meal of Chilean origin that was acutely toxic to chickens exhibited stomach abnormalities, including thinning of the mucosa and hemorrhage, and grew poorly, compared with those fed the nontoxic control diet. While the data seemed to confirm the results of Watanabe et al. (1987), additional studies required to determine the compounds responsible for the putative toxicity of the fish meal to coho salmon and to identify the processing conditions associated with their formation were not conducted.

Gizzard erosion and the resultant black vomit disease of chickens has long been associated with feeding thermally abused fish meal, but it was not until 1983 that Okazaki et al. recovered an active substance from fish meal. The active compound, identified as 2-amino-9-(4-imidazolyl)-7-azanonanoic acid (Figure 2.3) and named gizzerosine, was presumably formed via condensation of histamine with the epsilon amino group of lysine during high temperature processing of raw fish into fish meal. Mori et al. (1983, 1985) synthesized several forms of the compound, and established the L form as a potent inducer of gizzard erosion in chickens. In one study, Mori et al. (1983) observed severe gizzard erosion in chickens fed less than 50 μg gizzerosine/d (about 2.2 mg/kg diet) for one week. Subsequently, the compound was extensively studied by several researchers and its role in the genesis of gizzard erosion and ulceration in chickens elucidated.

Gizzard erosion and ulceration due to biogenic amine toxicity has widespread economic implications for the poultry industry worldwide. However, few studies have determined the threshold levels of toxicity or chronic effects of sublethal dosages on growth and mortality. In perhaps the most comprehensive study published to

date, Sugahara et al. (1988) examined the dose-dependent response of male broiler chickens to synthetic DL-gizzerosine. In one experiment, diets supplemented with gizzerosine at levels ranging from 0 to 18 mg/kg were fed to chickens for 7 d beginning 4 d post-hatch. In a second trial, diets were supplemented with gizzerosine at levels ranging from 0 to 1 mg/kg. In both, the authors measured the effects of gizzerosine on growth, mortality, and severity of gizzard erosion.

In the experiments of Sugahara et al. (1988), chickens fed diets containing less than 1 mg gizzerosine/kg grew as well as those fed the unsupplemented diet. Weight gain and feed consumption decreased linearly with increasing concentration above 1 mg/kg, and severe gizzard erosion and mortality were observed when chickens were fed diets containing gizzerosine at levels of 2 mg/kg or greater. However, moderate to severe gizzard erosion was detected in birds fed gizzerosine at dietary levels above 0.6 mg/kg, with mortality noted at levels of 0.8 mg/kg or greater. Based on these data, the authors recommended dietary levels of L-gizzerosine not exceed 0.4 mg/kg. Fish meals tested by Ito et al. (1985) were found to contain up to 20 mg gizzerosine/kg, and were therefore suggested to contain high enough concentrations of the compound to induce severe gizzard erosion when incorporated into feeds at the 10% level.

Considerable experimental evidence exists indicating that gizzerosine induces gizzard erosion in chickens by hyperstimulating gastric acid secreting cells of the proventriculus. Early work by Masumura et al. (1985) demonstrated that oral or intravenous administration of gizzerosine enhanced gastric acid secretion and induced gizzard erosion in chickens. They also found that cimetidine, an H₂ receptor antagonist of histamine, blocked its effect. These results were confirmed by Hino et al. (1987) using isolated mucosal cells of the chicken proventriculus.

The relative potencies of histamine and gizzerosine as stimulators of gastric acid secretion were examined by Ito et al. (1988), who studied the effect of gizzerosine on intracellular levels of cyclic-3',5'-adenosine monophosphate (cAMP), the main second messenger of histamine action through the H-2 receptor. They found that the potency of gizzerosine was approximately 1,000 times higher than that of histamine. Receptor binding studies demonstrated that gizzerosine was bound with higher affinity than histamine, a factor important in explaining its potency *in vivo*. Differences in the rate of metabolism of the two compounds were also suggested as factors in the higher potency of gizzerosine relative to histamine *in vivo*, although this hypothesis was not tested.

In summary, at least two amino acid derivatives often found in fish meal, histamine and gizzerosine, have demonstrated or predicted effects on important physiologic functions in animals. Whether these compounds represented sensitive and accurate indicators of the nutritive value of fish meal protein to aquatic species was unknown. Furthermore, available data were insufficient to definitively establish the relationship between histamine or other compounds causative of gizzard erosion in chickens and morphological changes in the digestive tissues of fish. A series of experiments were conducted to address the following specific objectives:

1. To examine the relationship between the results of toxicity bioassays on broiler chickens and the palatability and nutritive value of fish meal to rainbow trout.
2. To establish the effects of thermal abuse and histamine content on the palatability and nutritive value of casein and fish meal to rainbow trout.

3. To determine the effects of these factors on the digestive tract morphology of rainbow trout.

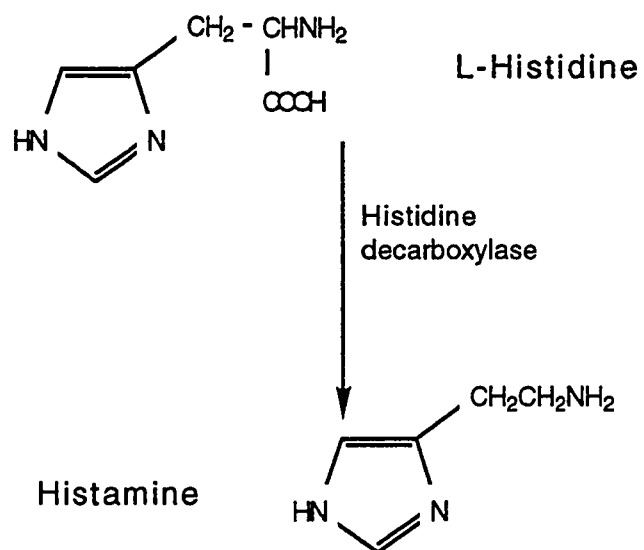


Figure 2.1. Formation of histamine by decarboxylation of L-histidine (Taylor, 1986).

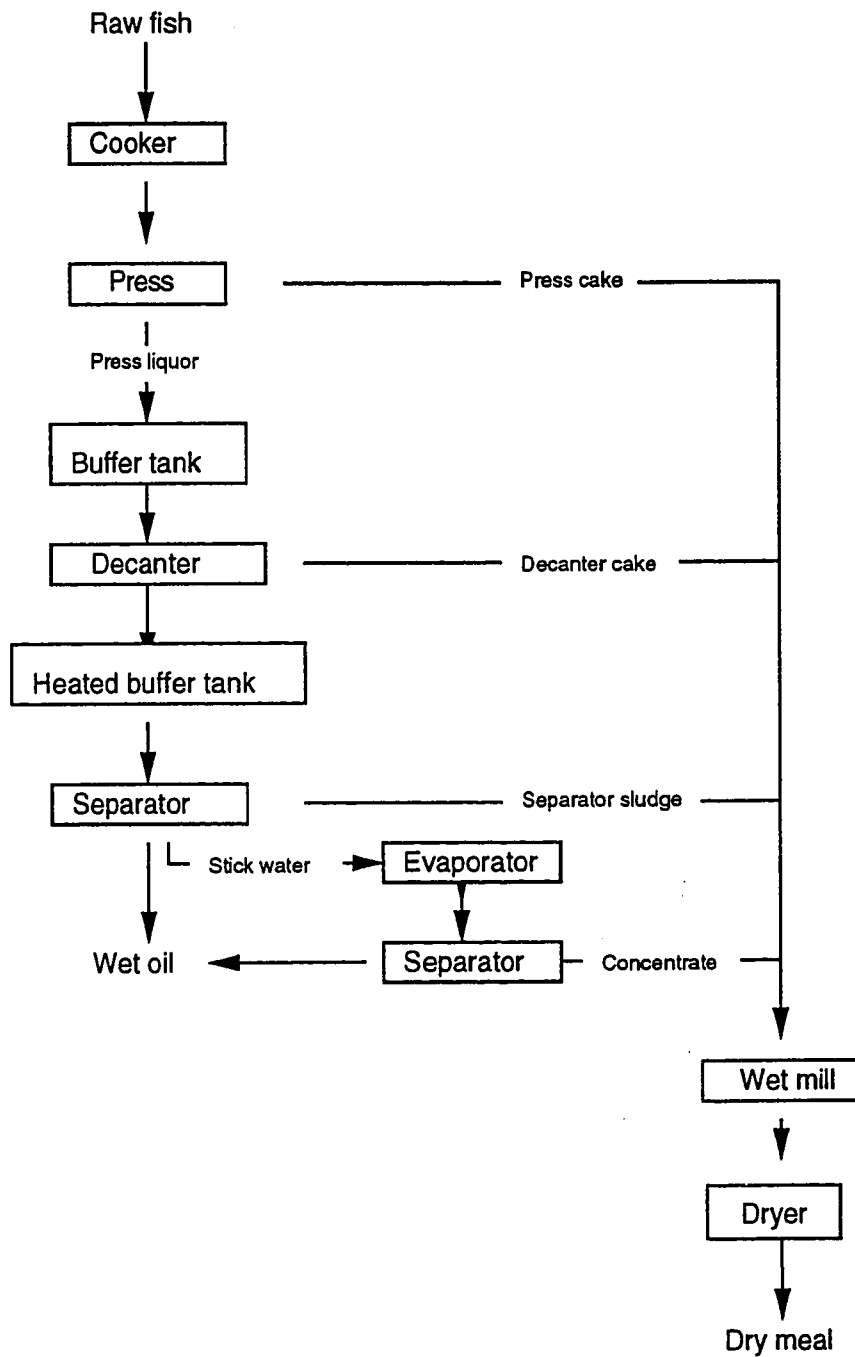


Figure 2.2. Schematic representation of the flow of raw material through a typical plant processing raw fish into whole fish meal.

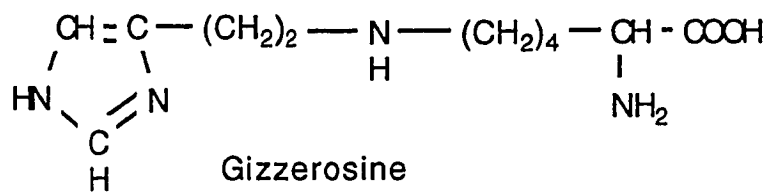


Figure 2.3. Chemical structure of gizzerosine (Okazaki et al., 1983).

Chapter 3

Preliminary Studies on the Palatability, Nutritional Value, and Toxicity of Chilean Fish Meals to Rainbow Trout.

3.1 INTRODUCTION

Gizzard erosion and the resultant black vomit disease of chickens has long been associated with feeding diets containing fish meals prepared from degraded raw materials (Johnson and Pinedo, 1971). Numerous studies have shown that excessive heating of these raw materials increases their toxicity (Umemura et al., 1980; Wessels and Post, 1988). At least one compound, gizzerosine, formed in this manner has been identified as a potent inducer of gizzard erosion (Okazaki et al., 1983). Research has shown that other free amines often found in high concentrations in degraded fish, including histamine and the diamines putrescine and cadaverine (Figure 3.1), also produce clinical changes in the gastric tissue morphology of chickens. Stuart et al. (1986) found that putrescine and cadaverine individually or in combination with histamine promoted proventricular dilation in broiler chickens. The wall of the enlarged proventriculus was thickened and stringy mucous was found adhered to the mucosa. Microscopically, mild to marked hyperplasia and hypertrophy of the glandular epithelium were observed. Gizzard enlargement accompanied the proventricular dilation, and multifocal erosions of the gizzard epithelium were occasionally present. Putrescine and cadaverine, as well tyramine, β -phenylethylamine, and tryptamine have been shown to potentiate histamine toxicity *in vivo* by inhibition of the histamine-metabolizing enzymes diamine oxidase and histamine-*N*-methyltransferase (Hui and Taylor, 1985). These data strongly suggest that fish meal toxicity to chickens is most likely related to complex interactions among many chemical compounds found in degraded fish products.

Although the determination of biogenic amine concentration in fish meal by chemical assay is possible, the relationship between the results of chemical assays and biotoxicity to chickens and other animals has not been definitively established. Therefore, fish meal produced in Chile that is intended for use in diets for poultry is often evaluated by bioassay using chickens as test animals. These assays have proven reliable and are well suited for this purpose. However, the use of the chicken bioassay has been extended to testing fish meal intended for use in the diets of salmonids, even though few data exist to justify this practice.

The purpose of this study was to collect preliminary information regarding the relationship between biotoxicity and the nutritive value and palatability of fish meal to rainbow trout, and to determine if fish meal causative of gizzard erosion in chickens could induce morphological changes in the gastric tissues of this species of fish.

3.2 METHODS AND MATERIALS

3.2.1 Test protein sources

Three fish meals: Norwegian LT-94 (LT-1-N) and two fish meals (C-1-T and C-2-N) prepared in by commercial processors in Chile from mackerel, were provided by Fundacion Chile (Santiago, Chile) during May 1990 for use in this preliminary study. The protein sources were evaluated by the Department of Marine Resources, Fundacion Chile using their standard chick bioassay (description below): two fish meals were identified as nontoxic (LT-1-N and C-2-N) and one as acutely toxic (C-1-T). The fish meals were packaged into airtight 20 L plastic pails and stored at -20°C for 5 months prior to incorporation into the test diets. Proximate composition, toxicity, and biogenic amine content of the fish meals are summarized in Table 3.1.

3.2.2 Diet preparation

Three practical diets were formulated to include fish meal as the major source of protein. Dietary alpha-cellulose levels were adjusted to accommodate differences in proximate composition of the various protein sources (Table 3.2). Proximate composition of the test diets is summarized in Table 3.3.

Experimental diets were prepared by mixing all ingredients except the herring oil in a Hobart Model N-50 mixer (The Hobart Mfg. Co., Troy, Ohio, U.S.A.) with a small amount of cold tap water (about 200 ml per kg dry ingredients) for 20 minutes. The mixtures were then compressed into 3.75 mm diameter pellets using a California laboratory pellet mill (California Pellet Mill Company, San Francisco, California) without steam injection. Pelleted feeds were spread on aluminum pans to a depth of about 3 cm, and allowed to dry at ambient temperatures overnight. Finally, the remaining oil was added to the pellets, and the mixture stirred carefully for 10

minutes to ensure even distribution. The diets were placed in plastic bags and stored frozen (-20°C) throughout the study.

3.2.3 Feeding regimen

Because the highest probability of obtaining an acute response to any toxic compounds in the fish meals was expected when feed intake was maximum, a to-satiation feeding regimen was chosen for this study. However, previous experience has shown that interpretation of nutritive value data from satiation or *ad libitum* feeding studies have been used is complicated by factors such as variable meal timing, duration of feed presentation, definition of feeding behavior that characterizes a satiation level of intake, feed wastage inherent in satiation or *ad libitum* feeding techniques, and inadequate measurement of feed intake. To avoid these factors, the following criteria were established: (1) on weekdays, fish were fed two times daily, morning and afternoon, with a period of 6 h between feedings. On weekends, fish were fed once, in the morning. Feed was withheld on weighing days. (2) Feed was given to the fish in small quantities over a period of 3 min at each feeding so that all feed presented was eaten. Feed was weighed at the start and end of each feeding period and the quantity consumed recorded. In practice, feeding activity diminished rapidly and consumption often ceased midway through the second daily feeding period.

3.2.4 Experimental facilities, fish and husbandry

The experiment described in this chapter was conducted in the wet laboratory facilities maintained by the National Marine Fisheries Service, Utilization Research Division, Seattle, Washington. Replicate groups of fish were reared in individual 200 L capacity circular polyethylene tanks, each equipped with a screened center drain. Thermoregulated ($15.5 \pm 0.7^\circ\text{C}$; Appendix 1) dechlorinated municipal water was supplied to each tank at a rate of 5 L per min. Water volume was fixed at 100 L by means of a

standpipe, and was not altered throughout the course of the experiment. Dissolved oxygen concentrations were determined at weekly intervals with a YSI Model 51B dissolved oxygen meter (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) and remained near saturation. An airstone placed into each tank provided supplemental aeration sufficient to prevent mortality in the event of a water supply failure. Tanks in this facility were exposed to natural daylight through translucent fiberglass roof panels and natural photoperiod was maintained.

Progeny of a single female-male mating of Donaldson strain rainbow trout were obtained from the University of Washington (Seattle, Washington) during October, 1990. After a 7 d acclimation period, a group of about 300 fish was selected for uniform size (26.4 ± 0.4 g , 126.5 ± 0.8 mm total length (TL)(mean \pm SD)), from which individual fish were distributed randomly among nine tanks until each contained 25 animals. Three experimental units were then assigned at random to each treatment.

In addition to feeding, routine daily activities included measuring water temperature, examining each tank for the presence of dead or moribund fish, and flushing fecal material from the tanks. No mortalities occurred during the course of the study.

3.2.5 Measurement of growth

On day 0 and at 7 d intervals for 42 d, the fish in each tank were weighed as a group (± 0.1 g), anesthetized in a 50 mg/L solution of tricaine methanesulfonate (Finquel, Argent Chemical Company, Redmond, Washington), and the total length (TL, ± 1.0 mm) of each fish measured and recorded. The fish were immediately returned to their respective tanks, and recovery from anesthesia was usually observed within 2 min. Fish were not fed for 16 h prior to sampling.

3.2.6 Collection and preparation of samples for histological evaluation

At the end of the trial, following 36 h of food deprivation, 5 fish from each group were individually anesthetized in a 100 mg/L solution of tricaine methanesulfonate, and killed by severing the spinal column with a scalpel immediately posterior to the cranium. Each fish was weighed, examined for external signs of infectious or noninfectious diseases, and the viscera exposed by incision along the ventral midline. Viscera were examined visually for gross abnormalities, including the presence of inflammation or lesions in the digestive organs, liver, and spleen. The liver and a portion of the stomach, beginning with its junction with the esophagus and extending posteriorly approximately 5 cm, were excised with scissors, placed into prenumbered cassettes, and immediately fixed in a freshly prepared solution of 10% buffered formalin. Since no gross abnormalities were noted, the liver from one fish, and the stomach tissues from three fish were randomly selected for further processing and histological evaluation. The sectioned tissues were stained with hematoxylin and eosin and examined under light microscope by M. Meyers (National Marine Fisheries Service, Seattle, Washington).

3.2.7 Bioassay of fish meals

Fish meals used in this study were collected by Fundacion Chile technicians following a routine sampling protocol which involves the collection of samples from a minimum of 20% of the bags in each 50 metric ton lot of bagged fish meal designated for evaluation by chick bioassay. The samples were thoroughly blended, and divided into progressively smaller aliquots until representative samples of the whole were obtained. A portion of each sample (about 3 kg) was then subjected to bioassay, while the remainder (about 5 kg) was shipped to Seattle.

Bioassay for compounds in fish meal responsible for gizzard erosion or avian black vomit disease was conducted under standard conditions of environment and animal husbandry. Each bioassay was conducted over a 10 d period, starting with 1 d old broiler chickens. For the first 3 d of the evaluation, 15 birds were fed a basal diet consisting of yellow maize, soybean meal, and vitamin and mineral premixes. During the subsequent 7 d, the birds were fed a test diet composed of equal quantities of fish meal and the basal diet. Feed and water were offered *ad libitum* throughout the experiment. The chickens were housed in individual cages and maintained under continuous lighting and at temperatures between 25 and 30°C.

At the conclusion of the trial, all birds were sacrificed and their gizzards examined visually for signs of inflammation, erosion or ulceration, hemorrhage, or necrosis. The degree of damage observed was assigned a numerical score, according to the following criteria:

Score 0: Complete absence of inflammation or lesions of any type.

Score 1: Generally free of lesions, although small, localized hemorrhage, ulceration, or necrosis may be observed. To receive a score of 1, lesions must not have penetrated the lining of the gizzard into the underlying musculature.

Score 2: Gizzard with many lesions, or more extensive ulceration, hemorrhage, and necrosis than observed in birds receiving a score of 1. Lesions must have penetrated into the musculature underlying the lining of the gizzard.

Score 3: The lining of the gizzard was severely ulcerated with some perforations present. Necrotic areas and hemorrhages were easily observed. Birds that died during the trial and exhibited signs of avian black vomit disease were assigned a score of 3.

An overall toxicological rating was calculated using only data from those birds assigned a score of 2 or 3, since scores of 0 or 1 were considered normal, by the following formula:

$$\text{Rating} = [(2 \cdot \text{number of birds with score 2}) + (3 \cdot \text{number of birds with score 3})] / 15$$

An overall rating of 0 to 0.5 was considered normal, and the fish meal nontoxic to chickens. Fish meal characterized by ratings ranging from 0.6 to 1.0 was regarded as marginally toxic, and was recommended for use in broiler diets at levels not exceeding 10 to 16%. Those receiving ratings of 1.1 to 1.5 were considered to be generally toxic to chickens and use in broiler diets at levels greater than 3 to 5% was not recommended. Fish meals receiving ratings exceeding 1.6 were acutely toxic to chickens and their use was not recommended. Toxic fish meals used in this and subsequent studies were classified in the latter category. Information regarding the bioassay protocol were provided by Emilio Castro (Fundacion Chile; personal communication).

3.2.8 Chemical analysis of fish meals and diets

Upon receipt of the fish meals in Seattle, five 20 g subsamples of each were collected. The respective subsamples were combined, placed into labeled plastic bags, and stored at -20°C until analyzed. Two to 3 d following preparation of the diets, 5 subsamples of about 20 g each were collected. The subsamples were combined, mixed together by gently stirring for 3 min and placed into a single plastic bag for storage at -20°C until analyzed.

Fish meal and diet samples were prepared for analysis by grinding about 20 g of each to a flour-like consistency (40 mesh) with a mortar and pestle. The ground materials were then analyzed for moisture, crude protein, crude lipid, and ash content according to AOAC (1984) methods. Methods of analysis used in this experiment are described in detail in Appendix 2.

3.2.9 Data analysis

Data on growth, feed intake, and efficiency of protein utilization were analyzed statistically using methods appropriate to completely randomized experimental designs. Fisher's Least Significant Difference (LSD) procedure was used following a significant "F" test ($P < 0.05$) to compare treatment means (Little and Hills, 1978); computed P values are shown. Statistical analysis was performed using STATVIEW version 2.0 (Brainpower, Inc., Calabasas, California).

Prior to initiation of the experiment, homogeneity of mean fish weight and body length among the various experimental units was examined by one-way analysis of variance (AOV). At the end of the experiment, arithmetic means of observed TL and wet weight data were again analyzed by AOV. Additionally, the effect of dietary treatment on rate of growth in length (mm TL/d) and weight (g wet weight/d) was assessed by least squares estimation of the unknown parameters a and b in the general linear model:

$$y = a + bx$$

The slope (b) of these lines, taken to represent the overall growth rate of the fish in each treatment replicate, were subjected to AOV.

Protein intake and weight gain data were used to estimate protein efficiency ratios (PER) for each dietary treatment, from which inferences regarding the nutritional value of each fish meal were made. This was done for each treatment replicate by regressing the dependent variable, cumulative weight gain (g), against the independent variable, cumulative protein intake (g) (Baker, 1984). The slope of these lines (b), was taken to represent PER. These data, as well as overall dry feed intake data, were subjected to AOV and mean comparison procedures described above.

Data on wet weight gain and dry feed intake were used to calculate feed efficiencies (F.E.) for each dietary treatment, as:

$$\text{F.E.} = \text{wet weight gain (g)} / \text{dry feed intake (g)}$$

Feed efficiency data were not analyzed statistically, since F.E. and PER are derived from the same data and are related by the protein content of the diet.

3.3 RESULTS

3.3.1 Effects of dietary protein source on growth

Growth in body length and wet weight gain of rainbow trout fed diets containing fish meal that was either acutely toxic or nontoxic to chickens are summarized in Table 3.4. During the 6 week feeding trial, fish in all treatments increased in weight nearly four-fold, from 26 g to 102-106 g, and increased in TL by about 64 mm. There was no significant ($P>0.05$) effect of protein source on final TL, final weight, rate of growth in TL, or rate of weight gain.

3.3.2 Feed intake and efficiency of protein utilization

Feed and protein intake, weight gain, and estimates of feed efficiency and PER are summarized in Table 3.5. In this study, degree of fish meal toxicity in chickens appeared to neither enhance nor decrease diet palatability to rainbow trout. First, fish consumed all feeds readily throughout the study. Second, on a dry matter basis, voluntary consumption of diets containing Chilean fish meal acutely toxic to chickens (C-1-T) was equal to those containing nontoxic Chilean fish meal (C-2-N). Fish fed the diet containing Norwegian LT-1-N fish meal consumed significantly less dry matter than those fed either Chilean fish meal ($P<0.05$). Since all diets were essentially isonitrogenous (Table 3.2), significant differences in dry matter intake also indicated significant differences in protein consumption.

Since rates of growth and final size attained were not significantly different among the various treatment groups, feed efficiency and PER for fish fed the diet consumed in the least quantity (LT-1-N) were somewhat higher than for those fed either Chilean fish meal. The differences were quite small, however, and analysis of variance showed that the mean PER values obtained were not significantly different among treatments ($P>0.05$).

3.3.3 Clinical findings

At each weekly sampling point, all fish in each tank were individually examined for external signs of infectious or noninfectious diseases. In all cases, the fish were robust, and except for some evidence of fin nipping associated with rearing under high densities, no external abnormalities were noted. No mortality occurred during the study (Appendix 3).

At trial termination, five fish from each treatment replicate (15 fish from each treatment) were sacrificed for gross and histological evaluation of the internal organs. *In situ* inspection of the visceral mass revealed no obvious inflammation or ulceration of the gastric or intestinal tissues, and no lesions or other abnormalities in the spleen, liver, or kidney. In most fish fed either Chilean fish meal, but in none fed LT-1-N, the visceral mass was light grey to slightly pink in color. Small quantities of ingesta were observed in the intestine of most fish, and the gall bladder of all fish examined was engorged with bile, a finding consistent with fish accustomed to regular feeding. Moderate quantities of visceral fat were observed in all fish, but the pyloric caecae were visible and distinct.

Following *in situ* examination, the stomach of each fish was excised and partially everted for visual evaluation of mucosal tissue morphology. In no case was inflammation or ulceration of the mucosa or muscularis noted. However, the general appearance of the internal structure of the stomach differed among treatment groups. In fish fed the LT-1-N diet, the longitudinal folding of the stomach lining was distinct, and the musculature contracted forcefully when stimulated with a sharpened probe. In contrast, a few fish fed either the toxic C-1-T or nontoxic C-2-N fish meal were found to have somewhat flaccid stomachs, and the distinct folding of the stomach lining noted in the control fish was greatly reduced, giving the mucosa a smooth appearance. Often, the stomach of affected fish

was filled with clear, viscous mucous, but as above, no inflammation or lesions were observed.

Histological evaluation of the cardiac stomach revealed no changes in the cellular structure of the gastric glands, mucosa, or underlying musculature. Liver tissues were histologically normal.

3.4 SUMMARY AND CONCLUSIONS

One hypothesis tested was that toxic fish meal is less palatable than nontoxic fish meal, and reduced growth resulting from reduced feed intake would be observed when diets containing toxic fish meal were fed to juvenile rainbow trout. This was clearly not the case in this study, since growth in weight and body length was equal among all treatment groups, and feed consumption was slightly higher in groups fed toxic fish meal. This conclusion is further strengthened when the dietary fish meal level is considered: diets contained Chilean fish meal at levels of about 66%, compared to 60% for LT-1-N. Moreover, given that the diets were nearly isonitrogenous and very similar in lipid content, the lower intake of diets containing LT-1-N was not likely correlated with energy intake.

The hypothesis that toxicity of fish meal to chickens is positively correlated with reduced nutritive value to rainbow trout was not supported by this study. This suggested that the assumptions underlying this hypothesis were at least in part incorrect. First, it was predicted that toxic fish meals would have reduced nutritional value proportional to the degree to which the essential amino acids in the raw material were degraded to their corresponding amines. Second, it was predicted that ingestion of the amines themselves would acutely alter the metabolic activity or function of the digestive tissues, thereby compromising the ability of the animal to absorb or otherwise optimally utilize the ingested protein. Together, these two factors were predicted to measurably reduce the nutritive value of the fish meal to the fish. The available data did not support this.

It is possible that real, albeit small, differences in the nutritive value of the various protein sources existed and that the use of the PER method was inadequate to detect them. PER indirectly estimates the nutritive value of protein. Since weight gain rather

than protein accretion (true growth) is used to calculate PER, small differences in the fate of ingested nutrients related to imbalances or deficiencies may not be detected. This is particularly true when fish are fed to satiation and intake may greatly exceed metabolic demand. In that case, dietary protein not used for true growth or energy will be converted to lipid.

Changes in gastric tissue morphology related to fish meal quality (toxicity to chickens) previously reported were not observed in this study. However, several factors must be considered when interpreting these data. First, the study included only one toxic fish meal that was manufactured from raw material of unknown quality under conditions not specified by the supplier. It is possible that the sample was not representative of toxic fish meal in general. Second, a toxic, volatile component may have been lost from the fish meal during storage. Third, the disease processes related to fish meal toxicity may be chronic, or may vary according to fish species or another unknown factor. For example, Watanabe et al. (1987) reported gross pathology in rainbow trout in fewer than 7 weeks, while Romero and Castro (1989) found pathology in coho salmon only after 4 months. The 6 week duration of the present study may have been insufficient for detectable morphological changes to develop, although given the available information, it was expected that some change at the histological level would be observed.

Data obtained in this study did not support a correlation between toxicity of fish meal to chickens and abnormal digestive tissue morphology in rainbow trout, and seemingly contradict the findings of both Watanabe et al. (1987) and Romero and Castro (1989). Also, too few data were available to establish whether biogenic amine content and nutritive value of fish meal to salmonids were correlated. Studies into specific factors related to the use of histamine content and fish meal toxicity bioassays as quality control criteria for Chilean fish meals used in the diets of

salmonids were clearly warranted. Three areas for additional research were to: (1) establish the role of histamine and thermal abuse on the genesis of gastric tissue abnormalities in rainbow trout; (2) examine the relationship between the results of toxicity bioassays in chickens and the nutritive value and palatability of fish meal to rainbow trout; and (3) describe the effects of histamine content and thermal abuse on the nutritive value of protein in the diets of rainbow trout.

Studies conducted to address each research area are described in the following chapters: Effects of histamine and thermal abuse on gastric morphology, Chapter 4; biotoxicity and the nutritive value and palatability of fish meal, Chapters 5, 6, 7; and the effects of added histamine and thermal abuse on nutritive value of proteins, Chapter 8.

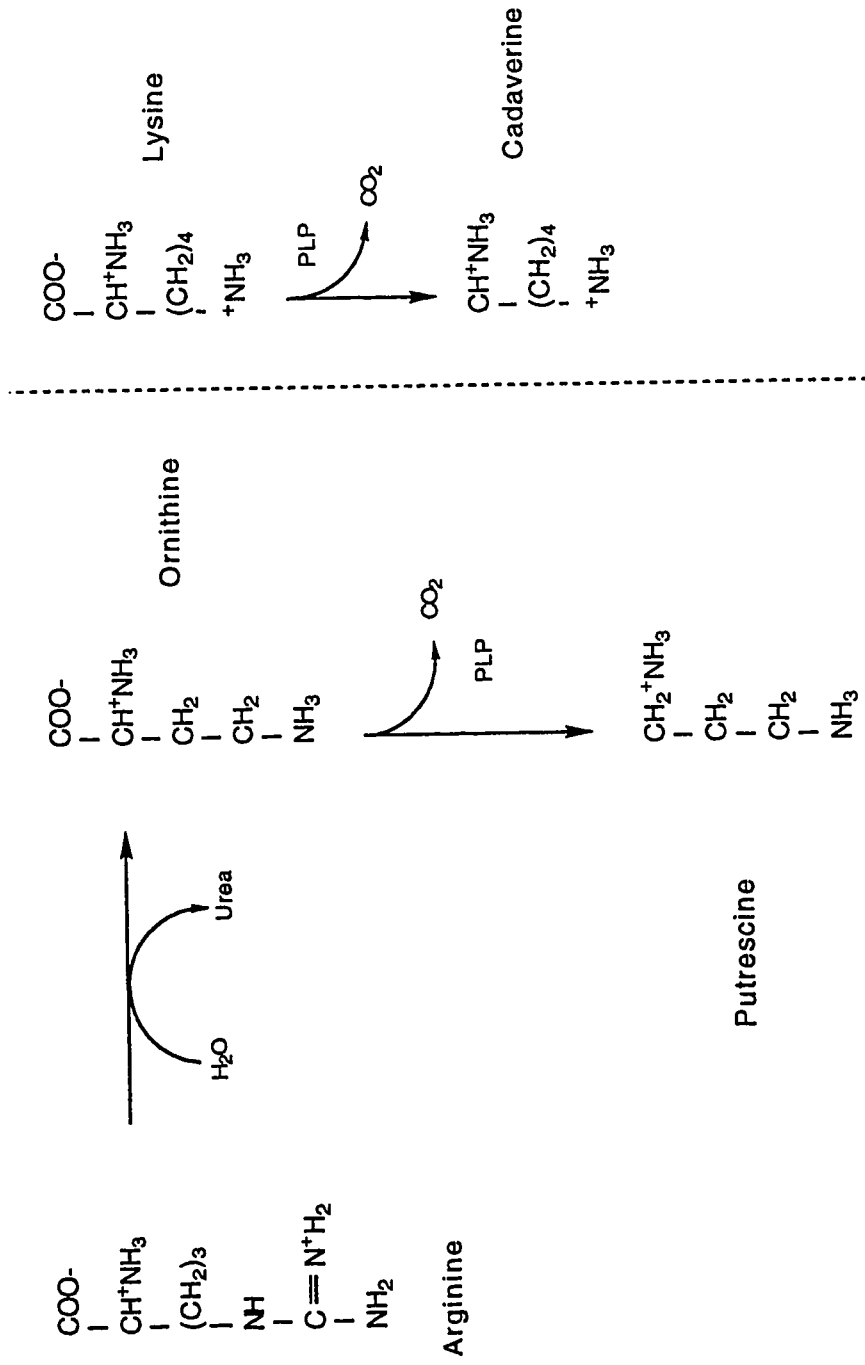


Figure 3.1. Formation of putrescine and cadaverine by decarboxylation of arginine and lysine.

Table 3.1. Proximate composition (% dry weight^a) and histamine content (mg/kg wet weight^b) of the fish meals.

Protein source	LT-1-N	C-1-T	C-2-N
Toxicity ^c	N	T	N
Origin	Norway	Chile	Chile
Dry matter	93.78	92.42	92.12
Protein	79.16	73.20	73.57
Lipid	9.24	8.05	7.84
Ash	11.20	16.88	16.64
Histamine	199	1321	ND ^d

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

^b Method of analysis not specified. Data provided courtesy of Department of Marine Resources, Fundacion Chile, Santiago, Chile.

^c Causative of gizzard erosion in chickens as determined by 7 d chick bioassay: T = toxic; N = nontoxic. Assay conducted by Department of Marine Resources, Fundacion Chile, Santiago, Chile.

^d Not determined.

Table 3.2. Formulation of the experimental diets (% dry ingredients).

Ingredient ^a	Diet		
	LT-1-N	C-1-T	C-2-N
LT-1-N fish meal	60.2		
C-1-T fish meal		66.0	
C-2-N fish meal			65.9
Herring oil	10.7	11.0	11.2
Wheat starch	15.0	15.0	15.0
Alpha-cellulose	11.9	5.7	5.7
Ascorbic acid (50% active)	0.1	0.1	0.1
Trace mineral premix ^b	0.1	0.1	0.1
Vitamin premix ^c	1.5	1.5	1.5
Choline Chloride (70% liquid)	0.5	0.5	0.5

^a Sources of ingredients were: fish meal, Fundacion Chile (Santiago, Chile); herring oil, B.C. Packers, Inc. (Richmond, B.C., Canada); wheat starch, Moore-Clark Co. (La Connor, Washington); alpha-cellulose, ICN Biomedicals, Inc. (Costa Mesa, California); ascorbic acid, Moore-Clark, Co. (La Connor, Washington); trace mineral premix, Moore-Clark Co. (La Connor, Washington); vitamin premix, Moore-Clark Co. (La Connor, Washington); choline chloride, Sigma Chemical Company. (St. Louis, Missouri).

^b Trace mineral premix supplied the following per kilogram of diet: ZnSO₄, 185 mg; MnSO₄, 55 mg; CuSO₄, 3.9 mg; KIO₃, 17 mg.

^c Vitamin premix supplied the following per kilogram of diet: Vitamin A acetate or palmitate, 6612 IU; Alpha tocopheryl acetate, 503 IU; menadione sodium bisulfite complex, 28 mg; thiamine mononitrate, 46 mg; riboflavin, 53 mg; pyridoxine HCl, 38 mg; d-calcium pantothenate, 115 mg; niacin, 220 mg; d-biotin, 1.45 mg; Vitamin B₁₂, 0.06 mg; myoinositol, 132 mg; folic acid, 16.5 mg; Vitamin D₃, 441 mg.

Table 3.3. Proximate compositions (% dry weight) of the experimental diets^a.

	Diet		
	LT-1-N	C-1-T	C-1-N
Dry matter	90.26	90.00	89.40
Protein	49.37	50.68	49.87
Lipid	17.41	17.82	18.33
Ash	10.21	13.07	13.10

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

Table 3.4. Mean growth of rainbow trout fed the experimental diets for 6 weeks^a.

Diet	Final length ^b (mm TL)	Growth rate ^c (mm TL/d)	Final weight (g)	Growth rate (g/d)
LT-1-N	190.0 (2.0)	1.56 (0.04)	102.3 (2.8)	1.83 (0.07)
C-1-T	190.3 (1.2)	1.55 (0.03)	106.1 (4.1)	1.92 (0.08)
C-1-N	189.7 (0.8)	1.55 (0.05)	105.1 (1.3)	1.92 (0.07)
	P = 0.867	P = 0.965	P = 0.331	P = 0.305

^a Data shown are means (\pm SD) of 3 replicates. Means were not significantly different ($P > 0.05$).

^b Initial total length (TL) and wet weight was 126.4 ± 0.8 mm and 26.4 ± 0.4 g (mean \pm SD), respectively.

^c Rates of growth shown are the slope coefficients from the regression of total length or weight (y) on time (x).

Table 3.5. Feed intake, weight gain, and efficiency of diet and protein utilization by rainbow trout fed the experimental diets for 6 weeks^a.

Diet	Intake (g)		Weight gain (g) ^b	F.E. ^c	PER ^d
	Dry feed	Protein			
LT-1-N	58.21 (1.73) ^X	28.76 (0.86) ^X	75.90 (3.12)	1.30 (0.02)	2.58 (0.02)
C-1-T	63.09 (1.83) ^Y	31.25 (0.90) ^Y	79.51 (3.66)	1.26 (0.03)	2.48 (0.07)
C-2-N	61.86 (0.67) ^Y	31.57 (0.34) ^Y	78.74 (1.29)	1.28 (0.04)	2.47 (0.11)
	P=0.017	P=0.007			P=0.205

^a Data shown are means (\pm SD) of three replicates. Values in columns followed by the same letter are not significantly different ($P>0.05$).

^b Initial weight: 26.4 ± 0.4 g (mean \pm SD).

^c Feed efficiency calculated as wet weight gain (g) / dry feed intake (g)

^d Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

Chapter 4

Effects of Histamine on the Development of Digestive Tract Abnormalities in Rainbow Trout.

4.1 INTRODUCTION

Gross morphological changes in the proventriculi and gizzards of chickens have been used as reliable indicators of toxicity by both researchers investigating the effects of various endogenous compounds in fish meal and by those seeking to guarantee the quality of products intended for use in poultry diets (Umemura et al., 1981; Castro, 1987). In general, chickens exhibit discernable lesions or ulcerations when fed toxic fish meal for 5 to 10 d, and in severe cases, the birds may succumb to black vomit disease (Johnson and Pinedo, 1971). In recent years, the relationship between degree of toxicity and severity of the resultant pathology has been well defined, permitting the development of standardized bioassays using chickens as test animals, similar to that described in Chapter 3.

Considerable experimental evidence now exists indicating that dietary histamine and its derivative, gizzerosine, stimulate gastric acid secretion in chickens by their action on the H-2 receptor of histamine (Masumura et al., 1985; Miyazaki and Umemura, 1987). In studies by Miyazaki and Umemura (1987), chickens fed diets containing histamine or a mixture of casein and histamine that had been exposed to heat (Umemura et al., 1981) exhibited significantly reduced gastric pH and increased incidence of gizzard erosion, compared to those fed the control diet. Administration of cimetidine, an H-2 receptor antagonist, or an antacid composed of aluminum hydroxide and magnesium hydroxide (0.9:1.0 w/w) elevated gastric pH and prevented lesion formation, thereby verifying the role of gastric acid in the development of gizzard erosion in chickens. Watanabe et al. (1987) suggested that gastric erosion in rainbow

trout may also be induced by a similar mechanism, although that hypothesis was not tested.

Regulation of gastric acid secretion in teleosts is not well understood, but the available data suggest that the mechanisms used by these animals are similar to those of other vertebrates. In humans and other higher animals, gastric acid secretion is stimulated by histamine via H-2 receptors, by acetylcholine via M₁ muscarinic receptors, and by gastrin via gastrin receptors in the membranes of parietal cells (Gespach et al., 1988; Ganong, 1989). In response to vagally-induced stimulation, ganglionic cholinergic neurons innervating the parietal cells release acetylcholine, which in turn stimulates the secretion of gastrin, histamine from non-mast cells concentrated in the gastric mucosa, pepsin, and gastric acid. Intracellular events are sequential, so that stimulation of one receptor potentiates the response of another (Ganong, 1989). Enzymatic systems responsible for neuromediator metabolism in man and other vertebrates are located close to their target tissues. They include histidine decarboxylase, histamine methyl transferase, choline acetyltransferase and acetylcholinesterase, tyrosine hydroxylase and monoamine oxidase (Bryant et al., 1982; Heitkemper and Marotta, 1983).

The teleostean stomach is innervated by the vagus nerve, and stomach distention stimulates gastric acid secretion (Smit, 1967). A dose-dependent increase in gastric acid secretion by the Atlantic cod (*Gadus morhua*) following administration of exogenous histamine was observed by Holstein (1975a). Histamine apparently acts through the H-2 receptor in this species, since its effect is blocked by the H-2 receptor antagonist, metiamide (Holstein, 1976). High concentrations of non-mast cell histamine have been found in the gastric mucosa of all teleosts studied (Reite, 1969; Lorenze et al., 1973). Together, these data provide indirect evidence that histamine is an important regulator of gastric acid secretion in fish. In

contrast to higher vertebrates, gastrin has no apparent effect on gastric acid secretion by Atlantic cod (Holstein, 1975a). In nonmammalian vertebrates (Larsson and Rehfeld, 1977), cerulein-like peptides may function as gastrin, but their effects in teleosts have not been fully evaluated. Further research is needed to fully elucidate the mechanisms used by teleosts for gastric acid regulation. Nevertheless, given the fact that gastric acid secretion can be induced by exogenous histamine and prevented by H-2 receptor antagonists in both chickens and fish, it is reasonable to suggest that toxic amines found in fish meal may have similar physiological effects on both species.

Two major pathways for the metabolism of histamine have been identified in humans and other species of animals (Figure 4.1). Histamine is primarily metabolized by two enzymes, diamine oxidase (DAO) and histamine-*N*-methyltransferase (HMT) (Douglas, 1980), but the relative importance of the two pathways vary among species. For example, the methylation pathway predominates in man, mice, rats, and hamsters, while the oxidative deamination pathway predominates in rats and guinea pigs (Wetterqvist (1978) cited by Taylor, 1986). The pathway of histamine metabolism also varies among tissues in the animal. In man, HMT is found in many tissues, including liver, spleen, lung, colon, small intestine, and stomach, while DAO is localized almost exclusively in the small intestine (Hesterberg et al., 1984). Interestingly, DAO, but not HMT, was found in crude extracts of intestinal tissues obtained from seven species of teleosts (Holstein, 1975b). Intestinal histamine metabolizing enzymes have considerable physiological significance, since they prevent the absorption of unmetabolized histamine into the circulation (Holstein, 1975b; Taylor, 1986).

The involvement of food-borne histamine in scombroid-fish poisoning of humans has been recently verified (Morrow et al., 1991). While oral administration of pure histamine to humans has produced a

toxic response, the severity was much less than expected (Motil and Scrimshaw, 1979) suggesting that histamine toxicity was potentiated by other compounds present in spoiled fish. Hui and Taylor (1985), demonstrated with female Sprague-Dawley rats that putrescine and cadaverine, as well as tyramine, β -phenylethylamine, and tryptamine potentiate the toxicity of histamine *in vivo* by inhibiting histamine-metabolizing enzymes. The administration of inhibitors increased the amount of unmetabolized histamine and decreased the amount of metabolites reaching the urine, and apparently prolonged the half-life of unmetabolized histamine.

The interactive effects of thermal abuse and histamine on the genesis of compounds in fish meal toxic to chickens (Umemura et al., 1981) and rainbow trout (Watanabe et al., 1987) have been studied extensively. However, little effort has been focused on the activity of other amines that may act as potentiators of histamine toxicity, especially putrescine and cadaverine, despite the fact that relatively high levels of these compounds are often formed concurrently with histamine during the spoilage of fish. For example, Salguero-Fernandez and Mackie (1987) found that herring (*Cupea harengus*) stored at 5°C for 7 d contained 531 mg histamine, 62.4 mg putrescine, and 366.6 mg cadaverine/kg wet weight. Whole pacific mackerel (*Scomber japonicus*) stored at 20°C for 3 d contained 3770 mg histamine, 229 mg putrescine, and 406 mg cadaverine/kg wet weight (Watts and Brown, 1982). These amines are not volatile at the temperatures of fish meal processing and are concentrated, along with histamine, during the manufacture of whole meal. Research with broiler chickens has shown that putrescine and cadaverine individually or in combination with histamine promoted proventricular dilation accompanied by hyperplasia of the glandular epithelium, gizzard enlargement, and in some cases, multifocal erosions of the gizzard epithelium (Stuart et al., 1986). Putrescine, cadaverine, and histamine are all substrates of intestinal DAO in teleosts (Holstein, 1975b). In the study by Holstein (1975b), DAO had

a higher affinity for histamine than for putrescine or cadaverine, but the two diamines were found to compete with histamine. Therefore, it was predicted that putrescine and cadaverine may potentiate histamine activity in fish by increasing the absorption of unmetabolized histamine, as described for rats. Given these data, it seemed reasonable to consider the effect of putrescine and cadaverine as potentiators of histamine activity during the development and evaluation of model protein sources in this study.

Few data were available regarding the duration of exposure to histamine and other amines found in toxic fish meal on the development of changes in gastric tissue morphology of aquatic species. As described in Chapter 2, studies by Watanabe et al. (1987), who used fish meals to which graded levels of histamine had been added, followed by heat treatment, indicated that the development of clinical pathology in rainbow trout required at least 6 weeks exposure to the toxic substances. In contrast, Romero and Castro (1989) observed overt pathology in coho salmon only after 4 months exposure to commercially prepared fish meal that was acutely toxic to chickens. Studies comparing the effects of toxic products prepared in the laboratory with those manufactured by commercial processors have not been conducted, and no effort has been made to determine the relationship between the level of amines present and the duration of exposure required to produce the observed pathology.

Data obtained in the preliminary study described in Chapter 3 were insufficient to either confirm or refute the findings of Watanabe et al. (1987) and Romero and Castro (1989) cited above. It was clear, therefore, that additional study of fish meal toxicity to aquatic species would require that suitable experimental treatments of fish meal and other protein sources be developed that would produce well-defined pathological changes in the digestive tissues of fish. Furthermore, data comparing the effects of experimentally

prepared materials and commercially manufactured products were needed. This experiment was designed to address these requirements.

The objectives of this study with rainbow trout were to: (1) determine the effects of histamine alone and in combination with putrescine and cadaverine on the development of gastric tissue abnormalities; (2) determine whether the response is altered by the type of protein treated with the various amines; (3) determine if the effects of the various model systems differ from those produced by toxic fish meal obtained from commercial sources; (4) determine the duration of exposure required to produce gross and microscopic changes in the target tissues; and (5) measure the effects of the various treatments on feed consumption, growth, and feed efficiency.

4.2 METHODS AND MATERIALS

4.2.1 Test protein sources

Seven sources of dietary protein, including vitamin-free casein and six different fish meals, were used in the present study. One fish meal, LT-1-N, was obtained during May 1990, as described in Chapter 3. The remaining fish meals, obtained from Fundacion Chile during April 1991 were: two samples of Norwegian LT-94 (LT-2-N and LT-3-N) and three fish meals prepared by commercial processors in Chile from Jack mackerel (*Trachurus murphyi*) (C-3-N, C-4-T, and C-5-T). (Note: LT-2-N and LT-3-N were separate subsamples obtained from a single bulk lot of Norwegian LT-94 fish meal. Similarly, C-4-T and C-5-T were separate subsamples obtained from a single bulk lot of Chilean mackerel meal). The protein sources were evaluated using the standard chick bioassay described in Chapter 3 and found to be either nontoxic (all LT fish meals and C-3-N) or acutely toxic (C-4-T and C-5-T). The fish meals obtained during 1991 were packaged into sealed plastic bags and stored at -20°C for 3 months prior to incorporation into the test diets, whereas the LT-1-N fish meal had been stored frozen for about 14 months prior to use in this trial. Proximate composition and biogenic amine content of the fish meals are summarized in Table 4.1.

In this study, it was hypothesized that (1) the effects of histamine, putrescine, and cadaverine on the genesis of gastric tissue pathology of rainbow trout would be independent of protein source and (2) the activity of histamine would be potentiated by the presence of putrescine and cadaverine. Based on the work of Watanabe et al. (1985), it was assumed that heating of the amine-treated protein sources was required to produce toxic derivatives. Therefore, the seven protein sources were incorporated into diets in their native state, or altered by the addition of various combinations of histamine, putrescine, and cadaverine and heat

treatment, yielding nine dietary protein sources. They were: (1) casein; (2) casein combined with histamine, then heated; (3) casein combined with histamine, putrescine, and cadaverine, then heated; (4) LT-1-N; (5) LT-2-N combined with histamine, then heated; (6) LT-3-N combined with histamine, putrescine, and cadaverine, then heated; (7) C-3-N mackerel meal; (8) C-4-T mackerel meal; and (9) C-5-T mackerel meal combined with histamine, putrescine, and cadaverine, then heated.

Dietary levels of histamine, putrescine, and cadaverine of 2,000, 500, and 500 mg/kg dry diet, respectively, were used. Since the protein sources were treated with the various combinations of biogenic amines and heat before incorporation into the test diets, the quantity of each amine added to the individual protein sources varied slightly according to its proximate composition and dietary inclusion levels. Sources and purity of the amines were: histamine dihydrochloride (60.384% as free base); putrescine dihydrochloride (54.7% as free base); and cadaverine dihydrochloride (58.4% as free base). All amines were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored at 0-5°C until used.

With one exception (LT-3-N, noted below), dietary protein sources to which amines were added were processed as follows: first, immediately prior to incorporation into the test proteins, the amines were weighed accurately to 0.1 mg, then blended with cool tap water at a rate of 2.0 L water per kg protein source. This solution was gradually added to the appropriate protein source, which was mixed with a Hobart Model N-50 blender at low speed for 10 min. The moistened material was spread to a depth of about 2 cm on aluminum cookie sheets, tightly covered with aluminum foil, and held at -40°C for 24 to 36 h prior to lyophilization.

Lyophilization of the test proteins was conducted using a Thermovac Industries, Inc. (Seattle, Washington) apparatus. After the frozen materials were dried for 3 d without heating, the

partially dried samples were weighed and estimated to contain about 50% moisture. Ambient temperature during lyophilization was -28°C. Drying and heat-treating of the protein sources was completed in a Precision model 625 mechanical convection oven (Precision Scientific Group, Chicago, Illinois) as follows:

The lyophilized protein sources were allowed to thaw at room temperature for about 2 h before being transferred to the convection oven. The uncovered trays were placed on racks spaced about 10 cm apart, and the samples dried at 50°C at minimum air flow for 3 to 4 h. The oven temperature was then increased to 130°C for an additional 5 to 6 h. The protein sources were stirred at 30 to 60 min intervals throughout the heating process to reduce clumping and promote even drying. The dried materials were cooled to room temperature overnight, then transferred to plastic bags for storage at -20°C until incorporation into the test diets.

The method used for drying the amine-treated LT-3-N fish meal was modified to eliminate the lyophilization step when the equipment was found to be ineffective in reducing the moisture content below about 50%. To do this, the amines were blended with 1.0 L cool tap water/kg fish meal. The fish meal was spread onto aluminum cookies sheets to a depth of about 2 cm, and dried in a Precision Model 625 mechanical convection oven at 50°C with minimum air flow for 9 h. The dried material was cooled to room temperature overnight, then reheated to 130°C for 3 h. As before, the protein source was stirred at 30 to 60 min intervals to reduce clumping and promote even drying. The final product was cooled to room temperature overnight and finally transferred to plastic bags for storage at -20°C until incorporation into the test diets.

4.2.2 Diet preparation

Eight test diets based on isonitrogenous substitution of each protein source for casein in the semipurified casein-gelatin control

diet (Shearer, 1988) were used in this study (Table 4.2). Alpha-cellulose and dextrin levels were adjusted to accommodate differences in proximate composition of the various protein sources and to replace macromineral and crystalline amino acid supplements not included in diets containing fish meal. Diet codes are shown in Table 4.2. Proximate composition of the test diets is summarized in Table 4.3.

Blending of the test ingredients into the final dry mix was done in a series of operations to ensure uniform distribution of each component. First, the protein sources were thawed at room temperature for 4 to 6 h, then ground with a hammer mill to a flour-like consistency (40 mesh). Next, the vitamin C, vitamin premix, alpha cellulose, dextrin, carboxymethyl cellulose, and when present, the macrominerals and amino acids were mixed together for 15 min in a twin shell dry blender (The Patterson-Kelley Co., Inc., East Stroudsburg, Pennsylvania). The dry ingredients were combined and mixed together for 15 minutes at low speed with a Hobart Model N-50 mixer. Uniform blending of the dry ingredients was ensured by scraping of the sides and bottom of the mixing bowl repeatedly during mixing and by visual examination of the final dry mix. Fish oil and choline were added simultaneously to the dry ingredients, and mixed for an additional 10 min.

The gelatin solution was prepared by mixing an appropriate volume of the trace mineral solution with boiling tap water, to which dry gelatin was gradually added. The solution was returned to boiling, and when the gelatin was completely dissolved, the solution was added with continuous mixing to the remainder of the ingredients. Finally, the dough was spread onto aluminum cookie sheets to a depth of about 3 cm and frozen at -20°C.

Diets were formed into 1.2 mm diameter pellets by cold extrusion of the partially thawed dough through a 3.2 mm die. Pellets were stored frozen at -20°C until used.

4.2.3 Feeding regimen

Two primary objectives of this feeding trial were to quantify the duration of exposure to toxic fish meals and amine-treated protein sources required to elicit gross and histologically detectable changes in gastric tissue morphology of rainbow trout, and to obtain preliminary information regarding the effects of added amines on feed intake. In order to maximize feed intake and prevent feed wastage, the timed-response feeding strategy described in Chapter 3 was used with the following modifications:

1. Fish were fed twice daily, morning and afternoon, with an interval of about 6 h between feedings. Feed was withheld for 2 d prior to collection of samples for histological evaluation. This period of time was adequate to allow nearly complete emptying of feed from the stomach and upper intestine.
2. Feed was given to the fish in small quantities over a period of 2 min at each feeding interval.
3. As discussed in Chapter 3, small differences in feed palatability were difficult to quantify using feed intake data alone. Therefore, at the end of each feeding period observations regarding feeding behavior or activity of fish in each tank were recorded and a numerical score assigned. Groups of fish that were very active and consumed feed readily received a score of 4, while those that were active, but for which feed intake diminished noticeably were given a score of 3. A score of 2 was given to groups of fish that consumed some feed, but exhibited little activity. Fish that refused feed entirely received a score of 1. While these data could not be analyzed statistically, experience has shown that information of this type is useful, especially where differences in palatability are small.

4. Cumulative feed intake was recorded weekly, rather than at daily intervals.

4.2.4 Experimental facilities, fish, and husbandry

The experiment described in this chapter was conducted in the wet laboratory facilities maintained at the School of Fisheries, University of Washington, Seattle, Washington. Eighteen 38 L glass aquaria, each supplied with ambient temperature ($17.3 \pm 2.1^{\circ}\text{C}$; Appendix 1) dechlorinated municipal water at a rate of 1.5 L/min were used in this study. Dissolved oxygen concentrations were determined at 14 d intervals with a YSI model 51B dissolved oxygen meter (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) and remained near saturation throughout the study. Supplemental aeration was provided by means of an airstone placed into each tank. The facility was illuminated by fluorescent lights in a 12 h light: 12 h dark photoperiod cycle.

Progeny of a single female-male mating (UW female x Nisqually male) of rainbow trout obtained from the School of Fisheries hatchery, University of Washington, were selected for uniform size (mean weight: 5.5 g), then randomly distributed among 18 aquaria (two tanks per dietary treatment) until each contained 30 fish. Randomization of experimental units to the dietary treatments was not required since the data collected were solely descriptive in nature and were not statistically evaluated. Instead, the nine dietary treatments were assigned to the tanks in sequence, since this arrangement was most convenient for feeding and sampling.

In addition to feeding, routine daily activities included measuring water temperature, examining each tank for the presence of dead or moribund fish, and flushing accumulated fecal material from the tanks.

4.2.5 Measurement of growth

On day 0 and at 14 d intervals thereafter for 98 d, the fish in each tank were weighed in bulk, counted, and returned to their respective tanks. Feed was withheld for 16 h prior to sampling.

4.2.6 Collection and preparation of samples for histological evaluation

One sample of 5 fish was collected on day 0. Then, since test diet was fed to fish in two tanks, fish from each treatment were collected for histological evaluation every 14 d by alternating between the two tanks, allowing individual groups of fish 26 d of uninterrupted exposure between sampling points. Fish were sampled in this manner for 98 d. Final samples were collected on day 105. On day 111, the remaining fish were photographed and the trial terminated. Feed was withheld for 40 h prior to collection of samples evaluated histologically.

Fish were selected for histological evaluation using two criteria. First, if no externally visible signs of noninfectious diseases consistent with toxicity were evident (bloating), fish were selected randomly from among each treatment group. Second, once externally visible signs were noted, only the most overtly affected fish were selected. This practice maximized the efficiency with which histologically significant changes in tissue morphology consistent with biotoxicity of the protein sources could be identified.

Fish selected for histological evaluation were individually anesthetized in a 100 mg/L solution of tricaine methanesulfonate, and killed by severing the spinal column with a scalpel immediately posterior to the cranium. The fish were examined for external signs of infectious and noninfectious diseases, and the viscera exposed by incision along the ventral midline. The viscera were examined

visually for gross abnormalities, then the liver, kidney, and the entire stomach were excised with scissors, placed into individual prenumbered cassettes, and immediately fixed in a freshly prepared solution of Bouin's solution (Humason, 1967). After 24 to 36 h, the tissues were removed from the fixative, and washed three times over a period of 72 h with a solution of 70% (v/v) ethanol in distilled water. After the final rinse, the tissues were stored in 70% ethanol under refrigeration (5°C) until submitted for further processing and histological evaluation.

Prior to sectioning, the tissues were dehydrated, cleared, and infiltrated with Polyfin (Triangle Biomedical Sciences, Durham, N.C.), using an Autotechnicon automated tissue processor, followed by embedment in Polyfin. The tissues were sectioned (5 µm thickness), stained with hematoxylin and eosin-phloxine (Luna, 1968), and examined under light microscope by M. Meyers (National Marine Fisheries Service, Seattle, Washington). Photomicrographs of selected tissues sections were obtained using a Microflex UFX-IIA camera mounted on a Nikon Optiphot microscope.

4.2.7 Bioassay of fish meals

Sample collection and bioassay of the fish meals were conducted by Fundacion Chile using the procedures detailed in Chapter 3.

4.2.8 Chemical analysis of fish meals and diets

Upon receipt of the fish meals in Seattle, a 100 g subsample of each was collected as described in Chapter 3, placed into labeled plastic bags, and stored at -20°C until analyzed. Two to 3 d following preparation of the diets, 5 subsamples of about 20 g each were collected. The subsamples were combined, mixed together by gently stirring for 3 min and placed into a single plastic bag for storage at -20°C until analysis.

Fish meal and diet samples were prepared for proximate analysis by oven drying about 20 g of each to constant weight, then grinding the dried material to a flour-like consistency (40 mesh) with a mortar and pestle. The ground materials were analyzed for crude protein, crude lipid, and ash content according to AOAC (1984) methods. Methods of analysis used in this experiment are described in detail in Appendix 2.

4.2.9 Data analysis

Statistical evaluation of growth and feed intake was not possible due to biases inherent in sampling procedures which required removal of fish from the rearing system over the course of the experiment. Fish numbers were reduced from 30 fish per tank to 15 or fewer, and sampling was not random. Weight gain and feed intake data, as well as calculated feed efficiencies [weight gain (g)/dry feed intake (g)] were tabulated for informational purposes only.

Feeding activity scores often varied substantially on a day-to-day basis, making graphs produced from the raw data difficult to interpret. The overall pattern of feeding activity was described for fish fed each dietary treatment by means of moving averages. In this study, moving averages were calculated by replacing each value in the series of equally spaced feeding activity data by the mean of itself and four of the values directly preceding it and following it. Graphs were produced from these data that showed trends in feed palatability among the various treatments. Trends in feeding activity, together with dry feed intake data, were used to make inferences regarding the effects of the various treatments on feed palatability.

4.3 RESULTS

4.3.1 Effects of amine treatment and protein source on growth, feed utilization, and feed palatability

Growth in weight, feed intake, and feed efficiency data for rainbow trout fed the experimental diets for 14 weeks are summarized in Table 4.4. Effects of protein source on feed palatability are shown in Figure 4.2.

As previously discussed, growth, feed intake, and feed efficiency data could not be analyzed statistically because fish were selectively removed from the tanks at 2 week intervals for gross and histological evaluation. However, the available data (Table 4.4) indicated that supplemental histamine, either alone or in combination with putrescine and cadaverine had no apparent effect on feed intake or growth of rainbow trout fed casein or LT fish meal based diets. Fish fed the toxic C-4-T fishmeal diet consumed about 20% more dry matter and gained about 42% more weight than those fed diet containing nontoxic C-3-N fish meal. Similar results were obtained when histamine, putrescine, and cadaverine were added to the toxic C-5-T fishmeal. Interestingly, feed efficiencies for fish fed diets containing the toxic fish meals were consistently better (10-17%) than those fed the nontoxic Chilean fish meal (C-3-N).

Feed palatability, as described by feeding response scores, was affected by amine supplementation, but the effect appeared to depend on the source of dietary protein (Figure 4.2). Among groups of fish fed the casein-based diets, feed acceptability, but not dry matter intake, was improved by amine supplementation. The effect of amine supplementation was especially apparent during the latter part of the trial. In contrast, neither feed acceptability nor feed intake by fish fed diets containing LT fish meals were apparently affected by amine supplementation. In general, the toxic Chilean fish

meals were consumed more readily than their nontoxic counterpart, but amine supplementation did not enhance this effect.

4.3.2 Clinical findings

At biweekly intervals for 98 d, and after 105 d, five fish from each dietary treatment were sacrificed for gross and histological examination of the internal organs. In this study, feeding of diets containing protein sources that were treated with histamine alone, histamine in combination with putrescine and cadaverine, or Chilean fish meal toxic to chickens were associated with the development of gross morphological changes in the stomachs of rainbow trout. The abnormalities developed gradually, and were first observed after different periods of exposure, depending on dietary protein source. During the first 28 d of the study, *in situ* inspection of the visceral mass revealed no obvious inflammation or ulceration of the gastric or intestinal tissues or other abnormalities in the spleen, liver, or kidney in fish fed any of the test diets. Very small quantities of ingesta were present in the lower intestine of most fish sampled, and the gall bladders of all fish examined were engorged with bile, a finding consistent with fish accustomed to regular feeding. Moderate quantities of visceral fat were observed, but the pyloric caecae were visible and distinct.

Effects of dietary amine supplementation first became evident after 42 d feeding with the experimental diets, and was first noted among groups of fish fed the CG(H), CG(HPC), and LT-2-N(H) diets. Severe distention of the stomachs, accompanied by the presence of partially digested feed and a thin, mucous-like liquid was observed in all fish examined. No inflammation, lesions, or ulcerations of the mucosa or muscularis were observed, but the tissues were stretched to the extent that longitudinal folding of the stomach lining consistent with normal structure was absent. In general, the severity of the condition worsened with continued feeding, but at the 56 d sampling, no additional treatment groups were affected.

After 70 d, all groups given diets containing amine-treated protein sources exhibited clinical signs consistent with those reported above. However, the number of fish affected and the severity of the observed condition varied among the treatment groups. The stomachs of fish fed the CG(H) and CG(HPC) diets were grossly distended with mucous and partially digested feed. In some cases, the pyloric caecae and intestine were also affected, but the condition was not noted among all fish examined. In contrast, fish fed the amine treated fish meals (LT-2-N(H), LT-3-N(HPC), C-5-T(HPC)) or the untreated Chilean toxic fish meal (C-4-T) generally exhibited mild to moderate clinical signs, although the observed abnormalities were clearly evident. Observations made after 98 d and 105 d were similar to those reported for the 70 d sample. Overall survival was 95-100% in all treatments groups (Appendix 3). Mortality did not appear related to dietary treatment.

At trial termination (111d), the fish were fed their respective diets and about 16 h later, three fish from each group were sacrificed. From each three fish sample, one fish that was selected for photographic documentation of the clinical signs described above. Figures 4.3, 4.4, and 4.5 depict the gross external and internal morphology of the fish fed the various dietary treatments.

In this study, samples of stomach, liver, and kidney were collected for histological evaluation at 14 d intervals for 98 d, and after 105 d feeding. These data were to be used to identify the duration of exposure to the various treatments required to elicit a histologically detectable response. Examination of the stained tissues commenced with those collected after 98 d feeding, since at that time, gross changes in stomach morphology were observed in all groups fed either the amine-treated protein sources or the untreated C-4-T fish meal. In this study, no erosion or exfoliation of the stomach mucosa or necrosis of the serous glands of the cardiac or transitional region of stomach was observed in any dietary

treatment group. In cases where the stomach was distended, the rugae were attenuated (flattened), but the underlying cellular structure was histologically normal. In short, no cellular changes consistent with impaired gastric function were observed. Typical cross sections of gastric tissues obtained from fish fed the various diets are shown in Figures 4.6, 4.7, and 4.8. Liver and kidney tissues in all fish examined were histologically normal.

4.4 SUMMARY AND CONCLUSIONS

Under the conditions of this 111 d study, rainbow trout fed diets containing fish meal of Chilean origin that was acutely toxic to chickens exhibited moderate to severe distention of the stomach, accompanied by the presence of a thin, mucous-like liquid. No inflammation, lesions, or ulcerations of the mucosa or muscularis were observed, but the tissues were stretched to the extent that the longitudinal folding of the stomach lining consistent with normal structure was greatly attenuated or absent. Cellular structure was found to be histologically normal, however, indicating that the toxic compounds did not exert a direct effect on cell morphology in this species of fish. In contrast, coho salmon reared in seawater netpens and fed diets containing toxic fish meals for 112 d developed severe gastric ulceration similar to that observed in chickens (Romero and Castro 1989). Direct comparisons between the results of these studies cannot be made, since different fish meals, species of fish, and rearing conditions were used in each. However, the fact that the response to the toxic fish meals differed in the two trials suggests that predictions of fish meal toxicity to aquatic species based on the results of toxicity bioassays in chickens may not be valid.

Considerable experimental evidence now exists confirming the role of dietary histamine and its related derivative, gizzerosine, in the development of gastric erosion and ulceration in chickens (Chapter 2). Watanabe et al. (1987), who used fish meals to which graded levels of histamine had been added, followed by heat treatment, indicated that histamine both directly and through heat-mediated chemical changes induced gross and cellular abnormalities in the gastric tissues of rainbow trout. From their data, they inferred that the compounds responsible for fish meal toxicity to chickens were also toxic to fish, but further studies required to test this hypothesis were not conducted.

One important factor not considered by Watanabe et al. (1987) was the possibility that the effects they attributed to the supplemental histamine treatment were influenced by endogenous compounds found in the fish meal used in their study. As discussed in Chapter 2, degradative amines with demonstrated or putative roles in fish meal toxicity to chickens are concentrated in the aqueous fraction of the press liquor during the manufacture of whole meal. As shown in Table 4.1, fish meals containing relatively high levels of these amines are not necessarily toxic to chickens. However, toxic derivatives can be formed from the precursor compounds by heating the fish meal under laboratory conditions, indicating that this factor must be considered when evaluating the effects of single compounds, such as histamine, in model protein systems.

Putrescine and cadaverine may act as potentiators of histamine activity *in vivo* by inhibiting intestinal histamine metabolizing enzymes, thereby increasing the absorption of unmetabolized histamine into the circulation. In the present study, it was predicted that the toxic effects of supplemental histamine and heat treatment observed by Watanabe et al. (1987) would be exacerbated by heat-mediated interactions between the added histamine and endogenous compounds, including putrescine and cadaverine, in the test fish meal. To test this hypothesis, two unrelated protein sources, casein and LT fish meal were used. Histamine treatment of the protein sources induced gross distention of the stomach after 42 d feeding, but the condition was more severe and involved a higher proportion of the fish in groups fed the casein-based diet than in those fed the diet containing fish meal. Supplemental putrescine and cadaverine did not hasten the development of stomach distention, nor increase the proportion of fish affected, irrespective of dietary protein source. In fact, groups of fish fed the LT diet supplemented with histamine, putrescine, and cadaverine exhibited stomach distention after 70 d feeding, whereas

groups fed the LT diet supplemented only with histamine developed stomach distention after 42 d feeding. These data suggest that histamine or its derivatives may exert their effects independently from other endogenous compounds in the test fish meal.

Treatment of the toxic fish meal C-5-T(HPC) with histamine, putrescine, and cadverine, followed by heat treatment, did not increase its toxicity, relative to the untreated toxic fish meal, C-4-T. On a dry weight basis, the C-4-T diet contained about 340 mg histamine/kg, 19 mg putrescine/kg, 370 mg cadaverine/kg, and 6 mg gizzerosine/kg from endogenous sources. Supplementation of the toxic fish meal with histamine (2,000 mg/kg dry diet), putrescine (500 mg/kg dry diet), and cadaverine (500 mg/kg dry diet) followed by heating, neither hastened the development nor increased the severity of gastric abnormalities, compared to the unsupplemented toxic fish meal (C-4-T). The data therefore suggest that the endogenous levels of toxic compounds in the fish meal were above the threshold level necessary to produce a discernable response.

Although gross changes in gastric tissue morphology were observed among all groups of fish fed diets containing either toxic Chilean fish meals, protein sources treated with histamine, or those treated with histamine, putrescine, and cadaverine, followed by heat treatment, physiological function of the gastric tissues were apparently unaffected. Gastric tissues from fish exhibiting severe stomach distention were histologically normal, indicating that these compounds did not have direct, detrimental effects on cellular function, and that the observed distention did not result from changes in cell morphology. The fact that neither feed intake, feed efficiency, or growth was impaired in groups exhibiting severe distention of the stomach provides additional evidence that cell function was not compromised by the observed condition.

One of the objectives of the research was to document the duration of exposure to toxic fish meals required to produce well defined gross and microscopic changes in the gastric tissues of rainbow trout. These data are needed to develop criteria for the evaluation of model protein systems and for the development of bioassays of fish meal toxicity to aquatic species. Data provided by Romero and Castro (1991) indicated that chronic exposure to toxic fish meal may be required to evoke detrimental changes in the gastric tissues of coho salmon, and that suggestion was confirmed by the present study. It is also possible species-specific differences exist in their sensitivity to these compounds, and that bioassay conditions may have to be adjusted to accommodate these differences. Because practical considerations in diet formulation limit the amount of fish meal that can be included, future research on this topic should include evaluation of fish meal extracts which may be incorporated into the diets at comparatively high levels, and fed to the fish for short periods of time. Considerable research will be required to validate dose-response relationships before the results of these assays could be applied to the routine evaluation of fish meal, however.

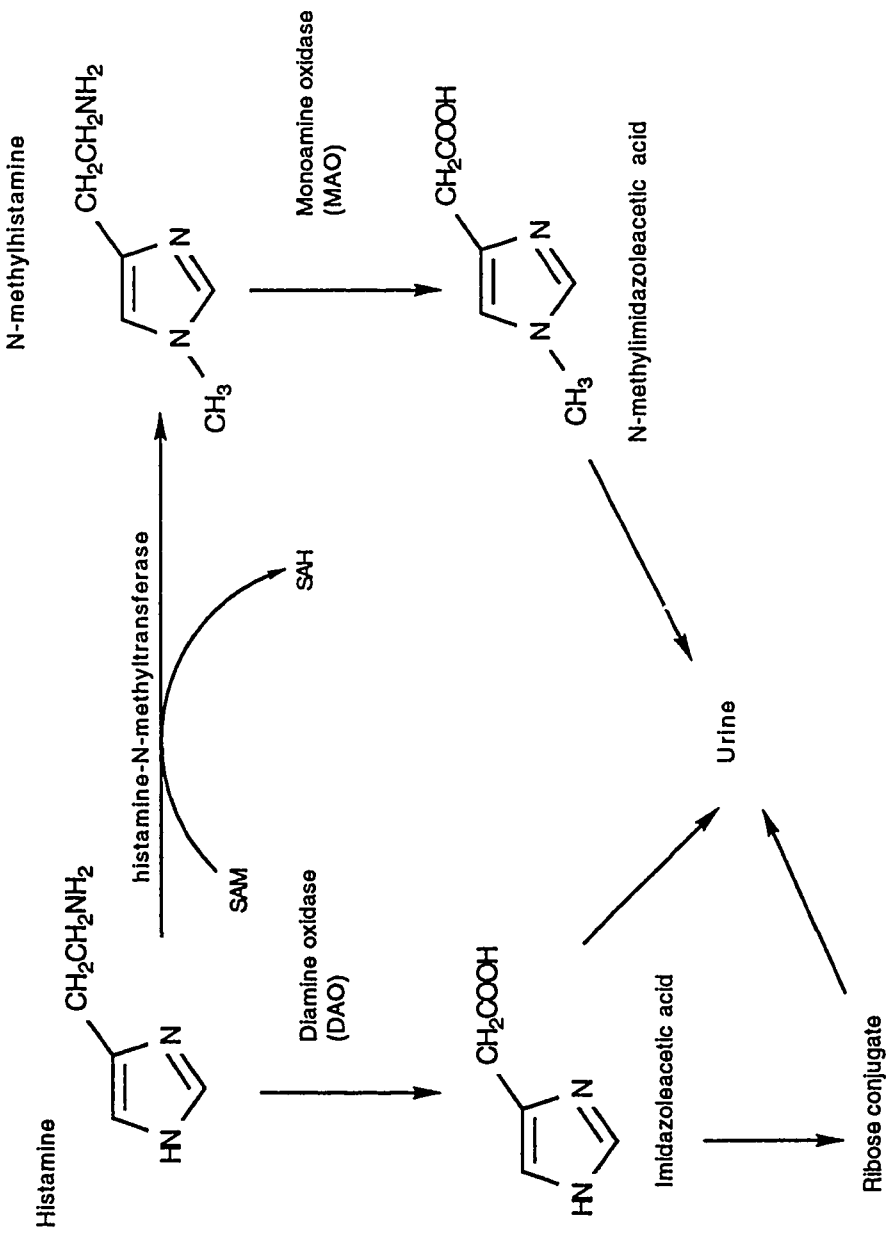


Figure 4.1. Pathways for the *in vivo* metabolism of histamine.

Figure 4.2. Feeding response scores for rainbow trout fed the experimental diets. Numerical scores were assigned following subjective evaluation of feeding response: (1) indicates little or no response, (2) indicates minimal feeding response, (3) indicates average response, and (4) indicates excellent response.

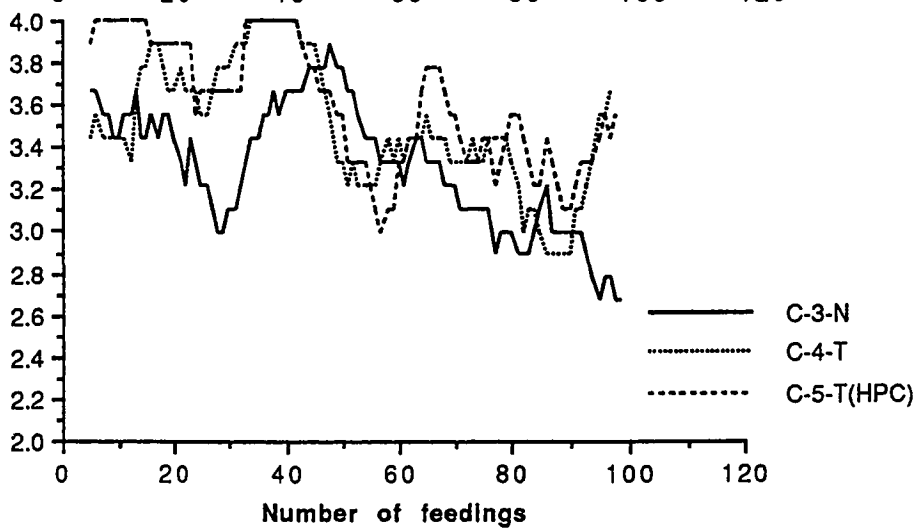
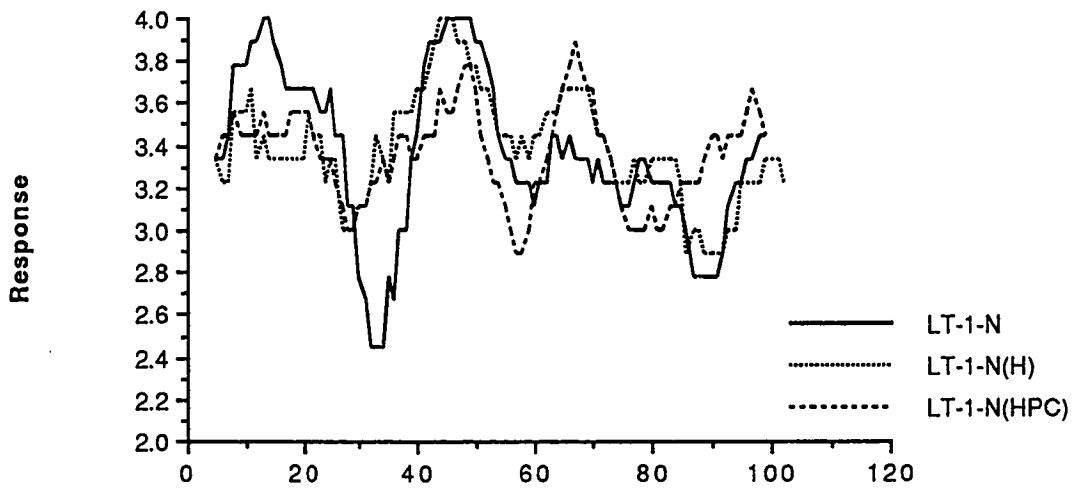
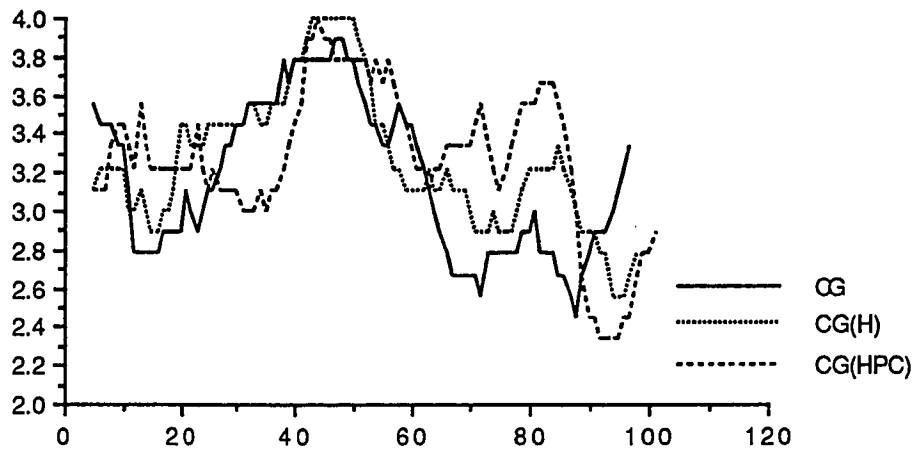


Figure 4.3. Gross external and internal morphology of rainbow trout fed the casein-based experimental diets for 111 d. Note grossly distended stomachs in fish fed diets containing the amine treated protein sources.

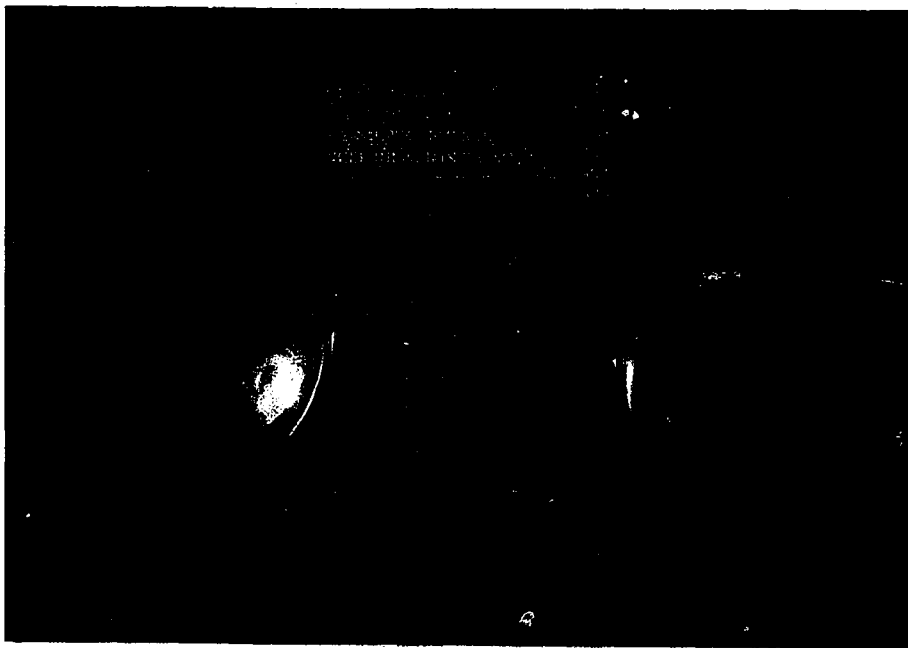


Figure 4.4. Gross external and internal morphology of rainbow trout fed the LT fish meal-based experimental diets for 111 d. Note grossly distended stomachs in fish fed diets containing the amine treated protein sources.

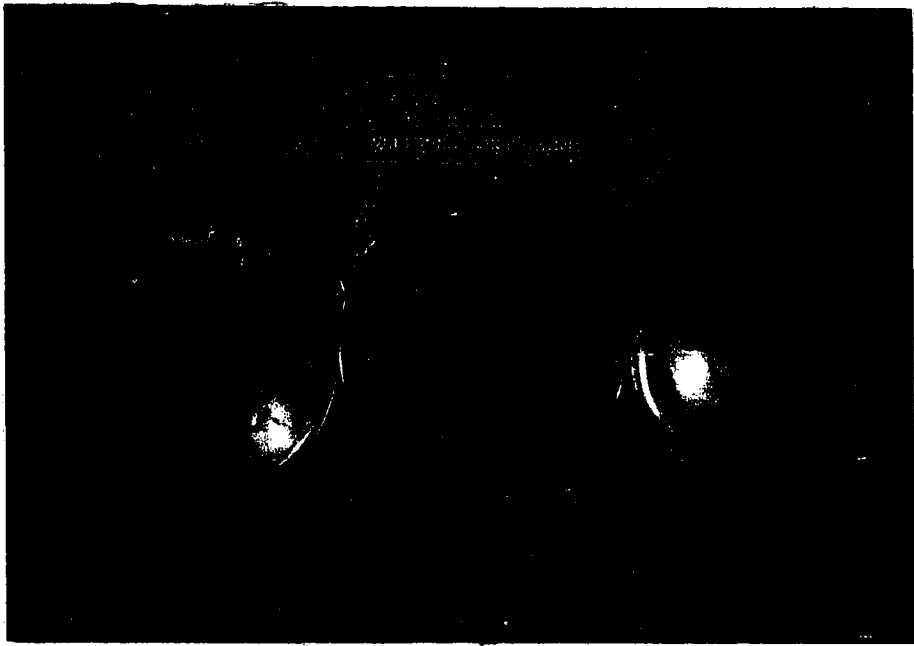
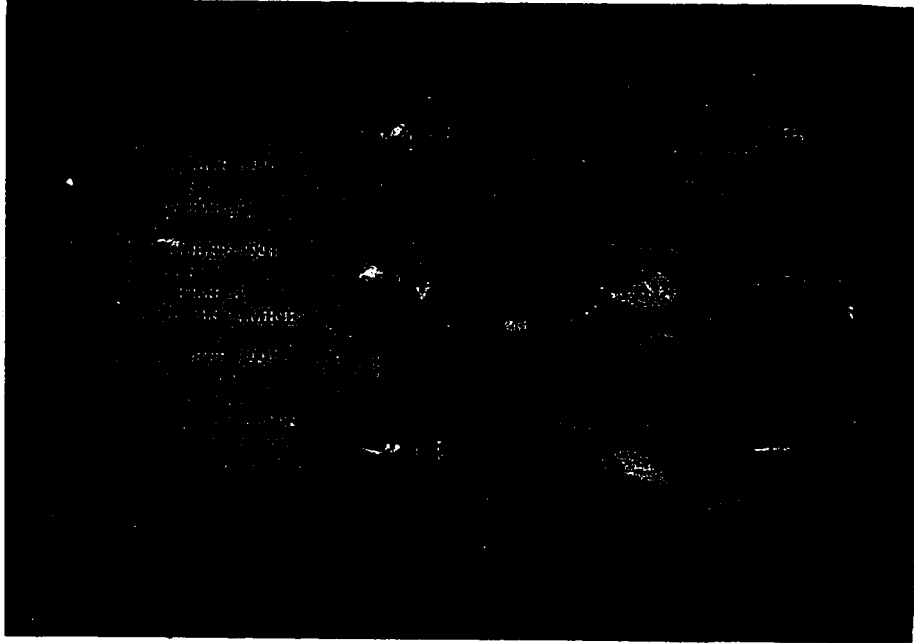


Figure 4.5. Gross external and internal morphology of rainbow trout fed the Chilean fish meal-based experimental diets for 111 d. Note grossly distended stomachs in fish fed diets containing the toxic fish meal and the toxic fish meal supplemented with histamine, putrescine, and cadaverine.

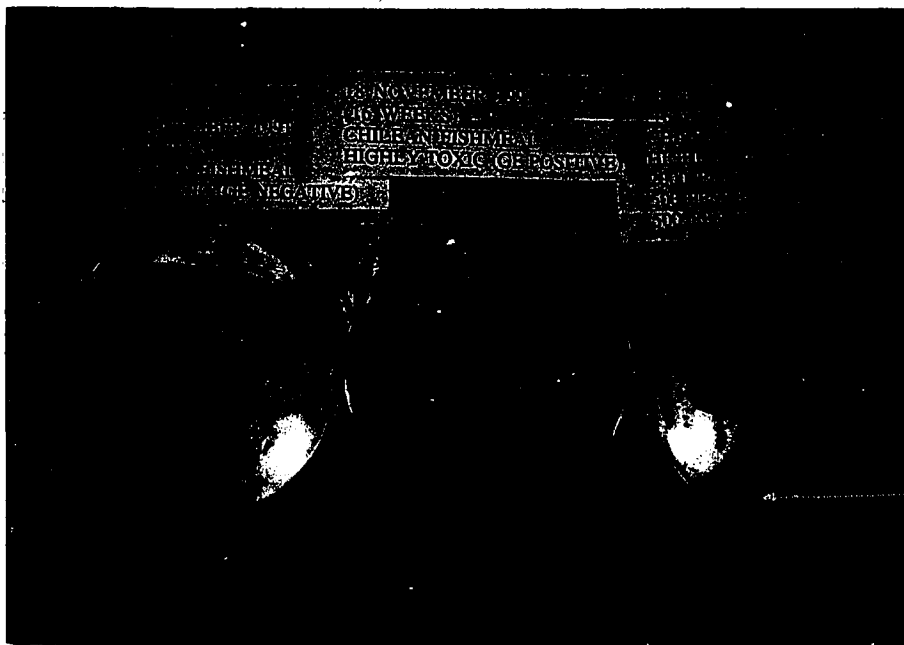
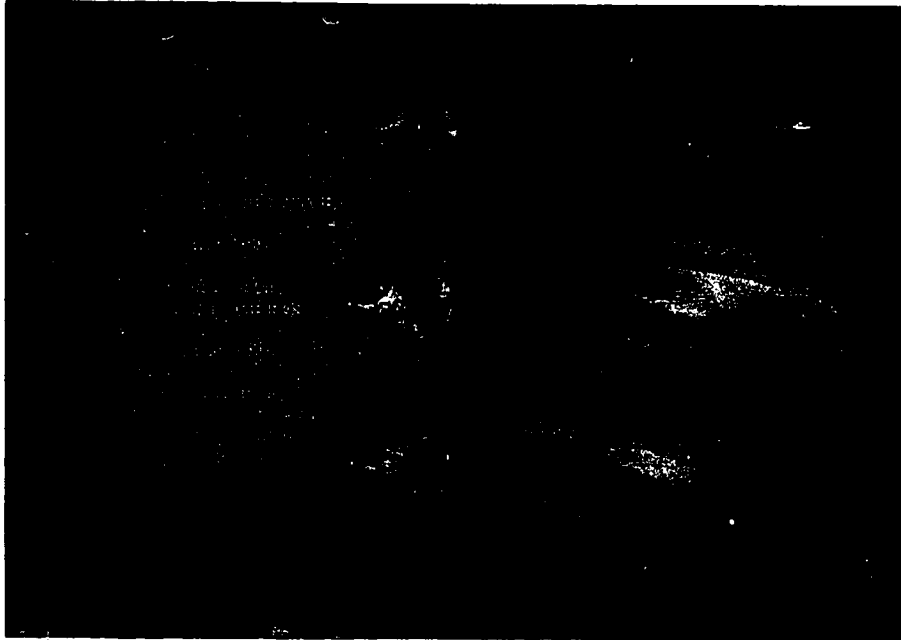


Figure 4.6. (LEFT) Cross section of transitional region of the stomach from a rainbow trout sampled after 42 d with the unsupplemented CG diet. Normal gastric mucosa (m), serous glands (arrow), submucosal tissues (sm), and a well developed mucosal fold or gastric ruga (r) are evident.

(RIGHT) Cross section of cardiac stomach from a rainbow trout sampled after 98 d feeding with the CG(HPC) diet. Normal gastric mucosa (m), serous cardiac glands (arrow), stratum compactum and granuloseum (sc) and muscular layers (M) are shown. Absence or attenuation of gastric rugae and reduction in thickness of the muscular layers were associated with stomach distention, rather than changes in cell morphology. Hematoxylin and eosin staining, 80x.

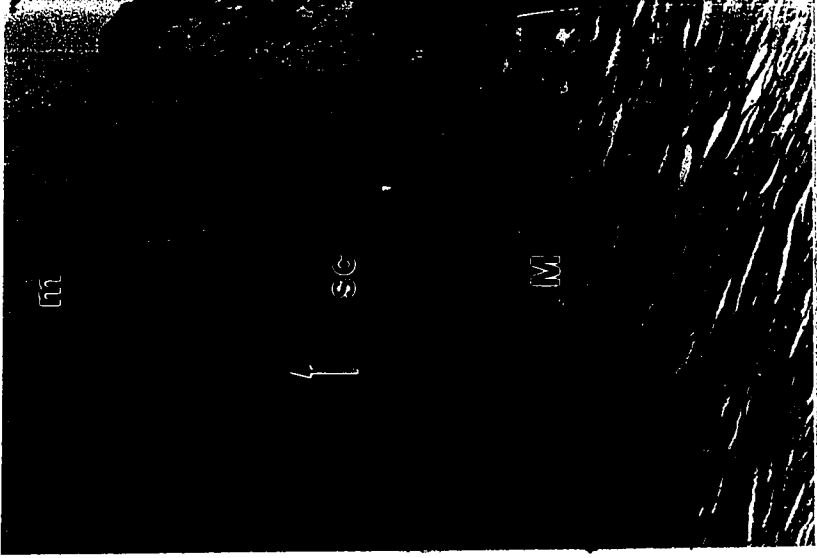


Figure 4.7. (LEFT) Cross section of cardiac region of the stomach from a rainbow trout after 42 d feeding with the unsupplemented LT-1-N fishmeal diet. Normal gastric mucosa (m), serous cardiac glands (small arrow), stratum compactum and granuloseum (sc), layers of the muscularis circularis (mc) and muscularis longitudinalis (large arrow), and gastric ruga (r) are shown. Hematoxylin and eosin staining, 100x.

(RIGHT) Cross section of cardiac stomach from a rainbow trout after 98 d feeding with the LT-3-N(HPC) diet. Photomicrograph indicates normal gastric mucosa (m), serous cardiac glands (s), stratum compactum and granuloseum (sc) and muscular layers (M). Absence or attenuation of gastric rugae and reduction in thickness of the muscular layers were associated with stomach distention, rather than changes in cell morphology. Hematoxylin and eosin staining, 160x.

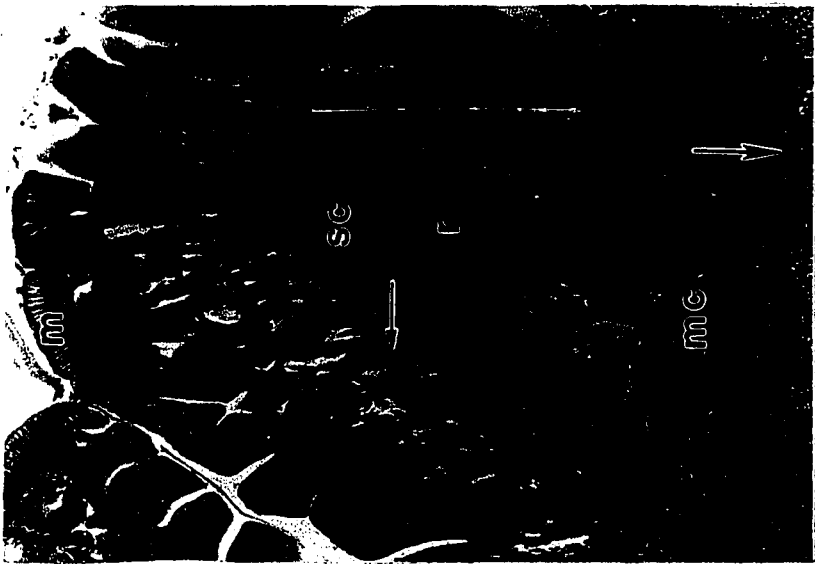
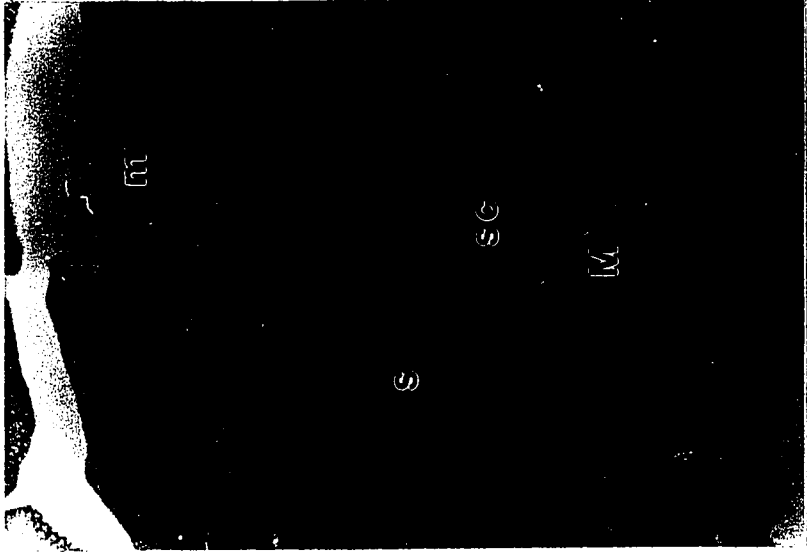


Figure 4.8. (LEFT) Cross section of cardiac stomach from a rainbow trout after 98 d feeding with the unsupplemented C-4-T diet. Normal gastric mucosa (m), serous cardiac glands (s), lamina propria (arrow), densely collagenous stratum compactum (sc), and muscular layers (M) are shown. Absence or attenuation of gastric rugae and reduction in thickness of the muscular layers was associated with stomach distention, rather than changes in cell morphology. Hematoxylin and eosin staining, 160x.

(RIGHT) Cross section of cardiac stomach from a rainbow trout after 98 d feeding with the C-5-T(HPC) diet. Normal gastric mucosa (m), serous cardiac glands (s), lamina propria (arrow), densely collagenous stratum compactum (sc), and muscular layers (M) are shown. Absence or attenuation of gastric rugae and reduction in thickness of the muscular layers were associated with stomach distention, rather than changes in cell morphology. Hematoxylin and eosin staining, 160x.

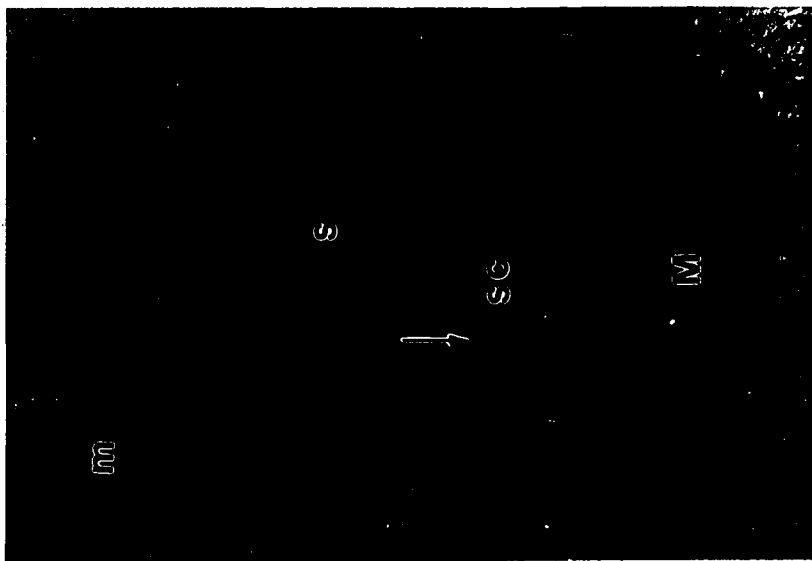
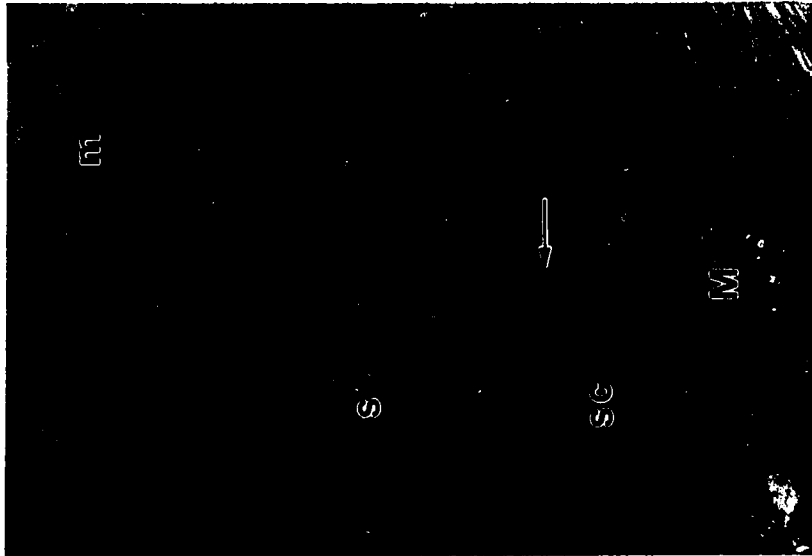


Table 4.1. Proximate composition (% dry weight^a) and selected biogenic amine content (mg/kg wet weight^b) of vitamin-free casein and fish meals included in experimental diets fed to rainbow trout to examine their effects on gastric tissue morphology.

Protein source	Casein	LT-1-N	LT-2-N	LT-3-N	C-3-N	C-4-T	C-5-T
Toxicity ^c	ND ^d	N	N	N	N	T	T
Origin		Norway	Norway	Norway	Chile	Chile	Chile
Dry matter	91.00	93.78	93.11	93.07	91.79	93.91	94.40
Protein	92.70	79.16	75.62	79.18	77.62	73.50	73.56
Lipid	0.70	9.24	13.74	12.05	12.50	11.49	14.38
Ash	2.40	11.20	11.22	11.43	14.38	15.44	15.49
Histamine	ND	199	209	209	83	516	516
Putrescine	ND	ND	21	21	9	28	28
Cadaverine	ND	ND	1101	1101	84	559	559
Tyramine	ND	ND	ND	ND	20	247	247
Agmatine	ND	ND	807	807	20	220	220
Gizzerosine	ND	ND	<1	<1	<1	9	9

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

^b Histamine, putrescine, cadaverine, tyramine, and agmatine assays conducted by Trouw Iberica, S.A. (Spain). Values represent the mean of duplicate determinations made on each fish meal. Gizzerosine assays were conducted by The Japan Food Research Laboratories (detection limit: 1 mg/kg). Data provided courtesy of Department of Marine Resources, Fundacion Chile, Santiago, Chile.

^c Causative of gizzard erosion in chickens as determined by 7 d chick bioassay: T = toxic; N = nontoxic. Assay conducted by Department of Marine Resources, Fundacion Chile, Santiago, Chile.

^d Not determined.

Table 4.2. Formulation of experimental diets (% dry ingredients).

Ingredient ^a	Diet ^b								
	CG	CG (H)	CG (HPC)	LT-1 N	LT-2 N(H)	LT-3 N(HPC)	C-3 N	C-4 T	C-5 T(HPC)
Casein	44.8	44.8	44.8						
LT-1-N fish meal				62.0					
LT-2-N fish meal					65.5				
LT-3-N fish meal						62.5			
C-3-N fishmeal							65.0		
C-4-T fishmeal								66.5	
C-5-T fishmeal									62.5
Gelatin	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Dextrin	12.0	12.0	12.0	8.5	8.5	8.5	8.5	8.5	8.5
Alpha-cellulose	4.6	4.6	4.6	3.3	3.0	4.6	2.7	2.4	4.6
Carboxymethylcellulose	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix ^c	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Choline chloride (70% liquid)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Herring oil	17.1	17.1	17.1	12.0	9.0	10.3	9.7	8.3	10.3
Amino acid mix ^d	4.1	4.1	4.1						
Mineral premix ^e	3.3	3.3	3.3						
Ascorbic acid (41% active)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water	300	300	300	300	300	300	300	300	300
Trace mineral solution ^f	100	100	100	100	100	100	100	100	100

^a Sources: vitamin-free casein, alpha cellulose, carboxymethylcellulose, dextrin, ICN Biomedicals, Inc. (Costa Mesa, Ca); fish meal, Fundacion Chile (Santiago, Chile); vitamin premix, Moore-Clark Co. (La Connor, Wa); herring oil, B.C. Packers (Richmond, B.C., Canada); gelatin, choline chloride, trace mineral solution, amino acid and mineral premixes, Sigma Chemical Company (St. Louis, Mo); ascorbic acid, Takeda Chemical Co. (Tokyo, Japan).

^b Histamine (H), Putrescine (P), Cadaverine (C) were incorporated at levels 2,000 mg/kg, 500 mg/kg, and 500 mg/kg of dry diet ingredients, respectively, as indicated. (Sigma Chemical Company, St. Louis, Mo)

^c Vitamin premix supplied the following per kg of dry diet ingredients: Vitamin A acetate or palmitate, 6612 IU; Alpha tocopheryl acetate, 503 IU; menadione sodium bisulfite complex, 28 mg; thiamine mononitrate, 46 mg; riboflavin, 53 mg; pyridoxine HCl, 38 mg; d-calcium pantothenate, 115 mg; niacin, 220 mg; d-biotin, 1.45 mg; Vitamin B₁₂, 0.06 mg; myoinositol, 132 mg; folic acid, 16.5 mg; Vitamin D₃, 441 mg.

^d Amino acid mix supplied the following per kg of dry diet ingredients: L-arginine, 10 g; L-histidine, 2 g; L-lysine, 10 g; L-methionine, 4 g; L-phenylalanine, 5 g; L-threonine, 10 g.

^e Mineral premix supplied the following per kg of dry diet ingredients: KCl, 15 g; CaHPO₄·H₂O, 12 g; MgO, 3 g; NaCl, 3 g.

^f Trace mineral solution supplied the following per kg of dry diet ingredients: KI, 1.9 mg; MnSO₄·4 H₂O, 32.5 mg; ZnSO₄·7 H₂O, 88.0 mg; NaHSO₃, 4.2 mg; CoCl₃·6 H₂O, 4.0 mg; CuSO₄·5H₂O, 11.8 mg.

Table 4.3. Proximate compositions (% dry weight^a) of experimental diets fed to rainbow trout to examine the effects of various biogenic amines and protein sources on gastric tissue morphology.

Diet	Dry matter	Protein	Lipid	Ash
CG	74.09	61.75	18.25	4.59
CG(H)	72.14	57.62	19.48	5.50
CG(HPC)	69.01	58.00	17.38	4.66
LT-1-N	68.70	59.94	19.29	7.82
LT-2-N(H)	73.79	63.19	15.11	8.27
LT-3-N(HPC)	72.77	54.62	17.03	7.93
C-3-N	69.48	61.69	17.29	9.90
C-4-T	68.67	58.87	16.76	10.78
C-5-T(HPC)	72.65	56.93	17.50	10.23

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

Table 4.4. Growth, feed consumption, and efficiency of feed utilization by rainbow trout fed the experimental diets for 14 weeks^a.

Diet	Final weight ^b (g)	Weight gain (g)	Feed intake (g dry weight)	F.E. ^c
CG	63.7	58.1	53.7	1.08
CG(H)	71.9	66.3	55.8	1.18
CG(HPC)	70.4	65.0	55.6	1.17
LT-1-N	75.8	70.4	63.1	1.12
LT-2-N(H)	71.1	65.3	58.4	1.11
LT-3-N(HPC)	77.9	72.6	66.0	1.10
C-3-N	63.6	57.8	59.9	0.97
C-4-T	88.4	82.1	71.7	1.14
C-5-T(HPC)	78.5	74.6	69.7	1.07

^a Data were not analyzed statistically because fish were selectively removed from the tanks throughout the study for histological evaluation.

^b Initial weight: 5.5 g.

^c Feed efficiency (F.E.) calculated as wet weight gain (g) / dry feed intake (g)

Chapter 5

Comparison of Toxic and Nontoxic Fish Meals as Protein Sources in the Diets of Rainbow Trout.

5.1 INTRODUCTION

The results of the preliminary study (Chapter 3) did not support the hypothesis that fish meal toxicity to chickens was correlated with reduced feed intake, growth, nutritive value, or occurrence of gastric tissue abnormalities in rainbow trout. However, since many key steps in the manufacturing process affect the freshness of raw material and the ultimate fate of toxic amines generated, the single sample tested could not have represented all of the many possible combinations. Also, it was possible that the methods used were insufficiently sensitive to detect important differences in protein quality and palatability of the fish meal. The purpose of this study was to analyze additional toxic and nontoxic fish meals of Chilean origin and to improve the sensitivity of the methods used to evaluate palatability and nutritive value of the protein sources.

5.2 METHODS AND MATERIALS

5.2.1 Test protein sources

Five sources of dietary protein, including vitamin-free casein and four different fish meals of Chilean origin, were evaluated in these studies. The fish meals, prepared by various processors from Jack mackerel, were evaluated for toxicity to chickens by a commercial laboratory in Chile using a proprietary assay technique similar to that described in Chapter 3. Other, unspecified criteria were used to grade these fish meals according to their suitability for use in feeds for salmonids. Two of the fish meals were acutely toxic to chickens, and rated unsuitable for salmonid feeds. The remaining two were nontoxic to chickens: one was identified as being of "average" quality, and the remaining fish meal as "good". The fish meals were packaged in airtight plastic bags, shipped by airfreight to Seattle, and then frozen at -20°C for 1 month before incorporation into the test diets. The proximate composition of the fish meals is summarized in Table 5.1.

5.2.2 Diet preparation

Five test diets based on isonitrogenous substitution of each fish meal for casein in a semipurified casein-gelatin basal (control) diet were prepared as described in Chapter 4. Diet codes and formulations are shown in Table 5.2. Proximate and amino acid composition of the experimental diets is summarized in Table 5.3.

5.2.3 Feeding regimen

Palatability and nutritive value of the protein sources were evaluated in separate, concurrent feeding trials. Two feeding strategies were used, depending upon the specific objective of each study. In the palatability trial, fish were fed using a timed-response feeding strategy, with the intent to obtain maximum voluntary intake of the various diets. Since all groups of fish were given equal

opportunity to feed, differences in dry matter intake and feeding behavior were expected to indicate the effects of the various protein sources on diet palatability. This approach was supported by the results reported in Chapter 3, in which a 4.7% reduction in mean dry matter intake (g/fish) was statistically significant ($P < 0.05$). Methods used in this palatability trial were identical to those described in Chapter 4, except that the fish were fed twice daily on weekdays and once daily on weekends. Feed was withheld for 24 h prior to sampling, and the fish were not fed on weighing days.

In the nutritive value trial, protein intake was restricted and quantitatively equal (g protein/fish/week) among the various dietary treatments. This was done because the metabolic fate of dietary protein can be interactively affected by level of intake and nutritional quality. Therefore, only under conditions of equal intake can differences in protein utilization be attributed more closely to protein quality. These factors have been discussed in detail in Chapter 7.

During the first 14 d of the trial, fish in all groups were pair-fed equal quantities of protein from the respective diets as dictated by the amount consumed voluntarily by those receiving the casein-gelatin control diet, since that diet was consumed in the least quantity. Those data were used to establish the quantity of protein to be fed during the remainder of the trial. Rather than directly reduce the feeding rate, restriction was imposed by holding the quantity of protein offered to each fish on a daily basis (g protein/fish/day) constant during the final 28 d of the trial. As the fish grew, the rate of protein intake decreased proportionately. Amounts of diet fed to each group of fish varied slightly depending on the protein content of the diets and the number of fish remaining during each weekly interval. Rates of protein consumption (g/fish/week and g/kg fish body weight/d) are shown in Table 5.4. As in the palatability trial, groups of fish in the nutritive value

study were fed 6 d each week, since feed was withheld on day 7 when the fish were sampled for growth.

5.2.4 Experimental facilities, fish, and husbandry

The experiment described in this chapter was conducted in the wet laboratory facilities of the School of Fisheries, University of Washington, Seattle, Washington. Replicate groups of fish were reared in individual 64 L circular polyethylene tanks, each equipped with a screened center drain. Ambient temperature ($16.5 \pm 1.2^\circ\text{C}$, Appendix 1) dechlorinated municipal water was supplied to each tank at a rate of 1.5 L/min. Water volume was fixed at 35 L by means of a standpipe, and was not altered throughout the course of the experiment. Dissolved oxygen concentrations were determined at weekly intervals with a YSI model 51B dissolved oxygen meter (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) and remained near saturation. An airstone placed into each tank provided supplemental aeration. The facility was illuminated by fluorescent lights in a 12 h light: 12 h dark photoperiod cycle.

Progeny of two separate female-male matings of rainbow trout obtained from the School of Fisheries hatchery, University of Washington, were used in this study. Eggs collected from two Donaldson strain rainbow trout were fertilized with the milt from either a single Donaldson strain or a single Nisqually strain male to produce two full-sib families, referred to hereafter as UW or Nisqually strains, respectively. Eggs, fry, and juvenile fish resulting from each cross were maintained in isolation. When the fish attained a mean weight of about 2.5 g, they were transferred from the hatchery to the wet laboratory facility and acclimated for 14 d prior to distribution to the experimental units.

Few experimental data were available regarding family related behavioral or physiological characteristics that might affect feed intake or fate of ingested nutrients. However, observations by

hatchery personnel suggested that progeny of Nisqually crosses exhibited behavioral characteristics that made them much more difficult to feed than those of pure UW parentage. Therefore, since it was expected that the Nisqually cross would be more sensitive to palatability differences among the various protein sources, these fish were assigned to the palatability trial. (Note: A separate study to investigate this observation was conducted concurrently with the feeding trials described in this chapter. The experimental protocol and results are detailed in Chapter 6.) Similarly, only fish of the UW parentage were used in the nutritive value trial since it was important for fish in that trial to consume a predetermined quantity of dietary protein, irrespective of palatability factors. Fish used in each study were first selected for uniform size, then randomly distributed among 15 tanks until each contained 25 animals. Three experimental units were assigned randomly among each of five dietary treatments. At the start of the study, fish of the Nisqually cross were 2.8 ± 0.1 g and 62.6 ± 0.3 mm TL while those of the UW cross were 2.8 ± 0.1 g and 63.3 ± 1.0 mm TL (mean \pm SD).

In addition to feeding, routine daily activities included measuring water temperature, examining each tank for the presence of dead or moribund fish, and flushing fecal matter from the tanks.

5.2.5 Measurement of growth

Growth in length and weight of the fish was measured on day 0 and at 7 d intervals for 42 d, as described in Chapter 3. Feed was withheld for 24 h prior to sampling.

5.2.7 Collection and preparation of samples for histological evaluation

Although fish involved in the nutritive value and palatability trials were fed identical diets, only fish from the palatability trial were sampled for histological evaluation, since it was assumed that

response to toxic compounds in the fish meals was most likely to occur in fish fed to satiation. Histology samples were not collected at the start of the trial. At trial termination, three fish were selected randomly from each treatment replicate and preserved for histological examination using the methods described in Chapter 4.

5.2.8 Chemical analysis of fish meal, diets, and fish carcasses

Procedures for sampling, preparation, and proximate analysis of fish meals and diets were described in detail in Chapter 3. Experimental diets used in this study were analyzed for amino acid composition by AAA Laboratories (6206 89th Ave. SE, Mercer Island, Washington 98040). One 50 g sample of each diet mixture was collected from the extruder barrel, placed into individual plastic bags, and stored at -20°C until analyzed. One subsample of each diet containing about 10 mg protein, hydrolyzed with 6N HCl/0.05% mercaptoethanol for 20 h at 115°C, was analyzed. Amino acid composition (% wet weight) was calculated using individual amino acid molecular weights: serine and threonine estimates were increased by 10% to compensate for losses during acid hydrolysis.

One sample of 10 fish from a pool common to each strain was collected at the beginning of the trial; at the end of the trial, 5 fish were randomly selected from each tank. The fish were not fed for 48 h prior to sampling. The fish were blotted dry with paper towels, placed into labeled plastic bags, and stored on ice for 2 to 3 h, then prepared for determination of whole-body levels of moisture, protein, lipid, and ash.

Fish samples were prepared for moisture determination by cutting the carcasses into pieces not larger than 1 to 2 cm on a side and placing the pieces in a single layer into aluminum pans. The samples were oven dried (16 h at 105°C) to constant weight, then ground to a flour-like consistency (40 mesh) with a mortar and pestle. The ground materials were analyzed for proximate

composition according to AOAC (1984) methods (described in detail in Appendix 2).

5.2.6 Data analysis

Feeding activity scores, rates of growth in wet weight and total length, feed efficiencies, and protein efficiency ratios were derived as described in Chapter 3. Additionally, protein intake and gain in whole body protein were used to calculate protein productive values (PPV) for fish from both the palatability and nutritive value trials, using the formula given by Pfeffer (1982):

$$\text{PPV (\%)} = 100 \cdot \text{protein fed} / \text{gain in carcass protein}$$

Initial and final weight and length, rates of growth in length and weight, PER, and PPV data collected in both the nutritive value and palatability trials were analyzed statistically using methods appropriate to completely randomized experimental designs. Feed intake (g dry matter) by groups of fish in the palatability trial were also subjected to statistical evaluation, since these data were indicative of feed palatability. Fisher's Least Significant Difference (LSD) procedure was used following a significant "F" test ($P < 0.05$) to compare treatment means (Little and Hills, 1978); computed P values are shown. Statistical analysis was performed using STATVIEW version 2.0 (Brainpower, Inc., Calabasas, California).

5.4 RESULTS

5.4.1 Effects of dietary protein source and toxicity on growth of rainbow trout fed to satiation and at restricted levels

Growth of rainbow trout fed diets containing either casein, toxic Chilean fish meal, or nontoxic Chilean fish meal to evaluate the relationship between toxicity and the palatability and nutritive value of the protein sources is summarized in Tables 5.5 and 5.6. In the palatability study, groups of fish fed the casein control diet increased in weight nearly five-fold, and increased in TL by about 31 mm, whereas fish fed the fish meal based diets increased in weight at least six-fold, and increased in TL by over 42 mm. Mean differences in final TL, rate of growth in TL, final wet weight, and rate of growth in weight were statistically significant ($P < 0.001$). In general, groups of fish receiving the toxic fish meals exhibited higher rates of growth and at the end of the trial were larger in both length and weight than fish fed the nontoxic fish meals or casein. Contrary to expectations, the C-9-N fish meal, which was identified by the supplier as of "good" quality, supported the lowest rate of growth of any fish meal tested.

Results of the nutritive value study (Table 5.6), in which groups of fish were fed equivalent quantities of protein, showed that the degree of toxicity to chickens was not consistently correlated with the capacity of fish meal to support growth. In this trial, one toxic fish meal (C-6-T) produced one of the highest observed rates of growth in both length and weight, while the remaining toxic fish meal (C-8-T) produced one of the lowest. As in the palatability study, differences in final TL, rate of growth in TL, final wet weight, and rate of growth in weight were statistically significant ($P < 0.001$).

5.4.2 Feed intake and efficiency of protein utilization

Feed and protein intake, gain in wet weight and body protein, and estimates of feed efficiency, PER, and PPV obtained using groups of fish fed to satiation (palatability trial) or given equivalent quantities of dietary protein (nutritive value trial) are summarized in Tables 5.7 and 5.8, respectively. Data on whole body composition used in PPV estimation are summarized in Tables 5.9 (palatability trial) and 5.10 (nutritive value trial).

Results of the palatability trial showed that toxic fish meals were not consistently more or less acceptable than those nontoxic to chickens. First, all diets were readily consumed by the fish throughout the trial. In general, the feeding protocol used in this study appeared to yield satiation levels of intake, since the fish usually ceased to feed before the end of the final daily feeding period. However, on a dry matter basis, voluntary consumption of diets containing fish meal was significantly higher ($P < 0.001$) in all cases than of the diet containing casein. Furthermore, the diet containing the toxic C-8-T fish meal was consumed in significantly greater quantities than any other diet tested. Observations on feeding behavior of the fish fed the various diets also indicated that differences in feed palatability were present (Figure 5.1). Overall, these data suggest that the toxic C-8-T fish meal was among the most highly palatable of the protein sources, a finding reflected in the dry matter intake data presented in Table 5.7. All diets except that containing casein and the C-8-T fish meal were consumed in statistically equivalent quantities (dry matter basis), however.

Data obtained in these two feeding trials did not support the hypothesis that compounds present in the fish meals toxic to chickens directly affected their nutritive values to rainbow trout. Despite rather large differences in the quantity of protein consumed by fish fed to satiation in the palatability trial, PER and PPV data demonstrated that toxic fish meals were not consistently of higher

or lower nutritive value than their nontoxic counterparts. For example, the nutritive value of the acutely toxic fish meal C-6-T was superior to all other protein sources tested, as indicated by both PER ($P < 0.001$) and PPV ($P < 0.01$) estimates. However, the remaining toxic fish meal, C-8-T, and the nontoxic fish meal, C-9-N, the quality of which was rated as "good" by the supplier, were equivalent to casein in nutritive value, even though the latter protein source produced the lowest rate of growth in both the nutritive value and the palatability trials.

Preliminary estimates of nutritional quality obtained in the palatability study were further clarified by the results of the nutritive value trial, in which all groups of fish received equal quantities of protein. First, all fish meals were superior to casein in nutritive value, whether assessed by the PER ($P < 0.001$) or PPV ($P < 0.001$) method. Second, rates of protein retention by fish fed the toxic C-6-T fish meal and the nontoxic C-7-N fish meal were not significantly different, whereas both were superior to the toxic C-8-T and nontoxic C-9-N fish meals. These data indicated that indices of fish meal toxicity to chickens were not reliable indicators of nutritional quality of fish meal for use in the diets of rainbow trout.

5.4.3 Clinical findings

At weekly intervals throughout both 6 week feeding trials, all fish were anesthetized and examined individually for external signs of infectious or noninfectious diseases. In all cases, the fish were robust, and except for occasional evidence of fin nipping associated with high rearing densities and feeding at restricted levels, no external abnormalities were noted. Mortality was very low in all treatments, and was in every case attributable to either handling stress or to jumping from the uncovered tanks during feeding or tank maintenance. Mortality data are summarized in Appendix 3.

At trial termination, three fish from each tank of fish in the palatability study (nine fish per treatment) were sacrificed for gross and histological evaluation of the internal organs. *In situ* inspection of the visceral mass revealed no obvious inflammation or ulceration of the gastric or intestinal tissues, and no lesions or other abnormalities in the spleen, liver, or kidney were observed. In the fish fed the fish meal based diets, but not the diet containing casein, the visceral mass was tinged light pink. Irrespective of dietary treatment, small quantities of ingesta were present in the intestines of most fish examined, and as before, the gall bladder was typically engorged with bile. Moderate quantities of visceral fat were observed in all fish examined, but the pyloric caecae were visible and distinct.

Following *in situ* examination, the entire visceral mass of each fish was excised and immediately preserved for histological evaluation. Examination of the sectioned and stained tissues under light microscope revealed no abnormalities in the cellular structure of the gastric glands, mucosa, or underlying musculature of the stomach. Liver tissues were histologically normal.

5.5 SUMMARY AND CONCLUSIONS

Two feeding studies were conducted to independently assess the relationship between the results of toxicity assays in chickens and the palatability and nutritive value of fish meal to rainbow trout. In the palatability study, fish were fed to satiation using a timed-response feeding protocol, since this method allowed direct evaluation of feed palatability from feed intake and feeding behavior observations. Conversely, in the nutritive value study, fish were fed equal quantities of protein at restricted levels of intake. This procedure allowed direct estimation of the relative nutritional quality of the protein sources from growth data, protein efficiency ratios, and protein retention estimates.

The results of these studies confirmed those reported previously (Chapter 3) and provided additional evidence that palatability and nutritive value of fish meal to rainbow trout could not be reliably predicted from results of toxicity bioassays in chickens. All possible combinations of raw material and concentrate quality were not tested, and the fish meals were selected using only the results of toxicity assays, without knowledge of raw material freshness or processing conditions. However, the finding that the toxic fish meals were equal or superior to their nontoxic counterparts in nutritional value and palatability indicated that compounds responsible for toxicity to chickens may not affect either of these quality attributes in rainbow trout. Furthermore, these rainbow trout were apparently insensitive to short term exposure to the toxic compounds in these fish meals, since neither gross nor microscopically detectable abnormalities in the digestive tissues of the fish were observed.

As predicted, feeding method had substantial influence on the sensitivity of growth and protein utilization assays in rainbow trout. Since feed intake and feeding behavior are two key indicators of feedstuff palatability, feeding trials designed to evaluate this

quality attribute must, by definition, utilize a satiation feeding regimen. However, because the nutritional quality of the feed and level of intake interactively affect growth and protein utilization, data on protein quality obtained from these studies may be difficult to interpret, especially when differences in feed consumption are large. In this study, feeding at restricted levels with equal quantities of dietary protein (nutritive value trial) revealed differences in growth and protein utilization that were not apparent with groups fed to satiation. Because all conditions of husbandry and environment were identical and protein intake was equal among all fish, the observed differences could be attributed directly to differences in the nutritional quality of the various protein sources.

The effects of intake level on the metabolic fate of dietary protein has been well documented (see Chapter 7), but few data presently exist in the scientific literature regarding differences in the ability of various strains of rainbow trout to absorb and utilize dietary protein. This factor may be important to consider when comparing the results of different feeding trials that have been conducted with various pure and hybrid strains of fish. Another important consideration may be the effect of behavioral differences relating to feeding activity on interpretation of feed palatability data. As stated previously, hatchery technicians observed considerable differences in feeding behavior between the interstrain Nisqually hybrid and pure strain UW rainbow trout that affected feed intake and, apparently, growth. If differences in feeding behavior by various strains of fish affect feed intake, standard comparison of palatability measurements using consumption as a criterion would be hampered. Also, it is possible that some strains of rainbow trout are more sensitive to differences in feedstuff palatability, and that results obtained would depend on the strain used in the experiment. To investigate this possibility, a pilot study was conducted comparing the feeding behavior of the pure UW strain with the

Nisqually interstrain hybrid of rainbow trout. The results of that study are reported in Chapter 6.

One of the primary objectives of the nutritive value study was to develop and validate a method for controlling and equalizing protein intake among various dietary treatments. While it is relatively easy to adjust feeding levels among treatment groups to achieve equal protein intake, *a priori* determination of the appropriate level requires some knowledge of the effects of intake level on the metabolic fate of the ingested protein. Data needed to identify an appropriate level of protein intake for fish used in the nutritive value trial were not available from the scientific literature, however. Thus, for the first 14 d of this study, all fish were pair-fed quantities of dietary protein equal to those consumed by the fish fed the casein-gelatin control diet. During the subsequent 28 d, all fish were given equal quantities of protein. As a result, protein intake levels relative to body size changed dramatically between the beginning and end of the study, a factor that had unquantifiable effect on nutrient fate and, consequently, on estimates of protein retention. Furthermore, the feeding protocol used did not provide adequate criteria for the selection of the appropriate level of protein intake and therefore the experimental conditions could not be precisely duplicated. A study designed to identify appropriate protein intake levels for use in the final study on the effects of histamine and heating on protein quality (Chapter 8) is detailed in Chapter 7.

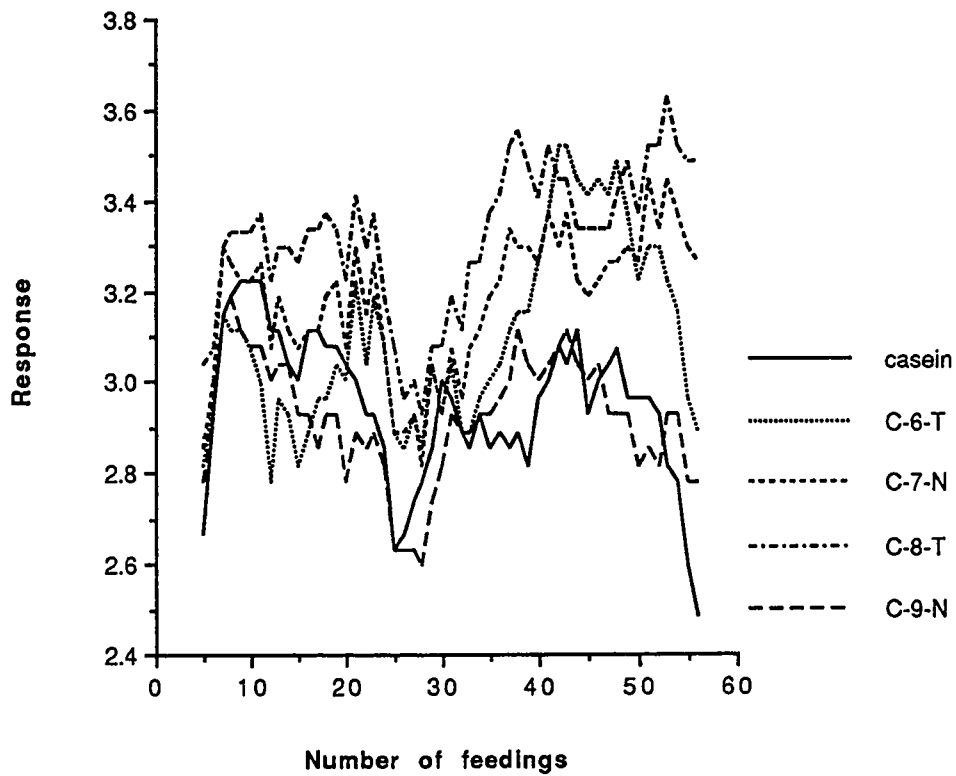


Figure 5.1. Feeding response scores for rainbow trout fed the experimental diets. Numerical scores were assigned following subjective evaluation of feeding response: (1) indicates little or no response, (2) indicates minimal feeding response, (3) indicates average response, and (4) indicates excellent response.

Table 5.1. Proximate composition (% dry weight^a) of casein and fish meals of Chilean origin evaluated for palatability, nutritive value, and toxicity to rainbow trout.

Protein source	Casein	C-6-T	C-7-N	C-8-T	C-9-N
Toxicity ^b	ND ^c	T	N	T	N
Origin		Chile	Chile	Chile	Chile
Dry matter	91.00	93.39	92.86	93.41	91.56
Protein	92.70	72.43	73.81	72.12	73.81
Lipid	0.70	11.98	12.16	11.89	11.79
Ash	2.40	14.36	15.41	14.22	14.84

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

^b Causative of gizzard erosion in chickens as determined by 7 d chick bioassay: T = toxic; N = nontoxic. Assay conducted by INUAL, Santiago, Chile.

^c Not determined.

Table 5.2. Formulation of the experimental diets (% dry ingredients).

Ingredient ^a	Diet				
	CG	C-6-T	C-7-N	C-8-T	C-9-N
Casein	44.8				
C-6-T fish meal		68.3			
C-7-N fish meal			68.3		
C-8-T fish meal				68.3	
C-9-N fish meal					68.0
Gelatin	10.0	10.0	10.0	10.0	10.0
Dextrin	12.0	8.2	8.2	8.2	8.2
Alpha-cellulose	4.6				
Carboxymethylcellulose	1.0	1.0	1.0	1.0	1.0
Vitamin premix ^b	2.0	2.0	2.0	2.0	2.0
Choline chloride (70% liquid)	1.0	1.0	1.0	1.0	1.0
Herring oil	17.1	9.4	9.4	9.4	9.7
Amino acid mix ^c	4.1				
Mineral premix ^d	3.3				
Ascorbic acid (41% active)	0.2	0.2	0.2	0.2	0.2
Water	300	300	300	300	300
Trace mineral solution ^e	100	100	100	100	100

^a Sources of ingredients were: vitamin-free casein, ICN Biomedicals, Inc. (Costa Mesa, California); fish meal, INUAL (Santiago, Chile); gelatin, Sigma Chemical Company (St. Louis, Missouri); dextrin, alpha cellulose, and carboxymethylcellulose, ICN Biomedicals, Inc. (Costa Mesa, California); vitamin premix, Moore-Clark Co. (La Connor, Washington); choline chloride, Sigma Chemical Company (St. Louis, Missouri); herring oil, B.C. Packers (Richmond, B.C., Canada); amino acid premix, Sigma Chemical Company (St. Louis, Missouri); mineral premix, Sigma Chemical Company (St. Louis, Missouri); ascorbic acid, Takeda Chemical Co. (Tokyo, Japan); trace mineral solution, Sigma Chemical Company (St. Louis, Missouri).

^b Vitamin premix supplied the following per kg of dry diet ingredients: Vitamin A acetate or palmitate, 6612 IU; Alpha tocopheryl acetate, 503 IU; menadione sodium bisulfite complex, 28 mg; thiamine mononitrate, 46 mg; riboflavin, 53 mg; pyridoxine HCl, 38 mg; d-calcium pantothenate, 115 mg; niacin, 220 mg; d-biotin, 1.45 mg; Vitamin B₁₂, 0.06 mg; myoinositol, 132 mg; folic acid, 16.5 mg; Vitamin D₃, 441 mg.

^c Amino acid mix supplied the following per kg of dry diet ingredients: L-arginine, 10 g; L-histidine, 2 g; L-lysine, 10 g; L-methionine, 4 g; L-phenylalanine, 5 g; L-threonine, 10 g.

^d Mineral premix supplied the following per kg of dry diet ingredients: KCl, 15 g; CaHPO₄·H₂O, 12 g; MgO, 3 g; NaCl, 3 g.

^e Trace mineral solution supplied the following per kg of dry diet ingredients: KI, 1.9 mg; MnSO₄·4 H₂O, 32.5 mg; ZnSO₄·7 H₂O, 88.0 mg; NaHSeO₃, 4.2 mg; CoCl₃·6 H₂O, 4.0 mg; CuSO₄·5H₂O, 11.8 mg.

Table 5.3. Proximate composition (% dry weight^a) and amino acid composition (%wet weight^b) of the experimental diets .

	Diet				
	CG	C-6-T	C-7-N	C-8-T	C-9-N
Dry matter	68.49	72.33	71.45	68.38	72.42
Protein	58.50	62.12	60.97	64.75	62.75
Lipid	16.08	18.08	17.95	16.89	18.27
Ash	3.97	9.97	10.70	9.82	10.19
Alanine	1.54	2.92	2.85	2.89	2.98
Arginine	2.40	2.86	2.78	2.82	2.97
Aspartic acid	2.84	4.12	4.08	4.08	4.37
Glutamic acid	8.48	6.08	6.09	6.03	6.60
Glycine	2.28	4.22	4.03	4.20	4.26
Histidine	1.15	1.46	1.36	1.46	1.11
Isoleucine	1.83	1.83	1.83	1.84	1.85
Leucine	3.39	3.22	3.22	3.19	3.29
Lysine	3.96	3.73	3.72	3.68	3.90
Methionine	1.14	1.06	1.08	1.04	1.15
Phenylalanine	2.30	1.75	1.75	1.74	1.80
Proline	5.45	3.41	3.32	3.39	3.46
Serine	2.26	1.94	1.91	1.89	2.07
Threonine	2.49	1.91	1.91	1.88	1.97
Tyrosine	1.92	1.38	1.36	1.37	1.41
Valine	2.31	2.16	2.16	2.16	2.15

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

^b Calculated using individual amino acid molecular weights. Serine and threonine levels increased by 10% to compensate for losses due to acid hydrolysis. Amino acid analysis was conducted by AAA Laboratory, Mercer Island, Washington.

Table 5.4. Protein intake (g/fish/week) and feeding rates (g protein consumed/kg fish body weight/d) used in the nutritive value trial.

Protein intake (g/fish/week) ^a						
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
	0.45 (0.01)	0.65 (0.01)	0.81 (0.01)	0.82 (0.01)	0.82 (0.01)	0.82 (0.01)
Feeding rate (g protein/kg body weight/d) ^b						
Diet	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Casein	31.73 (1.79)	33.17 (1.75)	29.84 (0.50)	23.06 (1.24)	19.27 (0.79)	15.76 (0.25)
C-6-T	31.57 (0.17)	28.72 (0.69)	27.51 (0.58)	18.67 (0.05)	15.83 (0.48)	13.28 (0.40)
C-7-N	31.93 (1.39)	28.57 (0.85)	25.08 (1.10)	19.48 (0.14)	16.02 (0.06)	13.31 (0.23)
C-8-T	34.36 (0.22)	31.30 (0.58)	25.74 (1.45)	20.60 (0.16)	16.86 (0.07)	14.41 (0.15)
C-9-N	31.46 (0.94)	29.76 (0.99)	26.07 (0.49)	20.00 (0.37)	16.56 (0.11)	14.02 (0.13)

^a All fish were fed equal quantities (g) of protein from their respective dietary treatments. Values shown are means (\pm SD) of protein (g) consumed by individual fish in 15 tanks.

^b Rate of protein intake by individual fish (g/kg fish body weight/d) in each dietary treatment group. Rate of intake decreased in proportion to amount of growth supported by equal quantities of protein from the respective diets. Values shown are means (\pm SD) of three replicates tanks of each dietary treatment.

Table 5.5. Mean growth of rainbow trout fed the experimental diets to satiation for 6 weeks in the palatability trial^a.

Diet	Final length ^b (mm TL)	Growth rate ^c (mm TL/d)	Final weight (g)	Growth rate (g/d)
CG	90.9 (1.5) ^W	0.76 (0.04) ^W	11.0 (0.7) ^W	0.20 (0.02) ^W
C-6-T	104.4 (0.7) ^{XY}	1.10 (0.02) ^{XY}	15.7 (0.4) ^{XY}	0.32 (0.01) ^{XY}
C-7-N	102.6 (1.9) ^{YZ}	1.06 (0.05) ^{YZ}	15.0 (1.1) ^{YZ}	0.30 (0.03) ^{YZ}
C-8-T	106.0 (1.5) ^X	1.12 (0.04) ^X	16.7 (0.8) ^X	0.34 (0.02) ^X
C-9-N	101.6 (1.1) ^Z	1.00 (0.03) ^Z	14.2 (0.4) ^Z	0.27 (0.01) ^Z
	P=0.0001	P=0.0001	P=0.0001	P=0.0001

^a Values in columns followed by the same letter are not significantly different ($P>0.05$). Values are means (\pm SD) of three replicates.

^b Mean initial weight (g) and total length (mm TL) were 2.3 ± 0.1 g and 59.5 ± 0.8 mm (mean \pm SD).

^c Rates of growth are the slope coefficients from the regression of total length or weight (y) on time (x).

Table 5.6. Mean growth of rainbow trout fed equal quantities of protein from the experimental diets for 6 weeks in the nutritive value trial^a.

Diet	Final length ^b (mm TL)	Growth rate ^c (mm TL/d)	Final weight (g)	Growth rate (g/d)
CG	91.1 (0.7) ^X	0.76 (0.01) ^X	10.1 (0.2) ^W	0.18 (0.01) ^W
C-6-T	98.0 (0.3) ^Y	0.93 (0.02) ^Y	11.9 (0.1) ^X	0.23 (0.01) ^X
C-7-N	97.7 (1.1) ^Y	0.92 (0.34) ^{YZ}	11.6 (0.2) ^{XY}	0.22 (0.00) ^{XY}
C-8-T	95.4 (0.4) ^Z	0.89 (0.02) ^{YZ}	11.0 (0.1) ^Z	0.21 (0.00) ^Z
C-9-N	96.1 (0.2) ^Z	0.89 (0.02) ^Z	11.3 (0.5) ^{YZ}	0.21 (0.01) ^{YZ}
	P=0.0001	P=0.0001	P=0.0001	P=0.0001

^a Values in columns followed by the same letter are not significantly different ($P > 0.05$). Values are means (\pm SD) of three replicates.

^b Mean initial weight (g) and total length (mm TL) were 2.4 ± 0.1 g and 59.7 ± 0.6 mm (mean \pm SD).

^c Rates of growth are the slope coefficients of the regression of total length or weight (y) on time (x).

Table 5.7. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout fed the experimental diets for 6 weeks in the palatability trial^a.

Diet	Intake (g)		Gain (g)			F.E. ^c	PER ^d	PPV ^e
	Dry feed	Protein	Weight ^b	Protein	Protein			
Casein	7.87 (0.49) ^X	4.60 (0.28)	8.69 (0.66)	1.31 (0.13)	1.10 (0.02)	1.82 (0.03) ^X	28.45 (1.14) ^X	
C-6-T	9.96 (0.47) ^Y	6.19 (0.29)	13.33 (0.38)	2.11 (0.19)	1.34 (0.03)	2.07 (0.05) ^Y	34.05 (2.28) ^Y	
C-7-N	9.98 (0.82) ^Y	6.08 (0.50)	12.67 (0.99)	1.90 (0.14)	1.27 (0.04)	1.97 (0.08) ^Z	31.32 (0.91) ^Z	
C-8-T	11.30 (0.52) ^Z	7.31 (0.34)	14.38 (0.80)	2.19 (0.18)	1.27 (0.01)	1.88 (0.03) ^X	29.91 (1.16) ^{XZ}	
C-9-N	9.49 (0.21) ^Y	5.95 (0.13)	11.76 (0.37)	1.80 (0.07)	1.24 (0.02)	1.87 (0.03) ^X	30.26 (0.74) ^{XZ}	

P=0.0003

P=0.0006 P=0.0056

^a Values in columns followed by the same letter are not significantly different (P>0.05). Values are means (±SD) of three replicates.

^b Initial weight: 2.3 ± 0.1 g (mean ± SD).

^c Feed efficiency (F.E.) calculated as wet weight gain (g) / dry feed intake (g)

^d Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

^e Protein productive value (PPV) calculated as: 100 · gain in body protein (g) / protein consumed (g).

Table 5.8. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout fed diets equal quantities of protein from the experimental diets for 6 weeks in the nutritive value trials^a.

Diet	Intake (g)		Gain (g)			PER ^d	PPV ^e
	Dry feed	Protein	Weight ^b	Protein	F.E. ^c		
Casein	7.50 (0.05)	4.39 (0.03)	7.66 (0.24)	1.20 (0.04)	1.02 (0.02)	1.70 (0.06) ^w	27.25 (0.73) ^x
C-6-T	7.03 (0.03)	4.37 (0.02)	9.49 (0.09)	1.54 (0.09)	1.35 (0.01)	2.06 (0.03) ^x	35.19 (1.81) ^y
C-7-N	7.15 (0.06)	4.36 (0.03)	9.28 (0.22)	1.53 (0.03)	1.30 (0.02)	2.01 (0.02) ^x ^y	35.01 (0.49) ^y
C-8-T	6.79 (0.04)	4.40 (0.03)	8.74 (0.12)	1.39 (0.02)	1.29 (0.02)	1.86 (0.04) ^z	31.65 (0.70) ^z
C-9-N	6.95 (0.06)	4.36 (0.03)	8.92 (0.52)	1.38 (0.12)	1.28 (0.07)	1.93 (0.1) ^y ^z	31.62 (2.50) ^z

P=0.0001 P=0.0004

^a Values in columns followed by the same letter are not significantly different (P>0.05). Values are means (±SD) of three replicates.

^b Initial weight: 2.4 ± 0.1 g (mean ±SD).

^c Feed efficiency (F.E.) calculated as wet weight gain (g) / dry feed intake (g)

^d Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

^e Protein productive value (PPV) calculated as: 100 · gain in body protein (g) / protein consumed (g).

Table 5.9. Whole body composition (% wet weight) of rainbow trout fed the experimental diets to satiation for 6 weeks in the palatability trial^{ab}.

Diet	Moisture	Protein	Lipid	Ash
CG	70.04 (0.36)	15.79 (0.21)	12.80 (0.23)	2.00 (0.08)
C-6-T	70.45 (0.65)	16.16 (0.91)	12.50 (0.70)	2.35 (0.02)
C-7-N	71.03 (0.22)	15.52 (0.27)	12.09 (0.10)	2.31 (0.01)
C-8-T	70.30 (0.29)	15.62 (0.41)	12.56 (0.21)	2.33 (0.03)
C-9-N	70.08 (0.36)	15.70 (0.07)	12.66 (0.26)	2.32 (0.10)

^a Values shown are means (\pm SD) of determinations made on fish from three replicate tanks. Duplicate determinations of protein, ash, and lipid content were made on each dried sample.

^b Proximate composition of the fish at the start of the trial was: Moisture, 78.47%; protein, 19.38 %; Lipid, 8.20%; and ash, 3.13%. Analysis was conducted on a pooled sample of fish common to all dietary treatment groups.

Table 5.10. Whole body composition (% wet weight) of rainbow trout fed equal quantities of protein from the experimental diets for 6 weeks in the nutritive value trial^{ab}.

Diet	Moisture	Protein	Lipid	Ash
CG	71.82 (0.03)	16.06 (0.23)	11.14 (0.07)	1.92 (0.03)
C-6-T	73.37 (0.52)	16.50 (0.61)	10.06 (0.61)	2.67 (0.09)
C-7-N	73.49 (0.35)	16.75 (0.19)	9.93 (0.28)	2.64 (0.05)
C-8-T	73.83 (0.83)	16.54 (0.13)	9.62 (0.20)	2.64 (0.14)
C-9-N	72.86 (0.32)	15.90 (0.37)	10.60 (0.50)	2.51 (0.02)

^a Values shown are means (\pm SD) of determinations made on fish from three replicate tanks. Duplicate determinations of protein, ash, and lipid content were made on each dried sample.

^b Proximate composition of the fish at the start of the trial was: Moisture, 78.79%; protein, 17.92 %; Lipid, 6.91%; and ash, 3.18%. Analysis was conducted on a pooled sample of fish common to all dietary treatment groups.

Chapter 6

Effects of Feeding Behavior by Interstrain Hybrids of Rainbow Trout on the Measurement of Feedstuff Palatability.

6.1 INTRODUCTION

Knowledge of interstrain differences in feeding behavior is important, since failure to account for this factor may lead to erroneous conclusions regarding feed ingredient palatability. Differences in feeding behavior among strains of rainbow trout have been observed, and are most apparent when fish from the wild are compared with domesticated stocks (Kindschi et al., 1991). Interstrain differences in feeding behavior of domesticated rainbow trout have also been observed. For example, hatchery workers at the University of Washington observed differences in the feeding behavior of pure UW strain and UW x Nisqually interstrain hybrid rainbow trout reared under identical conditions of environment and husbandry that affected feed intake, and apparently, growth (Chapter 5). If feeding behavior by different strains affects feed intake in a manner not correlated with feed palatability, general comparisons of palatability measurements made using consumption as a criterion would be hampered, even though each trial might include a standard control diet. Also, it is possible that some strains of rainbow trout are more sensitive to differences in feedstuff palatability, and that results obtained would depend on the strain used in the experiment.

In the preceding studies, protocols for the evaluation of feedstuff palatability focused on standardization of feed formulation, feeding regimen, and husbandry practices. In view of the foregoing, it may be necessary to consider the influence of feeding behavior characteristics of pure strain and interstrain hybrid rainbow trout when assessing the results of a particular feeding trial and, perhaps more importantly, when between-trial

comparisons are made. Therefore, this experiment was conducted to evaluate the effects of behavioral differences on feeding activity, feed intake, and growth by one pure strain and one interstrain hybrid of rainbow trout.

6.2 METHODS AND MATERIALS

6.2.1 Test protein source

Norwegian LT-94 (LT-1-N, described in Chapter 3) was used in this study. Proximate composition of this fish meal is presented in Table 6.1.

6.2.2 Diet preparation

One diet based on isonitrogenous substitution of fish meal for casein in a semipurified casein-gelatin basal diet was used in this study (Table 6.2). Procedures for preparation of the diet were described in Chapter 4. Proximate composition of the test diet is presented in Table 6.3.

6.2.3 Feeding practices

Groups of fish were fed using the timed-response feeding strategy as described in Chapter 5. Fish were fed twice daily on weekdays, and once daily on weekends for 6 weeks. All other conditions were identical to those previously described.

6.2.4 Experimental facilities, fish, and husbandry

The experiment summarized in this chapter was initiated 2 weeks after the beginning of the palatability study described in Chapter 5. Experimental facilities and husbandry were identical to those used in that trial. Mean daily water temperature was $17.4 \pm 0.9^{\circ}\text{C}$ (Appendix 1).

Progeny of the two separate female-male matings of rainbow trout obtained from the School of Fisheries hatchery, University of Washington, described in Chapter 5 were used in this study. Fish from each family were first selected for uniform size, then randomly distributed among three tanks until each contained 25 animals. At the start of the study, fish of the Nisqually cross were

2.3 ± 0.1 g and 59.5 ± 0.1 mm, while those of the UW cross were 2.4 ± 0.1 g and 59.7 ± 0.6 mm (mean ± SD).

6.2.5 Measurement of growth

Growth in length and weight were measured on day 0 and at 7 day intervals for 28 d, using the methods described in Chapter 5.

6.2.6 Chemical analysis of fish meal, diets, and fish carcasses

Procedures for sampling, preparation, and chemical analysis of fish meal and diet were described in detail in Chapter 3.

One sample of five fish from each strain was collected at the beginning of the trial; at the end of the trial, 10 fish were randomly selected from each tank. The samples were analyzed for proximate composition as described in Chapter 5.

6.2.7 Data analysis

Methods for estimation of growth rates, feed efficiencies, protein efficiency ratios, and protein productive values for rainbow trout fed the experimental diet were as described in Chapter 5. Those data were compared statistically by unpaired t-tests (Little and Hills 1978). Statistical analyses were performed using STATVIEW version 2.0 (Brainpower, Inc., Calabasas, California).

Feeding response scores collected during the study often varied substantially on a day-to-day basis, making graphs produced from the raw data difficult to interpret. Data were transformed by calculating moving averages over a nine-feeding interval from which graphs that showed trends in feeding response by the two strains were produced. Trends in feeding activity, together with dry feed intake data, were used to make inferences regarding the effects of feeding behavior on feed intake by different strains of rainbow trout.

6.3 RESULTS

6.3.1 Growth and survival

Growth in body length and wet weight of two strains of rainbow trout fed to satiation a diet in which LT-1-N fish meal was the primary protein source using a timed-response feeding regimen are summarized in Table 6.4. Survival data are summarized in Appendix 3.

Large differences in growth rate between fish of the two crosses were observed in this trial. Fish of the UW cross grew in weight at a rate nearly 63% higher and in TL at a rate more than 44% higher than those of the Nisqually cross. During the 6 week study, fish of the UW cross increased in weight about six-fold, from 2.8 g to 16.7 g, and increased in TL by 47.8 mm. Fish of the Nisqually cross increased in weight about four-fold, from 2.7 g to 11.3 g, and increased in TL by 32.7 mm. Observed final TL, rate of growth in TL, final weight, and rate of growth in weight were significantly different between the two crosses ($P < 0.05$).

Overall survival rates were $97.3 \pm 2.3\%$ and $90.7 \pm 6.1\%$ for the UW and Nisqually crosses, respectively. All mortality was associated with stress imposed by handling during periods of high water temperature; most occurred during the final two weighing periods when daily mean water temperature exceeded 18°C .

6.3.2 Efficiency of feed and protein utilization

Feed and protein intake, gain in wet weight and protein, and estimates of feed efficiency, PER, and PPV are summarized in Table 6.5. Carcass composition data used in PPV estimation are summarized in Table 6.6.

In this study, pure UW strain rainbow trout consumed almost 50% more dry matter and gained about 61% more weight than those

of the Nisqually interstrain hybrid. These results were reflected in the slightly more efficient utilization of dry matter by the UW cross (F.E.=1.10) than the Nisqually cross (F.E.=1.02). Despite the large between-family differences in dry matter intake and growth rate, no significant differences ($P>0.05$) in either PER or PPV were found.

6.3 Feeding behavior and its effect on feed intake

Observations on the feeding behavior of the two families of fish used in this study confirmed those reported by UW hatchery personnel. Pure strain UW fish consumed nearly 50% more dry matter than the Nisqually interstrain hybrid during the course of the study, even though all fish were given equal access to feed. Observations on feeding activity indicated that these differences were most likely related to differences in the ability of the two families to adapt to the laboratory rearing system. As shown in Figure 6.1, both the UW and Nisqually crosses accepted feed with similar, albeit only fair, enthusiasm during the first week of the study (about 10 feedings). Previous experience has shown that reduced feeding activity during this period is common when fish are not acclimated to the rearing tanks prior to the start of the study, even when a highly palatable diet is offered. Pure strain UW fish quickly became acclimated to the rearing system, and after the first week, exhibited good to excellent feeding response. The interstrain hybrids, however, continued to exhibit a strong fright response when the tank covers were removed at each feeding, and consequently, those fish proved extremely difficult to feed. While fish of the UW cross accepted feed readily throughout the study, feeding response by the Nisqually cross remained almost constant or declined. Changes in feeding activity observed in both groups between the thirtieth and fortieth feedings did not appear to be temperature related (Appendix 1), and could not be attributed to changes in other environmental conditions or husbandry practices.

6.4 SUMMARY AND CONCLUSIONS

The objective of this study was to determine whether differences in feeding behavior between different strains of rainbow trout must be considered when evaluating feedstuff palatability. Under the experimental conditions described, pure UW strain rainbow trout were found to consistently exhibit a higher level of feeding activity and consumed greater quantities of a single standard diet than fish of the Nisqually interstrain hybrid. The observed differences in feeding activity appeared to be related to the ability of the two strains to adapt to the rearing system, since the interstrain hybrid was observed to exhibit a strong fright response and refused feed immediately after the tank covers were removed, while the UW strain generally fed actively throughout the feeding period.

It may be reasonably argued that intrinsic differences in growth potential between the two strains influenced feeding behavior and therefore, feed intake. However, the available data strongly suggest that this was not a major factor. The present investigation was conducted under conditions of environment and husbandry virtually identical to those reported in Chapter 5. In that study, the Nisqually rainbow trout grew in length and weight at rates exceeding 1.1 mm TL/d and 0.3 g wet weight/d, respectively (Table 5.5); rates of growth reported in this chapter for the UW strain were similar (Table 6.4). The data therefore indicate that the Nisqually hybrids did not attain their maximum growth potential under the conditions of this study. The reasons for this result are not known, but may include differences in the nutritive value of the various fish meals or insufficient acclimation to the experimental rearing units.

Results of this trial showed that estimates of feedstuff palatability based on feed intake may be biased when the effects of feeding behavior on feed intake by different strains of rainbow trout

are not considered. The magnitude of this effect cannot be estimated from the results of this trial, but it seems reasonable to suggest that between-trial comparisons may not be valid unless a standard reference protein source to which all test ingredients can be compared is included in every trial. Additionally, it may be advantageous to use a single strain of fish when a series of experiments is to be conducted, since this practice may reduce between-trial variability. Finally, reliable data regarding the growth potential of the various strains of fish used in the trials is required, since this may be a factor governing rates of feed intake. In general, these data would be valuable in assessing the results of a particular feeding trial, and would be indispensable for between-trial comparisons.

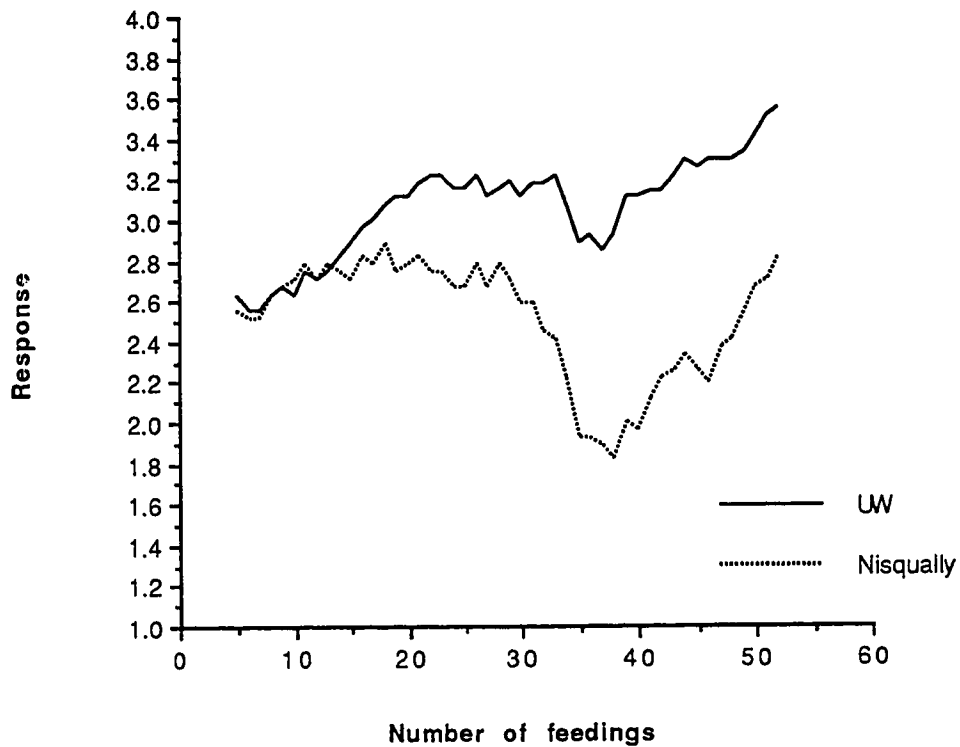


Figure 6.1. Feeding response scores for rainbow trout fed the experimental diets. Numerical scores were assigned following subjective evaluation of feeding response: (1) indicates little or no response, (2) indicates minimal feeding response, (3) indicates average response, and (4) indicates excellent response.

Table 6.1. Proximate composition (% dry weight^a) of the fish meal used in the experimental diet.

Protein source	LT-1-N
Toxicity ^b	N
Origin	Norway

Dry matter	93.78
Protein	79.16
Lipid	9.24
Ash	11.20

^a Protein, lipid and ash data are means of two determinations on the dried sample.

^b Causative of gizzard erosion in chickens as determined by 7 d chick bioassay: T = toxic; N = nontoxic. Assay conducted by Department of Marine Resources, Fundacion Chile, Santiago, Chile.

Table 6.2. Formulation of the experimental diet (% dry ingredients^a).

Ingredient ^a	Diet
	LT-1-N
LT-1-N fish meal	62.0
Gelatin	10.0
Dextrin	8.5
Alpha-cellulose	3.3
Carboxymethylcellulose	1.0
Vitamin premix ^b	2.0
Choline chloride (70% liquid)	1.0
Herring oil	12.0
Ascorbic acid (41% active)	0.2
Water	300
Trace mineral solution ^c	100

^a Sources of ingredients were: fish meal, Fundacion Chile (Santiago, Chile); gelatin, Sigma Chemical Company (St. Louis, Missouri); dextrin, alpha cellulose, and carboxymethylcellulose, ICN Biomedicals, Inc. (Costa Mesa, California); vitamin premix, Moore-Clark Co. (La Connor, Washington); choline chloride, Sigma Chemical Company (St. Louis, Missouri); herring oil, B.C. Packers (Richmond, B.C., Canada); ascorbic acid, Takeda Chemical Co. (Tokyo, Japan); trace mineral solution, Sigma Chemical Company (St. Louis, Missouri).

^b Vitamin premix supplied the following per kg of dry diet ingredients: Vitamin A acetate or palmitate, 6612 IU; Alpha tocopheryl acetate, 503 IU; menadione sodium bisulfite complex, 28 mg; thiamine mononitrate, 46 mg; riboflavin, 53 mg; pyridoxine HCl, 38 mg; d-calcium pantothenate, 115 mg; niacin, 220 mg; d-biotin, 1.45 mg; Vitamin B₁₂, 0.06 mg; myoinositol, 132 mg; folic acid, 16.5 mg; Vitamin D₃, 441 mg.

^c Trace mineral solution supplied the following per kg of dry diet ingredients: KI, 1.9 mg; MnSO₄·4 H₂O, 32.5 mg; ZnSO₄·7 H₂O, 88.0 mg; NaHSeO₃, 4.2 mg; CoCl₃·6 H₂O, 4.0 mg; CuSO₄·5H₂O, 11.8 mg.

Table 6.3. Proximate composition (% dry weight^a) of the experimental diet.

Diet	
LT-1-N	
Dry matter	69.17
Protein	59.43
Lipid	19.99
Ash	13.27

^a Protein, lipid, and ash data are means of two determinations on the dried sample.

Table 6.4. Mean growth of two strains of rainbow trout fed the experimental diet to satiation for 6 weeks^a.

Strain	Final length ^b (mm TL)	Growth rate ^c (mm TL/d)	Final weight (g)	Growth rate (g/d)
UW x UW	111.0 (3.0)	1.20 (0.05)	16.7 (0.9)	0.35 (0.02)
UW x Nisqually	95.4 (1.1)	0.83 (0.03)	11.4 (0.9)	0.22 (0.01)
	P=0.001	P=0.0003	P=0.002	P=0.0004

^a Mean differences in growth were significant ($P < 0.05$). Values are means (\pm SD) of three replicates.

^b Mean (\pm SD) initial weight (g) and total length (mm TL) of the UW x UW cross were: 2.8 ± 0.1 g and 63.2 ± 1.0 mm, respectively. Mean (\pm SD) initial weight (g) and total length (mm TL) of the UW x Nisqually cross were: 2.8 ± 0.1 g and 62.6 ± 0.3 mm, respectively. Differences in length and weight between families were not significant at the beginning of the trial by unpaired t-test ($P > 0.05$).

^c Rates of growth are the slope coefficients from the regression of total length or weight (y) on time (x).

Table 6.5. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by two strains of rainbow trout fed the experimental diet to satiation for 6 weeks^a.

Strain	Intake (g)			Gain (g)			PER ^d	PPV ^e
	Dry feed	Protein	Weight ^b	Protein	F.E. ^c	PER ^d		
UW x UW	12.59 (0.70)	7.48 (0.42)	13.86 (0.82)	2.12 (0.15)	1.10 (0.01)	1.87 (0.01)	28.32 (1.44)	
UW x Nisqually	8.40 (0.22)	5.00 (0.13)	8.60 (0.82)	1.37 (0.09)	1.02 (0.10)	1.71 (0.11)	27.42 (2.40)	
						P=0.071	P=0.608	

^a Values are means (±SD) of three replicates.

^b Initial weight of the UW x UW and the UW x Nisqually strains were, respectively, 2.8 ± 0.1 g and 2.8 ± 0.1 g (mean ± SD).

^c Feed efficiency (F.E.) calculated as wet weight gain (g) / dry feed intake (g)

^d Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

^e Protein productive value (PPV) calculated as: 100 · gain in body protein (g) / protein consumed (g).

Table 6.6. Whole body composition (% wet weight^a) of two strains of rainbow trout fed the experimental diet to satiation for 6 weeks^b.

	UW female x UW male		UW female x Nisqually male	
	Initial	Final	Initial	Final
Moisture	79.14	70.94 (1.54)	77.85	69.63 (0.81)
Protein	14.10	15.09 (0.59)	14.05	15.49 (0.92)
Lipid	4.70	11.63 (0.78)	5.62	12.77 (0.73)
Ash	2.47	2.38 (0.13)	2.47	2.47 (0.17)

^a At the beginning of the trial, proximate analysis was conducted on a pooled sample of fish from each strain. At trial termination, determinations were made on fish from three replicate tanks: values shown are means (\pm SD). Duplicate determinations of protein, ash, and lipid content were made on each dried sample.

^b Mean (\pm SD) initial weight (g) and total length (mm TL) of the UW x UW cross were: 2.82 ± 0.13 g and 63.19 ± 1.02 mm, respectively. Mean (\pm SD) initial weight (g) and total length (mm TL) of the UW x Nisqually cross were: 2.75 ± 0.09 g and 62.61 ± 0.28 mm, respectively.

Chapter 7

Effects of Protein Intake Level on Nutrient Partitioning in Rainbow Trout

7.1 INTRODUCTION

Many procedures for measurement of the nutritional value of feed proteins to higher animals have been devised. In the broadest sense, biological assays of protein quality attempt to measure how closely the pattern of amino acids in the protein meet the requirements of the growing animal. The results of these assays depend to a large extent on total protein intake, whether a function of feeding level or dietary protein content, relative to the optimum requirement level for growth or nitrogen retention. In general, protein intake is usually restricted to slightly below the optimum requirement, since those conditions tend to accentuate quality differences. For example, borderline deficiencies or imbalances in essential amino acids that reduce the nutritional value of a protein at low levels of intake may be overcome when total protein intake is increased above the requirement level. Furthermore, the capacity for protein to support weight gain and protein retention have been shown to be dose dependent, indicating that standard comparisons of protein sources must be made at the same level of protein intake. These topics have been critically reviewed by Hegsted and Chang (1965), Sammons and Hegsted (1977), and Evans and Witty (1978).

As in higher animals, level of protein intake also affects protein utilization and retention by fish. For example, Steffens (1981), using the data of Huisman (1976) estimated maximum PER and PPV to be 1.26 and 23.2%, respectively, when rainbow trout reared at 15°C were fed a 49% crude protein diet at a rate of 2% body weight/d. A feeding rate of 1% body weight/d yielded a PER of 1.22 and a PPV of 11.5%, whereas feeding at a rate of 3% body

weight/d yielded a PER of 1.04 and a PPV of 17.1%. Nose (1971) fed rainbow trout diets containing graded levels of either casein or white fish meal as the sole sources of protein at a rate of 2.5% body weight/d for 15 d, and determined PER, net protein utilization (NPU), and biological value (BV) for each. In his studies, efficiency of protein utilization was directly related to total protein intake. Furthermore, the intake level at which maximum response was noted differed depending on the protein source tested. For example, the PER maxima for casein and fish meal were 2.4 and 2.8 at 130 and 110 mg absorbed nitrogen/100 g body weight/d, respectively, and apparently declined when higher or lower rates were used. Similar trends were observed for NPU. Apparent digestibility and thus BV of the protein sources also seemed to be affected by protein intake level, though no clear maxima could be identified. These data underscore the importance of evaluating protein quality under conditions of submaximal and equal intake, if reasonable estimates of protein utilization appropriate for standard comparisons are to be obtained.

While it is relatively easy to adjust feeding levels among treatment groups to achieve equal protein intake, *a priori* determination of the appropriate level requires some knowledge of the effects of intake level on the metabolic fate of the ingested protein. Insufficient data were available from the literature to permit selection of appropriate rates of protein intake for growth and protein retention assays in rainbow trout reared under the experimental conditions present in this laboratory. Therefore, this study was conducted to: (1) determine how rate of protein intake from graded levels of a single standard diet affected dietary protein utilization by rainbow trout and (2) evaluate the sensitivity of the assay to changes in the nutritional quality of fish meal protein quality resulting from thermal abuse. The data were necessary to identify the level or range of feeding levels to be used in future studies conducted under similar conditions.

7.2 METHODS AND MATERIALS

7.2.1 Test protein sources

High quality Canadian herring meal (White Crest, Fisherman's Cooperative Federation, Prince Rupert, British Columbia, Canada), was incorporated into diets in either its native state or following thermal abuse by autoclaving, a process expected to measurably reduce the nutritive value of the protein source through structural alteration or chemical changes in the constituent amino acids. The dry fish meal was spread to a depth of about 3 cm on aluminum cookie sheets, placed on racks within an autoclave (AMSCO model 3021 gravity autoclave, Erie, Pennsylvania) and heated with steam to 123°C at 103-117 kPa for 3.5 h. The heat treated fish meal was then removed from the autoclave, and was stirred at 5 min intervals for 1 h to facilitate the evaporation of residual moisture. The fish meal was then placed into plastic bags and stored at room temperature for 2 d before incorporation into the test diets. Proximate composition of the fish meal is presented in Table 7.1.

7.2.2 Diet preparation

Two diets based on isonitrogenous substitution of the heated or unheated protein source for casein in a semipurified casein-gelatin basal diet were used in this study (Table 7.2). Procedures for preparation of the diets were described in Chapter 4. Proximate composition of the test diets is summarized in Table 7.3.

7.2.3 Feeding practices

In this study, groups of fish were pair-fed the two experimental diets at graded levels of protein intake ranging from unfed to satiation. Feeding levels for fish fed the diet containing the unheated (control) fish meal were established as follows: (1) 1.3 g protein/kg body weight/d, based on the maintenance requirement for protein reported by Ogino (1980); (2) arbitrarily set levels of 3.0,

6.0, and 11.0 g protein/kg body weight/d; and (3) 16.0 g protein/kg body weight/d, based on voluntary rates of feed intake by rainbow trout fed fish meal based diets described in Chapter 3. Groups of fish fed diets containing the autoclaved fish meal were paired to and, on a per-fish basis, received identical quantities of protein as their respective control groups, without regard to differences in weight gain over each weekly rearing interval. In addition to these treatments, two groups of fish received no feed, and two groups were fed each of the diets to satiation in each of two daily feeding intervals of 2 min duration, as previously described. Feed was weighed and intake recorded at weekly intervals. Protein consumption data are summarized in Table 7.4.

Quantities of feed to be given to each treatment pair were adjusted at 7 d intervals, based on the gross weight of the fish in each tank receiving the control diet and the wet weight protein level of the respective test and control diets. To accomplish this, fish in each tank were first sampled for changes in weight and body length (see 7.2.4). Protein intake for each control group during the subsequent 6 d feeding interval was then calculated as:

$$p = 6 * (r * w)$$

where: p = protein to be fed, in g per tank; r = rate of protein intake, in g/kg body weight/d; w = gross wet weight of the fish in the control tank (kg).

Next, the average protein intake (g/tank/week) was calculated for the two control tanks at each feeding level. Gross wet feed intake (g/tank/week) was then calculated for each dietary treatment by dividing the protein intake value by the protein content of the respective diets. Only two mortalities were observed during the experiment (Appendix 3), and since their influence on the overall rate of protein intake was very small, the number of fish in each tank was not considered in the feeding rate calculations.

Previous observations on feeding behavior of fish fed at restricted levels indicate that the most aggressive fish in a population often obtain a disproportionate amount of the feed presented. In order to equalize feed intake among fish in each tank, groups pair-fed at the lowest level (1.3 g protein/kg fish body weight/d) were fed once per week, on the day immediately following weighing. Groups pair-fed at the next highest level (3 g protein/kg fish body weight/d) were fed three times per week. All other groups were fed on a daily basis. Fish were not fed on weighing days.

7.2.4 Experimental facilities, fish, and husbandry

Experimental facilities and husbandry were as described in Chapter 4. Mean water temperature was $17.8 \pm 0.5^{\circ}\text{C}$. A profile of daily water temperature recorded during the experiment is depicted in Appendix 1.

Progeny of single female-male mating of Donaldson strain rainbow trout obtained from the School of Fisheries hatchery, University of Washington were selected for uniform size, then randomly distributed among 26 tanks until each contained 20 animals. Two experimental units were then assigned at random to each treatment (diet-feeding level combination).

7.2.5 Measurement of growth

Growth in length and weight was measured on day 0 and at 7 d intervals for 28 d, using the methods described in Chapter 5.

7.2.6 Chemical analysis of fish meal, diets, and fish carcasses

Procedures for sampling, preparation, and chemical analysis of fish meals and diets were described in detail in Chapter 3. One sample of five fish were collected at the beginning of the trial; at the end of the trial, five fish were randomly selected from each tank. The samples were analyzed as described in Chapter 5

7.2.7 Data analysis

PER and PPV for fish in each treatment group were calculated from data on weight gain, protein intake, and nitrogen retention, as described in Chapter 5. Statistical analysis of PER and PPV data was limited to protein intake levels that consistently resulted in growth in both length and weight and yielded positive values for both estimates of protein utilization. This was done since only intake levels that met these conditions are valid measures of protein utilization. No attempt was made to statistically compare heated (control) and unheated (test) protein sources as this approach failed to yield additional information relevant to the stated purpose of this experiment.

7.4 RESULTS

7.4.1 Relationship between protein intake level and growth in length and weight

Table 7.5 summarizes mean TL and wet weight data for groups of rainbow trout pair-fed at rates of protein intake ranging from 0 g/kg body weight/d to satiation. Statistical analysis of these data was not performed, since the information provided by these measurements was used only to identify levels of protein intake that would result in growth in length and weight gain by the fish, whether or not the protein source had been heated prior to incorporation into the test diets.

Groups of fish that were unfed, or were pair-fed at the rate of 1.3 g protein/kg body weight/d lost weight. Fish pair-fed 3.0 g protein/kg body weight/d grew slightly in body length, but only fish fed diet containing the heated protein source gained weight. Groups of fish pair-fed at protein intake levels of 6.0 g/kg body weight/d or higher consistently gained weight and increased in body length.

Feeding levels were assigned with the intent of providing evenly spaced increments between maintenance and satiation. Satiation intake was greatly underestimated, and fish fed at this level consumed nearly 2.5 times more dry feed and gained about 2.4 times more weight than those fed at the next lowest level. Nevertheless, the effects of dietary treatment on growth in length and weight at the satiation level were apparent, in that weight gain and growth in length were substantially lower in groups fed the heat-treated fish meal. The nature of this relationship could not be fully evaluated from the available data, however.

7.4.2 Relationship of protein intake level to efficiency of diet and protein utilization

Feed and protein intake, gain in wet weight and protein, and estimates of feed efficiency, PER, and PPV for all feeding levels tested are summarized in Table 7.6. Statistical analyses of PER and PPV data for intake levels that consistently resulted in growth in both length and weight and yielded positive values for both PER and PPV are summarized in Table 7.7. Carcass composition data used in PPV estimation are summarized in Table 7.8.

PER and PPV data (Table 7.6) indicated that all groups of fish pair-fed at protein intake levels ranging from 6.0 g/kg body weight/d to satiation were in positive nitrogen balance, and that measurable protein accretion had occurred at the highest feeding levels. Data for each protein source were then examined separately by AOV to identify the rates of protein intake that would yield equivalent PER and PPV values (Table 7.7). The results of this analysis were markedly different for the two protein sources. Protein efficiency ratios for the diet containing unheated fish meal increased significantly ($P < 0.001$) as rate of protein intake increased from 6.0 to 16.0 g protein/kg body weight/d. Satiation feeding of the diet containing the unheated fish meal did not improve the PER, compared to the highest controlled intake level (16.0 g protein/kg body weight/d). In contrast, estimated protein efficiency ratios for the diet containing the heated fish meal were statistically equivalent ($P > 0.05$) at all levels of protein intake tested. Overall, protein efficiency ratios were about 15% higher for groups fed the unheated fish meal than those fed the heated protein source.

As predicted, efficiency of protein retention (Table 7.7) was affected by rate of protein intake, and the rate that resulted in the highest retention differed between the two protein sources. In this study, protein retention from the unheated source was maximized ($P < 0.05$) when the fish were fed at a rate of 11.0 g/kg body

weight/d: higher intake levels, including satiation, did not affect PPV. In contrast, there was no significant difference ($P>0.05$) in protein retention by fish fed the heated protein source, irrespective of the level of protein intake, although retention seemed to increase in proportion to intake up to the highest controlled level of feeding (16.0 g protein/ kg body weight/d) and to decline at the satiation level.

The largest differences in PPV between the two dietary treatments were observed at the lowest level of intake (6.0 g protein/kg body weight/d). Surprisingly, the PPV of the heated protein source was about 3 times higher than that of the unheated fishmeal. However, the overall nutritive value of the fish meal was diminished by heating. When only the three highest levels of protein intake were considered, the PPV of the unheated fishmeal was about 30% better than that of the heated protein source.

7.5 SUMMARY AND CONCLUSIONS

The primary objective of this study was to identify which rates of restricted protein intake would yield near-maximal efficiencies of protein retention from a standard test diet by rainbow trout. Under these experimental conditions, rainbow trout pair-fed at rates of protein intake of 3.0 g/kg body weight/d lost body protein, while those pair-fed 6.0 g protein/kg body weight/d were clearly in positive nitrogen balance. However, rates of protein intake below 11.0 g/kg body weight/d appeared to be unacceptable for assessing protein quality, since the apparent rate of protein retention was, in the case of the unheated fishmeal fed at 6.0 g protein/kg body weight/d, unrealistically low. Importantly, the level of intake that resulted in maximum retention of protein from a single standard diet differed depending on whether the fish meal was heated or used in its native state. Thus, it can be concluded that feeding trials conducted specifically to determine the relative quality of various protein sources under conditions of equal intake cannot at the same time measure their true nutritional values, since the optimum level of intake resulting in maximum protein retention may differ among the various sources. Nevertheless, the results of this study indicated that in future experiments, valid comparisons of protein quality could be made over a wide range of controlled protein intake levels, assuming that environmental conditions, sizes of fish, and husbandry practices would be nearly identical to those used in this experiment.

Table 7.1. Proximate composition (% dry weight^a) of fish meal used in the experimental diets.

Protein source	HM-1
Toxicity ^b	N.D.
Origin	British Columbia, Canada

Dry matter	93.62
Protein	76.20
Lipid	9.43
Ash	13.94

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

^b Not determined.

Table 7.2. Formulation of the experimental diets (% dry ingredients^a).

Ingredient	Diet
	HM-1
HM-1 fish meal	65.0
Gelatin	10.0
Dextrin	7.0
Alpha-cellulose	1.8
Carboxymethylcellulose	1.0
Vitamin premix ^b	2.0
Choline chloride (70% liquid)	1.0
Herring oil	12.0
Ascorbic acid	0.2
Water	200
Trace mineral solution ^c	100

^a Sources of ingredients were: fish meal, White Crest, Fisherman's Cooperative Federation (Prince Rupert, B.C., Canada); gelatin, Sigma Chemical Company (St. Louis, Missouri); dextrin, alpha cellulose, and carboxymethylcellulose, ICN Biomedicals, Inc. (Costa Mesa, California); vitamin premix, Moore-Clark Co. (La Connor, Washington); choline chloride, Sigma Chemical Company (St. Louis, Missouri); herring oil, B.C. Packers (Richmond, B.C., Canada); ascorbic acid, Takeda Chemical Co. (Tokyo, Japan); trace mineral solution, Sigma Chemical Company (St. Louis, Missouri).

^b Vitamin premix supplied the following per kg of dry diet ingredients: Vitamin A acetate or palmitate, 6612 IU; Alpha tocopheryl acetate, 503 IU; menadione sodium bisulfite complex, 28 mg; thiamine mononitrate, 46 mg; riboflavin, 53 mg; pyridoxine HCl, 38 mg; d-calcium pantothenate, 115 mg; niacin, 220 mg; d-biotin, 1.45 mg; Vitamin B₁₂, 0.06 mg; myoinositol, 132 mg; folic acid, 16.5 mg; Vitamin D₃, 441 mg.

^c Trace mineral solution supplied the following per kg of dry diet ingredients: KI, 1.9 mg; MnSO₄·4 H₂O, 32.5 mg; ZnSO₄·7 H₂O, 88.0 mg; NaHSeO₃, 4.2 mg; CoCl₃·6 H₂O, 4.0 mg; CuSO₄·5H₂O, 11.8 mg.

Table 7.3. Proximate composition (% dry weight^a) of diets.

	Diet	
	HM-1(unheated)	HM-1 (heated)
Dry matter	68.72	67.56
Protein	62.87	62.15
Lipid	18.43	17.97
Ash	9.21	8.90

^a Protein, lipid, and ash are means of two determinations on each dried sample.

Table 7.4. Protein consumption (g/fish/week^a) by rainbow trout fed diets containing either herring meal or herring meal heat treated by autoclaving.

Paired treatments ^b	Feeding rate	Heating	Protein intake (g/fish/week) ^c			
			Week 1	Week 2	Week 3	Week 4
	Not fed	NA	0	0	0	0
Control	1.3	N	0.06	0.05	0.05	0.05
Test		H	0.06	0.05	0.05	0.05
Control	3.0	N	0.15	0.14	0.14	0.14
Test		H	0.15	0.14	0.14	0.14
Control	6.0	N	0.29	0.29	0.30	0.32
Test		H	0.29	0.29	0.30	0.32
Control	11.0	N	0.52	0.57	0.62	0.69
Test		H	0.52	0.57	0.62	0.69
Control	16.0	N	0.77	0.87	1.00	1.17
Test		H	0.77	0.87	1.03	1.20
Control	Satiation	N	1.35	2.29	3.24	3.66
Control	Satiation	N	1.04	2.03	2.87	3.24
Test	Satiation	H	0.87	1.96	3.01	3.33
Test	Satiation	H	0.80	2.00	3.09	3.35

^a Groups of fish were either not fed, fed to satiation, or pair-fed at various fixed rates of protein intake for 4 weeks to determine the effect of level of protein intake on protein retention from a standard test diet.

^b N = 2 tanks per treatment. Protein intake (g/fish/week) between replicate tanks of fish in the fixed feeding treatments was equal, therefore only one value for each is given. Fish fed to satiation consumed different quantities of protein: data is shown for each replicate tank.

^c Rate of protein intake (g/kg fish body weight/d) established for control groups fed diet containing unheated herring meal. Quantity of feed given to both the control and test groups was adjusted at 7 d intervals, based on the weight of fish in the control group of each feeding rate pair. Heating codes are: N = not heated; H = heated in an autoclave for 3.5 h at 103-117 kPa.

Table 7.5. Mean growth of rainbow trout pair-fed the experimental diets at various rates of protein intake for 4 weeks^a.

Paired treatments ^b	Feeding rate	Heat	Initial length (mm TL)	Final length (mm TL)	Initial weight (g)	Final weight (g)
	Not fed		85.5 (0.3)	83.2 (0.0)	6.4 (0.1)	5.2 (0.6)
Control	1.3	N	87.6 (2.9)	87.5 (2.4)	7.4 (0.5)	6.5 (0.6)
Test		H	87.3 (0.3)	85.9 (0.4)	6.8 (0.1)	6.1 (0.1)
Control	3.0	N	90.7 (2.4)	92.0 (2.4)	8.3 (0.3)	8.0 (0.4)
Test		H	86.8 (0.7)	88.3 (0.6)	6.7 (0.2)	7.1 (0.3)
Control	6.0	N	88.9 (0.2)	94.9 (0.7)	8.2 (0.1)	9.3 (0.2)
Test		H	86.1 (0.3)	90.6 (0.5)	6.5 (0.0)	8.0 (0.1)
Control	11.0	N	89.7 (0.0)	100.3 (1.2)	7.9 (0.1)	11.6 (0.0)
Test		H	87.3 (0.5)	96.6 (0.4)	6.7 (0.0)	10.0 (0.2)
Control	16.0	N	90.0 (2.1)	106.0 (2.0)	8.1 (0.3)	14.1 (0.6)
Test		H	86.8 (0.5)	101.1 (0.4)	6.68 (0.03)	12.4 (0.1)
Control	Satiation	N	89.5 (2.1)	122.2 (4.4)	8.1 (1.1)	24.7 (2.5)
Test	Satiation	H	86.6 (0.8)	112.5 (0.9)	6.5 (0.2)	18.4 (0.5)

^a Data shown are means (\pm SD) of two replicates. Groups of fish were either not fed, fed to satiation, or pair-fed at various fixed rates of protein intake for 4 weeks to determine the effect of level of protein intake on protein retention from a standard test diet. Data shown are means of two replicate tanks.

^b Rate of protein intake (g/kg fish body weight/d) established for control groups fed diet containing unheated herring meal. Quantity of feed given to both the control and test groups was adjusted at 7 d intervals, based on the weight of fish in the control group of each feeding rate pair. Heating codes are: N = not heated; H = heated in an autoclave for 3.5 h at 103-117 kPa.

Table 7.6. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout pair-fed for 4 weeks at various rates of protein intake^a.

Paired treatment ^b	Feeding rate	Intake (g)			Gain (g)			PER ^e	PPV ^f
		Heat	Dry feed	Protein	Weight ^c	Protein	F.E. ^d		
	Not fed				-1.21 (0.01)	-0.31 (0.00)			
Control	1.3	N	0.34 (0.00)	0.22 (0.00)	-0.90 (0.06)	-0.23 (0.01)	-2.61 (0.16)	-2.77 (0.40)	-109.69 (3.55)
Test	1.3	H	0.35 (0.00)	0.22 (0.00)	-0.69 (0.14)	-0.21 (0.03)	-1.98 (0.41)	-2.61 (0.57)	-96.87 (15.87)
Control	3.0	N	0.91 (0.00)	0.58 (0.00)	-0.36 (0.12)	-0.12 (0.04)	-0.40 (0.13)	0.31 (0.15)	-21.51 (7.18)
Test	3.0	H	0.93 (0.01)	0.58 (0.00)	0.39 (0.44)	-0.04 (0.01)	0.51 (0.47)	0.85 (0.26)	-6.66 (1.52)
Control	6.0	N	1.92 (0.00)	1.21 (0.00)	1.12 (0.07)	0.07 (0.06)	0.59 (0.04)	1.33 (0.04)	5.72 (4.96)
Test	6.0	H	1.94 (0.00)	1.21 (0.00)	1.49 (0.12)	0.21 (0.05)	0.77 (0.06)	1.36 (0.19)	17.87 (4.64)
Control	11.0	N	3.83 (0.00)	2.41 (0.00)	3.68 (0.11)	0.56 (0.06)	0.96 (0.03)	1.58 (0.03)	23.23 (2.62)
Test	11.0	H	3.88 (0.00)	2.41 (0.00)	3.36 (0.20)	0.45 (0.03)	0.87 (0.05)	1.41 (0.07)	18.62 (1.06)
Control	16.0	N	6.09 (0.00)	3.83 (0.00)	6.05 (0.34)	0.87 (0.13)	0.99 (0.06)	1.66 (0.02)	22.71 (3.42)
Test	16.0	H	6.29 (0.18)	3.91 (0.11)	5.68 (0.12)	0.80 (0.01)	0.90 (0.01)	1.39 (0.15)	20.68 (0.84)
Control	Satiation	N	15.70 (1.53)	9.87 (0.96)	16.64 (1.40)	2.44 (0.13)	1.06 (0.01)	1.69 (0.00)	24.79 (1.07)
Test	Satiation	H	14.82 (0.09)	9.21 (0.06)	11.87 (0.35)	1.38 (0.39)	0.80 (0.03)	1.26 (0.05)	15.05 (4.12)

^a Data shown are means (±SD) of two replicates. Groups of fish were either not fed, fed to satiation, or pair-fed at various fixed rates of protein intake for 4 weeks to determine the effect of level of protein intake on protein retention from a standard test diet. Data shown are means of two replicate tanks.

^b Rate of protein intake (g/kg fish body weight/d) established for control groups fed diet containing unheated herring meal. Quantity of feed given to both the control and test groups was adjusted at 7 d intervals, based on the weight of fish in the control group of each feeding rate pair. Heating codes are: N = not heated; H = heated in an autoclave for 3.5 h at 103-117 kPa.

^c Initial weights shown in Table 7.5.

^d Feed efficiency (F.E.) calculated as wet weight gain (g)/dry feed intake (g)

^e Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

^f Protein productive value (PPV) calculated as: $100 \cdot \text{gain in body protein (g)} / \text{protein consumed (g)}$.

Table 7.7. Efficiency of protein utilization by rainbow trout pair-fed at various rates of protein intake for 4 weeks^a.

Feeding rate ^b	HM-1 (unheated)		HM-1 (heated)	
	PER ^c	PPV ^d	PER	PPV
6.0	1.33 (0.04) ^X	5.72 (4.96) ^X	1.36 (0.19)	17.86 (4.64)
11.0	1.58 (0.03) ^Y	23.23 (2.62) ^Y	1.41 (0.07)	18.62 (1.06)
16.0	1.66 (0.02) ^Z	22.71 (3.42) ^Y	1.40 (0.15)	20.68 (0.85)
Satiation	1.69 (0.00) ^Z	24.79 (1.06) ^Y	1.26 (0.05)	15.05 (4.12)
	P=0.0005	P=0.0129	P=0.6491	P=0.4546

^a Groups of fish were either fed to satiation or pair-fed at various fixed rates of protein intake for 4 weeks to determine the effect of level of protein intake on protein retention from a standard test diet. Data shown are means of two replicate tanks. Data shown are means (\pm SD) of two replicates. Values in columns followed by the same letter are not significantly different ($P>0.05$).

^b Rate of protein intake (g/kg fish body weight/d) established for control groups fed diet containing unheated herring meal. Quantity of feed given to both the control and test groups was adjusted at 7 d intervals, based on the weight of fish in the control group of each feeding rate pair. Heating codes are: N = not heated; H = heated in an autoclave for 3.5 h at 103-117 kPa.

^c Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

^d Protein productive value (PPV) calculated as: $100 \cdot \text{gain in body protein (g)} / \text{protein consumed (g)}$.

Table 7.8. Whole body composition (% wet weight^a) of rainbow trout pair-fed at various rates of protein intake for 4 weeks.

Paired treatments ^b	Feeding rate	Heat	Moisture	Protein	Lipid	Ash
	Not fed		80.40 (1.16)	13.98 (0.14)	3.23 (0.97)	3.14 (0.19)
Control	1.3	N	78.78 (0.03)	14.85 (0.14)	4.19 (0.03)	2.98 (0.04)
Test	1.3	H	78.70 (0.95)	14.63 (0.20)	4.57 (0.57)	2.97 (0.09)
Control	3.0	N	77.34 (0.59)	15.43 (0.32)	5.05 (0.43)	2.93 (0.02)
Test	3.0	H	78.52 (0.21)	15.45 (0.21)	4.20 (0.12)	2.90 (0.11)
Control	6.0	N	76.67 (0.06)	15.03 (0.75)	5.80 (0.28)	2.61 (0.07)
Test	6.0	H	76.92 (0.09)	15.90 (0.47)	6.00 (0.13)	2.76 (0.14)
Control	11.0	N	75.46 (0.89)	15.92 (0.40)	7.18 (0.66)	2.40 (0.02)
	11.0	H	76.26 (0.18)	15.27 (0.05)	6.19 (0.05)	2.57 (0.05)
Control	16.0	N	74.83 (0.90)	15.43 (0.57)	8.22 (1.18)	2.21 (0.10)
Test	16.0	H	75.38 (0.63)	15.32 (0.23)	7.31 (0.36)	2.40 (0.03)
Control	Satiation	N	71.71 (0.28)	15.20 (0.28)	11.08 (0.33)	1.93 (0.02)
Test	Satiation	H	74.48 (0.62)	13.33 (2.34)	8.93 (0.05)	2.17 (0.11)

^a Proximate composition (%) of the fish at the start of the trial was: Moisture, 74.83 ± 0.60 ; protein, 15.92 ± 0.46 ; lipid, 6.51 ± 0.20 ; and ash, 2.75 ± 0.28 . Data shown are means (\pm SD) of two treatment replicates. Duplicate determinations of protein, lipid, and ash were made on each dried sample.

^b Rate of protein intake (g/kg fish body weight/d) established for control groups fed diet containing unheated herring meal. Quantity of feed given to both the control and test groups was adjusted at 7 d intervals, based on the weight of fish in the control group of each feeding rate pair. Heating codes are: N = not heated; H = heated in an autoclave for 3.5 h at 103-117 kPa.

Chapter 8

Effects of Dietary Histamine Level and Heating on the Nutritive Value of Fish Meal and Casein to Rainbow Trout

8.1 INTRODUCTION

Histamine content and degree of toxicity to chickens have been used as key quality criteria in the selection of fish meal suitable for use in diets for salmonids. Previous studies reported in Chapters 3 and 5 have shown that the toxicity of fish meal to chickens is not an adequate indicator of either nutritive value or palatability to rainbow trout. Furthermore, short term feeding studies involving fish meals high in endogenous histamine and other putrefactive amines have suggested that these compounds do not have a negative effect on either protein quality or palatability. This was especially apparent from the results of the feeding trial described in Chapter 5, in which two toxic and two nontoxic fish meals were fed to rainbow trout for 6 weeks. However, the long term feeding trial described in Chapter 4, in which casein or various fish meals were treated with histamine or histamine, putrescine, and cadaverine, then subjected to heat treatment, indicated that these compounds could induce gross morphological changes in the digestive tissues of rainbow trout. Although the data were biased by the continual removal of fish for histological evaluation, the results of that trial seemed to indicate that growth and feed efficiency were not adversely affected by the treatments imposed. The objective of this study was to investigate the direct and interactive effects of protein source, level of added histamine, and heat treatment on the nutritive value and palatability of fish meal and casein to rainbow trout.

8.2 METHODS AND MATERIALS

8.2.1 Test protein sources

Two sources of dietary protein, vitamin-free casein and Norwegian LT-1-N (described in detail in Chapter 3) were used in this study. The two protein sources were incorporated into diets in their native state, or altered by the addition of various levels histamine, with or without subsequent heat treatment. Histamine dihydrochloride (60.384% histamine as free base: Sigma Chemical Co, St. Louis, Missouri) was added to the protein sources in quantities calculated to yield either 500, 1,000, 1,500, or 2,000 mg histamine/kg dry diet.

Dietary protein sources that were heated following addition of histamine were treated as previously reported for LT-3-N in Chapter 4, with the following modifications: the protein sources were oven dried for 9 h at 60°C instead of lyophilized, then heated for an additional 3 h at 130°C. Heating was discontinued and the dried materials allowed to cool in the oven for 12 h. Air circulation was maintained at the lowest setting throughout the drying and cooling sequence. Proximate composition of the test protein sources is summarized in Table 8.1.

The addition of histamine to dietary protein sources that were not subsequently heated was done with small modifications to the procedure described above. The amine was weighed, then blended with the trace mineral premix, which was added directly to the appropriate diet. Details of this procedure are reported below.

8.2.2 Diet preparation

Twenty test diets, one for each combination of protein source (fish meal or casein), histamine level (0, 500, 1,000, 1,500, 2,000 mg/kg dry diet), and heat treatment (heated or unheated), based on isonitrogenous substitution of the protein source for casein in the

semipurified basal diet described in Chapter 4 were used in this study. Diet codes, formulations, and proximate compositions are summarized in Tables 8.2 and 8.3.

Blending of the test ingredients into the final dry mix was as described in Chapter 4. The gelatin solution was prepared in two different ways. In cases where histamine had been added to the protein source, and the combination heat treated, the trace mineral mix was blended into the gelatin solution before addition to the diet. When heat treatment was not used, the appropriate amounts of histamine were blended with only the trace mineral premix at room temperature, and that solution mixed with the dry ingredients just prior to the addition of the fish oil. In order to minimize the possible effects of heat on the histamine added to these diets, the gelatin solution was allowed to cool slightly from boiling before it was added to the mix. The dough was then spread onto aluminum cookie sheets to a depth of about 3 cm and frozen at -20°C . Pelleting and storage of the diets was as reported in Chapter 4.

8.2.3 Feeding practices

Two feeding regimens were used, depending on the specific objectives of each part of the study. In order to evaluate the effects of histamine level and heat treatment on the nutritive value of vitamin-free casein and LT-1-N fish meal (Objective 1), all groups were fed identical quantities of dietary protein, based on the average weight of fish in the control treatment (LT0) and a fixed rate of protein intake of 11 g/kg fish body weight/d. All other procedures used in this portion of this study were identical to those described in Chapter 7, except protein intake level (g/tank) was adjusted on a weekly basis to account for mortalities.

The effects of histamine and heat treatment on the palatability of the LT-1-N fish meal to rainbow trout (Objective 2) was evaluated by feeding groups of fish twice daily on weekdays,

and once daily on weekends using the timed-response feeding regimen described in Chapter 4. Feed intake by fish in each tank was recorded on a weekly basis.

8.2.4 Experimental facilities, fish, and husbandry

Experimental facilities and husbandry were as described in Chapter 4. Mean (\pm SD) water temperature was $13.1 \pm 0.8^{\circ}\text{C}$. A profile of daily water temperature recorded during the experiment is depicted in Appendix 1.

Nisqually strain rainbow trout were selected for uniform size, then randomly distributed among 48 tanks until each contained 20 animals. Forty tanks were designated for use in the nutritive value trial, and the remaining eight tanks were designated for use in the palatability trial. Two experimental units were then assigned at random to each treatment. At the beginning of the trial, the fish used in the pair-feeding study were 8.6 ± 0.2 g and 91.6 ± 1.0 mm TL (mean \pm SD), whereas fish used in the palatability study were 8.6 ± 0.2 g and 91.6 ± 1.0 mm TL.

8.2.5 Measurement of growth

Body length (mm TL) and wet weight (g) was measured on day 0 and at 7 d intervals for 28 d, using the methods described in Chapter 5.

8.2.6 Collection and preparation of samples for histological evaluation

Since previous studies described in Chapters 3, 4, and 5 revealed no histologically significant morphological changes in digestive tract tissues of rainbow trout fed diets containing 2,000 mg/kg added histamine or fish meals acutely toxic to chickens for 4 to 6 weeks. In the current study, samples were not collected for histological examination.

8.2.7 Chemical analysis of fish meal, diets, and fish carcasses

Procedures for sampling, preparation, and proximate analysis of the protein sources and diets were described in detail in Chapter 3. Selected protein sources and diets were analyzed for histamine content by Dr. James Hungerford (U.S. Food and Drug Administration, Bothell, Washington) by flow injection analysis (Hungerford et al., 1990). Samples were prepared for analysis following the method outlined in Section 18.001 and paragraph 1, 18.066 (AOAC, 1984). Briefly, about 0.5 g of each wet sample was blended (Sorvall OM 1150; Ivan Sorvall, Inc., Norwalk, Connecticut) at high speed with about 25 ml HPLC grade methanol for 2 min, and then heated in a water bath (Model 1220 PC; Sheldon Manufacturing, Inc., Cornelius, Oregon) to 60°C for 15 min. The homogenates were cooled to room temperature, gravity filtered through Whatman #4 filter paper, and the filtrates diluted to 50.0 ml with methanol. The extracts were stored at 4°C until analyzed. Results of the assays are reported in Table 8.4

One sample of 10 fish was collected at the beginning of the trial; at trial termination, all fish remaining in each tank were collected. Fish samples from the beginning of the trial were prepared for moisture determination using the method described in Chapter 5. Fish collected at trial termination were prepared for moisture analysis by twice grinding them through a 6.35 mm die into a homogeneous mixture. Approximately 100 g of each homogenate was oven dried, then analyzed for lipid, protein, and ash as described in Chapter 5.

8.2.8 Data analysis

Rates of growth in wet weight and total length, feed efficiencies, and protein efficiency ratios were derived as described in Chapter 3. Protein productive values were calculated for fish in both the palatability and nutritive value trials using the methods

described in Chapter 5. All data were analyzed statistically using methods appropriate for completely randomized experimental designs (Little and Hills, 1978). The nutritive value trial was 2 x 2 x 5 factorial in treatments with main effects from protein source (A: casein or fishmeal), heat treatment (B: heat treatment or no heat treatment), and histamine level (C: 0, 500, 1,000, 1,500, or 2,000 mg histamine/kg dry diet). The nutritive value trial was 2 x 2 factorial in treatments, with main effects from histamine level (A: 0 and 2,000 mg histamine/kg dry diet) and heat treatment (B: heat treatment or no heat treatment). Fisher's Least Significant Difference (LSD) procedure was used following a significant "F" test to compare treatment means (Little and Hills, 1978). Statistical analysis was performed using Statview version 2.0 (Brainpower, Inc., Calabasas, California).

8.3 RESULTS

8.3.1 Effects of histamine supplementation and heating of casein and fish meal on growth and length and weight

Growth data are summarized for fish in the palatability study (Table 8.6) and nutritive value trial (Tables 8.8 and 8.9). Results of statistical evaluation of these data are summarized in Tables 8.5 and 8.7.

In the palatability study, to-satiation feeding of rainbow trout for 4 weeks revealed no significant ($P>0.05$, Table 8.5) interactive or direct effects of histamine supplementation or heat treatment of fish meal prior to incorporation into the test diets on final weight, rate of growth in weight, final TL, or rate of growth in TL (Table 8.6). Similarly, the results of the nutritive value study, in which fish were pair-fed equal quantities of protein from either fish meal or casein, revealed no direct, overall effects of histamine level or interactive effects between histamine level and protein source or heat treatment ($P>0.05$, Table 8.7) on final TL, growth in TL, or final weight (Tables 8.8 and 8.9). However, histamine level was found to significantly ($P<0.05$) affect rate of growth in weight (Table 8.8). There was a trend toward increased growth in weight with increasing histamine concentration, although significant ($P<0.05$) differences were only observed between the lowest two concentrations (0 and 500 mg/kg) and the highest (2,000 mg/kg). Interestingly, heat treatment of fish meal had almost no effect on weight gain or final TL, whereas fish fed diets containing heat-treated casein gained substantially more weight and were of greater final TL than those fed diet containing unheated casein (Table 8.9).

8.3.2 Effects of histamine and heat treatment on feed intake and efficiency of protein utilization

Feed and protein intake, gain in wet weight and protein, and estimates of feed efficiency, PER, and PPV obtained in the palatability trial are summarized in Table 8.10. Results of the nutritive value trial are shown in Tables 8.8 and 8.11. Data on whole body composition used in PPV estimation are given in Tables 8.12 (palatability trial) and 8.13 (nutritive value trial).

In the palatability trial, voluntary consumption of the fish meal based diet by rainbow trout was greatly affected by the addition of histamine, and the effect was found to be dependent on whether or not the protein source had been heated (histamine x heat interaction, $P < 0.01$; Table 8.10). On a dry matter basis, average consumption of the LT2000 diet was about 7.7% lower than that of the LT0 diet. Conversely, average consumption of the diet containing heat-treated fish meal, LT2000H, was about 15% higher than that of the LT0H diet. Observations on feeding behavior revealed that differences in feed acceptability developed early and persisted throughout the trial (Figure 8.1).

The results of these two feeding trials did not support the hypothesis that histamine directly or through heat-mediated chemical changes affects the nutritive value of dietary protein to rainbow trout. In the palatability trial, feed consumption by rainbow trout was significantly improved by the combined effects of histamine and heat treatment of the fish meal, but neither growth nor PER or PPV were significantly ($P > 0.05$) affected (Tables 8.5 and 8.10). These results were supported and further clarified by those obtained from the nutritive value trial, in which two unrelated protein sources, casein and fish meal, were treated with graded levels of histamine, then either heat treated or used without heat treatment. In that study, histamine content had no significant effect on either PER or PPV, irrespective of protein source or heat

treatment ($P>0.05$, Table 8.7). While PER appeared to improve with increasing histamine concentration (Table 8.8), direct determination of protein retention by carcass analysis, expressed as PPV, provided definitive evidence that histamine concentration did not affect protein utilization by rainbow trout.

Results of the nutritive value trial indicated that heat-treatment could have significant effects on the nutritive value of the protein sources, and that the nature of the effects were different for casein and fish meal (Table 8.7). As stated above, heat treatment had almost no effect on weight gain by fish fed fish meal-based diet, while feeding diet containing heat-treated casein dramatically improved growth. PPV data (Table 8.11) showed the positive effect of heat treatment on the nutritive value of casein to rainbow trout. In this study, mean retention of protein from diet containing unheated casein was 26.2%, compared to 34.4% for the heated material. Heat treatment had virtually no effect on the retention of protein from diet containing fish meal, however (unheated, 28.1%; heated, 26.5%).

8.3.3 Clinical findings

At the beginning of the trial, and at weekly intervals throughout the 4 week feeding period, all fish were anesthetized and individually examined during measurement of body length for external signs of infectious or noninfectious diseases. Since the fish had been exposed to *Flexibacter columnaris* prior to initiation of these feeding trials, fish were maintained at low rearing densities and treated with nitrofurazone (25 mg/L for 1 h) at weekly intervals to reduce the possibility of disease epizootics during the course of the studies. Nevertheless, stress imposed by weekly sampling and attempts of regulate water temperature during the fourth week of the study induced the development of clinical illness and some random mortality occurred. Those data are summarized in Appendix

3. No external signs associated with histamine toxicity were noted in any experimental group.

8.4 SUMMARY AND CONCLUSIONS

The objectives of this study were to measure the direct and interactive effects of histamine level and heat treatment on the palatability and nutritive value of protein from fish meal and casein to rainbow trout. The protein treatment protocol used in this experiment was designed to model commercial processing conditions under which high quality raw material was combined with concentrate of various quality, ranging from fresh (low in amines) to decomposed (high in amines). Under these experimental conditions, it was determined that the palatability of fish meal could be improved by adding histamine followed by heat, but neither factor alone had this effect. Furthermore, heat treatment and histamine supplementation had no effect on growth, PER, or PPV by fish fed to satiation in the palatability study. The results of the nutritive value study also showed that histamine supplementation had no significant effect on PER or PPV, irrespective of protein source or heat treatment. Together, these data supported previous observations (Chapter 4) which suggested that feed intake and growth were not adversely affected by heat treatment of fish meal or casein treated with histamine alone or a combination of histamine, putrescine, and cadaverine.

These studies have provided direct evidence that histamine, either directly or through heat-mediated chemical changes, does not affect the nutritive value of dietary protein to rainbow trout. These data must be interpreted with care, since the experimental conditions used for processing the model protein sources did not duplicate the drying conditions found in commercial fish meal processing plants. Commercial drying of fish meal may be done by direct or indirect heating (Barlow and Windsor, 1984). In the direct drying process, hot air at a temperature of up to 500°C is passed over the raw material as it is tumbled in the dryer drum. Indirect drying uses much lower temperatures, since the raw material is

heated in a steam-jacketed cylinder, or by steam-heated disks. With either method, the overall temperature of the wet raw material remains slightly below boiling (85-90°C), since evaporation tends to cool the product. Near the end of the drying process, when moisture levels are very low, the temperature of the fish meal approaches that of the heat applied, and the potential for heat-damage is high if the process is not carefully controlled. Further, material adhering to the walls of the dryer may be exposed to high temperatures for long periods of time. This condition was not duplicated. Second, the effects of putative potentiators of histamine toxicity were not considered by the model system, despite the fact that under commercial conditions, other amines would undoubtedly be present in decomposed concentrate high in histamine. Third, it is possible that chemical reactions related to the development of toxicity occur only during the drying of the raw material, and cannot be duplicated by rewetting and drying the fish meal. Finally, additional heating of the fish meal may not have the same effect as prolonged heating during the commercial drying process. Despite these limitations, the results of this study have direct application to the the development of criteria for grading of fish meal quality that include specifications for maximum histamine level, especially when fresh raw material is used.

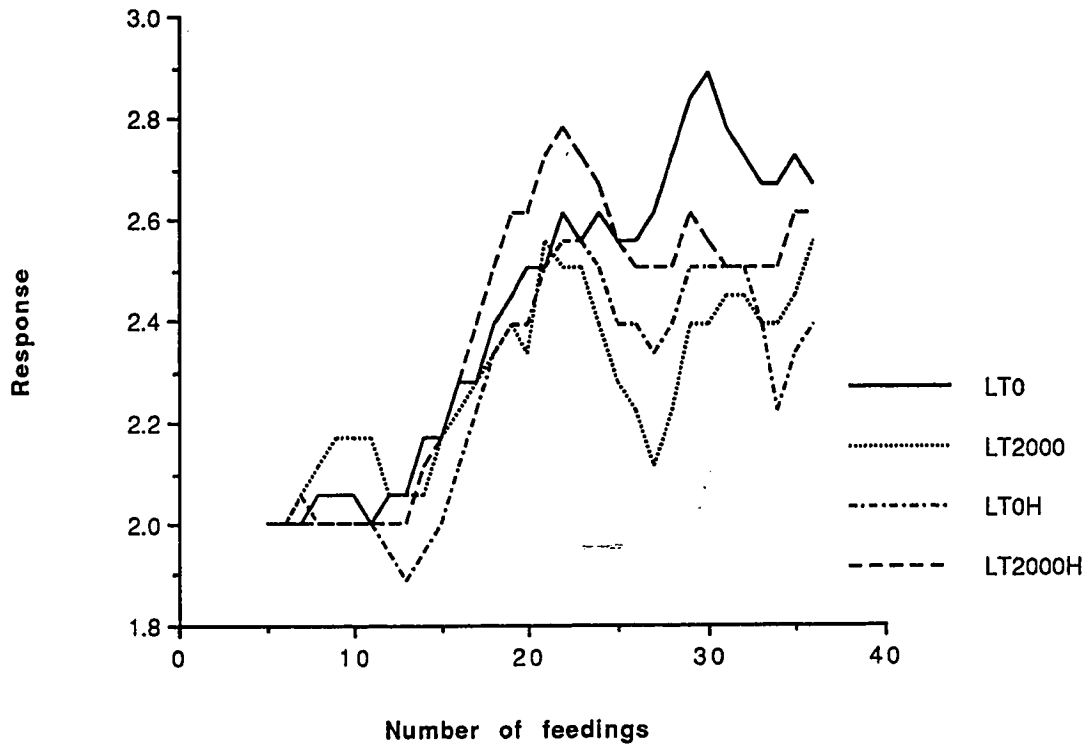


Figure 8.1. Feeding response scores for rainbow trout fed the experimental diets. Numerical scores were assigned following subjective evaluation of feeding response: (1) indicates little or no response, (2) indicates minimal feeding response, (3) indicates average response, and (4) indicates excellent response.

Table 8.1. Proximate composition (% dry weight) and histamine content (mg/kg wet weight) of protein sources used in the experimental diets^a.

Protein source	Casein	LT-1-N
Toxicity ^b	ND ^c	N
Origin		Norway
Dry matter	91.00	93.78
Protein	92.70	79.16
Lipid	0.70	9.24
Ash	2.40	11.20
Histamine		199

^a Protein, lipid, and ash data are means of two determinations on each dried sample. Data on histamine level of the fish meal was provided courtesy of Department of Marine Resources, Fundacion Chile, Santiago, Chile.

^b Causative of gizzard erosion in chickens as determined by 7 d chick bioassay: T = toxic; N = nontoxic. Assay conducted by Department of Marine Resources, Fundacion Chile, Santiago, Chile.

^c Not determined.

Table 8.2. Formulation of experimental diets (% dry ingredients).

Ingredient ^a	Diet	
	Casein based diets	Fish meal based diets
Casein	44.8	
LT-1-N fish meal		62.0
Gelatin	10.0	10.0
Dextrin	12.0	8.5
Alpha-cellulose	4.6	3.3
Carboxymethylcellulose	1.0	1.0
Vitamin premix ^b	2.0	2.0
Choline chloride (70% liquid)	1.0	1.0
Herring oil	17.1	12.0
Amino acid mix ^c	4.1	
Mineral premix ^d	3.3	
Ascorbic acid (41% active)	0.2	0.2
Water	300	300
Trace mineral solution ^e	100	100

^a Sources of ingredients were: vitamin-free casein, ICN Biomedicals (Costa Mesa, California); fish meal, Fundacion Chile (Santiago, Chile); gelatin, Sigma Chemical Company (St. Louis, Missouri); dextrin, alpha cellulose, and carboxymethylcellulose, ICN Biomedicals, Inc. (Costa Mesa, California); vitamin premix, Moore-Clark Co. (La Connor, Washington); choline chloride, Sigma Chemical Company (St. Louis, Missouri); herring oil, B.C. Packers (Richmond, B.C., Canada); amino acid mix, mineral premix, Sigma Chemical Company (St. Louis, Missouri); ascorbic acid, Takeda Chemical Co. (Tokyo, Japan); trace mineral solution, Sigma Chemical Company (St. Louis, Missouri).

^b Vitamin premix supplied the following per kg of dry diet ingredients: Vitamin A acetate or palmitate, 6612 IU; Alpha tocopheryl acetate, 503 IU; menadione sodium bisulfite complex, 28 mg; thiamine mononitrate, 46 mg; riboflavin, 53 mg; pyridoxine HCl, 38 mg; d-calcium pantothenate, 115 mg; niacin, 220 mg; d-biotin, 1.45 mg; Vitamin B₁₂, 0.06 mg; myoinositol, 132 mg; folic acid, 16.5 mg; Vitamin D₃, 441 mg.

^c Amino acid mix supplied the following per kg of dry diet ingredients: L-arginine, 10-g; L-histidine, 2 g; L-lysine, 10 g; L-methionine, 4 g; L-phenylalanine, 5 g; L-threonine, 10 g.

^d Mineral premix supplied the following per kg of dry diet ingredients: KCl, 15 g; CaHPO₄·H₂O, 12 g; MgO, 3 g; NaCl, 3 g.

^e Trace mineral solution supplied the following per kg of dry diet ingredients: KI, 1.9 mg; MnSO₄·4 H₂O, 32.5 mg; ZnSO₄·7 H₂O, 88.0 mg; NaHSeO₃, 4.2 mg; CoCl₃·6 H₂O, 4.0 mg; CuSO₄·5H₂O, 11.8 mg.

Table 8.3. Proximate composition (% dry weight^a) of diets used to evaluate the effects of histamine level and heat treatment on the nutritive value of fish meal and casein to rainbow trout.

Diet ^b	CG0H	CG500H	CG1000H	CG1500H	CG2000H	CG0	CG500	CG1000	CG1500	CG2000
Dry matter	74.57	73.96	68.50	70.96	70.10	71.35	70.73	69.48	71.62	72.15
Protein	56.55	59.23	57.25	57.65	57.08	57.36	57.57	57.57	58.04	57.61
Lipid	18.36	15.61	17.13	17.24	16.78	16.76	16.09	16.55	17.06	16.13
Ash	4.70	4.80	5.02	4.52	4.41	4.70	4.61	4.52	4.48	4.67
Diet	LT0H	LT500H	LT1000H	LT1500H	LT2000H	LT0	LT500	LT1000	LT1500	LT2000
Dry matter	70.67	70.81	71.37	72.09	70.84	67.86	69.82	68.56	67.59	67.93
Protein	60.04	58.80	58.49	58.21	59.35	58.56	59.60	59.94	58.36	59.29
Lipid	20.13	20.26	20.82	21.48	20.95	19.13	18.93	18.42	17.57	18.33
Ash	8.06	8.09	8.05	7.96	8.07	7.98	8.05	7.82	7.97	8.15

^a Protein, lipid, and ash data are means of two determinations on each dried sample.

^b Diet codes indicate protein source, dietary level of added histamine, and whether protein source had been heat treated prior to incorporation into test diets. Protein sources were casein (CG) and LT-1-N fish meal (LT). Dietary histamine concentrations (mg/kg dry diet) were: 0, 500, 1,000, 1,500, 2,000. Heat treatments of protein source after addition of histamine were: not heated (N) or heated (H) for 9 h at 60 °C, followed by 3 h at 130 °C. Histamine was added as histamine dihydrochloride (60.384% histamine).

Table 8.4. Histamine concentration (mg/kg dry weight^a) of selected protein sources and diets used to evaluate the effects of histamine level and heat treatment on the nutritive value of fish meal and casein to rainbow trout.

Protein source ^b	CG0H	CG500H	CG2000H	CG0		
Determined	44	504	3190	0		
Formulated ^c	0	1116	4464	0		

Diet	CG0H	CG500H	CG2000H	CG0	CG500	CG2000
Determined	216	305	1607	79	349	1647
Formulated	0	500	2000	0	500	2000

Protein source	LT0H	LT500H	LT2000H	LT0		
Determined	269	990	2736	317		
Formulated	199	1005	3424	199		

Diet	LT0H	LT500H	LT2000H	LT0	LT500	LT2000
Determined	241	625	1498	204	576	2362
Formulated	123	623	2123	123	623	2123

^a Histamine concentration was determined by flow injection analysis (Hungerford et al., 1990). Data are means of two determinations on each extract.

^b Codes indicate protein source, dietary level of added histamine, and whether protein source had been heat treated prior to incorporation into test diets. Protein sources were casein (CG) and LT-1-N fish meal (LT). Formulated dietary histamine concentrations (mg/kg dry diet) were: 0, 500, 1,000, 1,500, 2,000. Heat treatments of protein source after addition of histamine were: not heated (N) or heated (H) for 9 h at 60 °C, followed by 3 h at 130 °C. Histamine was added as histamine dihydrochloride (60.384% histamine).

^c Formulated histamine levels (mg/kg dry matter) indicate the expected concentration in the protein source or diet, based on the combined level of endogenous and supplemental histamine

Table 8.5. Results of analysis of variance on growth, dry feed intake, and efficiency of protein utilization and retention by rainbow trout fed fish meal based experimental diets to satiation for 4 weeks in the palatability study^a.

Source of variation ^b	Final length (mm TL)	Growth ^c rate (mm TL/d)	Final weight (g)	Growth rate (g/d)	Feed intake (g)	PER ^d	PPV ^e
Histamine level (A)	NS	NS	NS	NS	NA	NS	NS
Heat (B)	NS	NS	NS	NS	NA	NS	NS
AB	NS	NS	NS	NS	< 0.01	NS	NS

^a Significance levels shown: NS = not significant ($P>0.05$), NA = not applicable due to significant interaction.

^b Protein source: LT-1-N fish meal ; level of supplemental histamine (A): 0 or 2000 mg/kg dry diet; heat treatment (B) after addition of histamine: not heated or heated at 60°C for 9 h followed by 3 h at 130°C;. Experimental design: Completely randomized, 2 x 2 factorial in treatments, 2 replicates per treatment.

^c Rates of growth are the slope coefficients from the regression of total length (TL) or wet weight (y) on time (x).

^d Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on protein intake (x).

^e Protein productive value (PPV) calculated as: $100 \cdot \text{gain in body protein (g)}/\text{protein consumed (g)}$.

Table 8.6. Mean growth of rainbow trout fed the fish meal based diets to satiation for 4 weeks in the palatability study^a.

Diet	Final length ^b (mm TL)	Growth rate ^c (mm TL/d)	Final weight (g)	Growth rate (g/d)
LTOH	105.6 (3.2)	0.53 (0.11)	14.2 (1.2)	0.21 (0.04)
LT2000H	108.4 (2.2)	0.68 (0.08)	16.0 (1.0)	0.27 (0.03)
LT0	107.5 (0.7)	0.60 (0.03)	14.7 (0.0)	0.23 (0.00)
LT2000	107.2 (0.3)	0.60 (0.08)	14.9 (0.1)	0.23 (0.01)

^a Data shown are means (\pm SD) of two replicates. Main effects (histamine level and heat treatment) and interaction were not significant ($P>0.05$).

^b Mean initial weight (g) and total length (mm TL) were 8.6 ± 0.2 g and 91.6 ± 1.0 mm (mean \pm SD), respectively.

^c Rates of growth are the slope coefficients from the regression of total length (TL) or wet weight (y) on time (x).

Table 8.7. Results of analysis of variance on growth and efficiency of protein utilization and retention by rainbow trout pair-fed equal quantities of protein from the experimental diets for 4 weeks in the nutritive value trial^a.

Source of variation ^b	Final length (mm TL)	Growth ^c rate (mm TL/d)	Final weight (g)	Growth rate (g/d)	PER ^d	PPV ^e
Protein source (A)	NA	< 0.05	NA	NA	NA	NA
Heat (B)	NA	< 0.01	NA	NA	NA	NA
AB	<0.05	NS	<0.01	<0.01	<0.01	<0.001
Histamine level (C)	NS	NS	NS	<0.05	NS	NS
AC	NS	NS	NS	NS	NS	NS
BC	NS	NS	NS	NS	NS	NS
ABC	NS	NS	NS	NS	NS	NS

^a Significance levels shown: NS = not significant ($P>0.05$), NA = not applicable due to significant interaction.

^b Protein sources (A): vitamin free casein and LT-1-N fish meal ; heat treatment (B) after addition of histamine: not heated or heated at 60°C for 9 h followed by 3 h at 130°C; level of supplemental histamine (C): 0, 500, 1,000, 1,500, 2,000 mg/kg dry diet. Experimental design: Completely randomized, 2 x 2 x 5 factorial in treatments, 2 replicates per treatment.

^c Rates of growth are the slope coefficients from the regression of total length (TL) or wet weight (y) on time (x).

^d Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on protein intake (x).

^e Protein productive value (PPV) calculated as: $100 \cdot \text{gain in body protein (g)}/\text{protein consumed (g)}$.

Table 8.8. Effects of dietary histamine supplementation level on growth, protein efficiency ratio, and protein productive value by rainbow trout pair-fed equal quantities of protein for 4 weeks in the nutritive value trial^a.

Histamine (mg/kg dry diet)	Final ^b length (mm TL)	Growth ^c rate (mm TL/d)	Final weight (g)	Growth rate (g/d)	PER ^d	PPV ^e
0	103.0	0.42	13.8	0.18 ^X	2.02	29.17
500	103.3	0.43	13.8	0.18 ^X	2.00	27.65
1000	103.8	0.44	14.0	0.19 ^{XY}	2.14	29.62
1500	103.7	0.45	14.1	0.19 ^{XY}	2.13	28.54
2000	104.1	0.46	14.3	0.20 ^Y	2.19	31.57
	P=0.4037	P=0.3916	P=0.2313	P=0.0403	P=0.1640	P=0.1500

^a Data shown are the means of the 8 tanks of fish fed each histamine level. Main effects of histamine level were significant ($P < 0.05$) only for growth in weight. Results of analysis of variance are summarized in Table 8.6.

^b Mean initial weight (g) and total length (mm TL) were 8.6 ± 0.2 g and 91.6 ± 1.0 mm (mean \pm SD), respectively.

^c Rates of growth are the slope coefficients from the regression of total length (TL) or wet weight (y) on time (x).

^d Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on protein intake (x).

^e Protein productive value (PPV) calculated as: $100 \cdot \text{gain in body protein (g)}/\text{protein consumed (g)}$.

Table 8.9. Mean growth of rainbow trout pair-fed casein or fish meal based diets for 4 weeks in the nutritive value trial^a.

Diet	Final length ^b (mm TL)	Growth rate ^c (mm TL/d)	Final weight (g)	Growth rate (g/d)
CG0H	103.7 (1.3)	0.43 (0.04)	14.3 (0.7)	0.21 (0.02)
CG500H	103.0 (2.0)	0.44 (0.01)	14.3 (0.5)	0.20 (0.02)
CG1000H	104.3 (0.6)	0.46 (0.01)	14.5 (0.1)	0.21 (0.00)
CG1500H	104.5 (0.7)	0.46 (0.02)	14.2 (0.3)	0.19 (0.00)
CG2000H	105.4 (0.4)	0.48 (0.07)	15.0 (0.2)	0.22 (0.01)
CG0	101.0 (1.6)	0.37 (0.04)	13.0 (0.9)	0.16 (0.03)
CG500	102.6 (2.8)	0.39 (0.11)	13.3 (0.7)	0.16 (0.03)
CG1000	102.5 (0.3)	0.38 (0.02)	13.5 (0.2)	0.17 (0.01)
CG1500	102.4 (0.6)	0.41 (0.03)	13.7 (0.0)	0.18 (0.01)
CG2000	101.1 (1.7)	0.35 (0.05)	13.3 (1.2)	0.17 (0.02)
LT0H	104.2 (0.7)	0.44 (0.03)	14.1 (0.2)	0.19 (0.00)
LT500H	103.7 (0.4)	0.45 (0.06)	13.9 (0.1)	0.19 (0.02)
LT1000H	105.2 (0.6)	0.47 (0.06)	14.4 (0.2)	0.20 (0.01)
LT1500H	104.3 (0.5)	0.49 (0.01)	14.2 (0.2)	0.20 (0.00)
LT2000H	104.7 (0.9)	0.52 (0.02)	14.3 (0.1)	0.21 (0.01)
LT0	103.2 (1.7)	0.40 (0.08)	13.8 (0.7)	0.18 (0.03)
LT500	103.8 (1.7)	0.45 (0.01)	13.5 (0.3)	0.18 (0.01)
LT1000	103.3 (0.5)	0.46 (0.07)	13.8 (0.2)	0.20 (0.00)
LT1500	103.4 (0.3)	0.45 (0.05)	14.3 (0.4)	0.20 (0.00)
LT2000	105.3 (0.8)	0.51 (0.02)	14.4 (0.1)	0.21 (0.00)

^a Data shown are means (\pm SD) of two replicates.

^b Mean initial weight (g) and total length (mm TL) were 8.6 ± 0.2 g and 91.6 ± 1.0 mm (mean \pm SD).

^c Rates of growth are the slope coefficients from the regression of total length or weight (y) on time (x).

Table 8.10. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout fed fish meal based diets to satiation for 4 weeks in the palatability study^a.

Diet	Intake (g)		Gain (g)				PPV ^f
	Dry feed ^b	Protein	Weight ^c	Protein	F.E. ^d	PER ^e	
LT0H	5.10 (0.09)	3.06 (0.05)	5.53 (1.19)	0.77 (0.16)	1.09 (0.25)	1.72 (0.33)	25.09 (5.56)
LT2000H	5.87 (0.26)	3.48 (0.16)	7.19 (1.19)	1.00 (0.16)	1.22 (0.15)	1.95 (0.01)	28.62 (3.40)
LT0	5.67 (0.04)	3.32 (0.02)	6.06 (0.12)	0.86 (0.00)	1.07 (0.01)	1.86 (0.09)	25.94 (0.20)
LT2000	5.23 (0.22)	3.10 (0.13)	6.27 (0.18)	0.86 (0.01)	1.20 (0.02)	2.03 (0.03)	27.66 (1.00)

^a Data shown are means (\pm SD) of two replicates.

^b Statistically significant interaction ($P < 0.01$) of histamine level and heat: see Table 8.4 for details.

^c Initial weight: 8.6 ± 0.2 g (mean \pm SD).

^d Feed efficiency (F.E.) calculated as wet weight gain (g)/dry feed intake (g)

^e Protein efficiency ratios (PER) are the slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

^f Protein productive value (PPV) calculated as: $100 \cdot \text{gain in body protein (g)} / \text{protein consumed (g)}$.

Table 8.11. Feed and protein intake, gain in weight and protein content, and efficiency of diet and protein utilization by rainbow trout pair-fed equal quantities of protein from casein or fish meal based diets for 4 weeks in the nutritive value trials^a.

Diet	Intake (g)		Gain (g)					PPV ^f
	Dry feed ^b	Protein	Weight ^c	Protein	F.E. ^d	PER ^e		
CG0H	4.44 (0.00)	2.51 (0.00)	5.95 (0.70)	0.93 (0.14)	1.34 (0.16)	2.24 (0.22)	36.89 (5.76)	
CG500H	4.24 (0.00)	2.51 (0.00)	5.57 (0.49)	0.83 (0.10)	1.31 (0.12)	2.15 (0.14)	33.01 (4.06)	
CG1000H	4.39 (0.00)	2.51 (0.00)	5.99 (0.06)	0.88 (0.03)	1.37 (0.01)	2.21 (0.08)	34.90 (1.19)	
CG1500H	4.36 (0.00)	2.51 (0.00)	5.40 (0.11)	0.76 (0.02)	1.24 (0.03)	2.14 (0.04)	30.27 (0.79)	
CG2000H	4.40 (0.00)	2.51 (0.00)	6.37 (0.14)	0.93 (0.06)	1.45 (0.03)	2.26 (0.09)	37.18 (2.45)	
CG0	4.38 (0.00)	2.51 (0.00)	4.37 (0.72)	0.63 (0.10)	1.00 (0.16)	1.76 (0.27)	27.97 (3.85)	
CG500	4.36 (0.00)	2.51 (0.00)	4.47 (0.76)	0.63 (0.16)	1.02 (0.17)	1.67 (0.34)	25.10 (6.38)	
CG1000	4.36 (0.00)	2.51 (0.00)	4.79 (0.22)	0.69 (0.01)	1.10 (0.05)	1.83 (0.06)	27.40 (0.45)	
CG1500	4.33 (0.00)	2.51 (0.00)	5.08 (0.21)	0.67 (0.02)	1.17 (0.05)	2.00 (0.11)	26.60 (0.84)	
CG2000	4.36 (0.00)	2.51 (0.00)	4.72 (0.94)	0.68 (0.10)	1.08 (0.22)	1.85 (0.19)	27.00 (3.89)	
LT0H	4.18 (0.00)	2.51 (0.00)	5.37 (0.03)	0.70 (0.00)	1.28 (0.01)	2.09 (0.04)	27.77 (0.16)	
LT500H	4.27 (0.00)	2.51 (0.00)	5.36 (0.51)	0.68 (0.07)	1.26 (0.12)	2.19 (0.11)	27.00 (2.81)	
LT1000H	4.29 (0.00)	2.51 (0.00)	5.59 (0.22)	0.71 (0.00)	1.30 (0.05)	2.20 (0.23)	28.36 (0.12)	
LT1500H	4.31 (0.00)	2.51 (0.00)	5.56 (0.05)	0.73 (0.04)	1.29 (0.01)	2.18 (0.08)	29.09 (1.58)	
LT2000H	4.23 (0.00)	2.51 (0.00)	5.95 (0.11)	0.76 (0.01)	1.41 (0.03)	2.35 (0.06)	30.42 (0.27)	
LT0	4.29 (0.00)	2.51 (0.00)	5.01 (0.85)	0.68 (0.10)	1.17 (0.02)	2.01 (0.36)	27.04 (4.04)	
LT500	4.21 (0.00)	2.51 (0.00)	4.98 (0.39)	0.64 (0.09)	1.18 (0.09)	2.01 (0.02)	25.50 (3.62)	
LT1000	4.19 (0.08)	2.51 (0.05)	5.36 (0.33)	0.70 (0.06)	1.28 (0.10)	2.31 (0.00)	27.81 (2.79)	
LT1500	4.33 (0.05)	2.53 (0.03)	5.50 (0.24)	0.71 (0.04)	1.27 (0.04)	2.18 (0.02)	28.22 (1.37)	
LT2000	4.24 (0.00)	2.51 (0.00)	5.94 (0.12)	0.80 (0.02)	1.40 (0.03)	2.29 (0.06)	31.67 (0.98)	

^a Data shown are means (±SD) of two replicates.

^b Initial weight: 8.6 ± 0.2 g (Mean ±SD).

^c Feed efficiency (F.E.) calculated as wet weight gain (g) / dry feed intake (g)

^d Protein efficiency ratios (PER) are slope coefficients from the regression of cumulative weight gain (y) on cumulative protein intake (x).

^e Protein productive value (PPV) calculated as: 100 · gain in body protein (g) / protein consumed (g).

Table 8.12. Whole body composition (% wet weight^a) of rainbow trout pair-fed equal quantities of protein from casein from fish meal based diets for 4 weeks in the nutritive value trial.

Diet	Moisture	Protein	Lipid	Ash
CG0H	74.32 (0.49)	15.39 (0.25)	8.53 (0.35)	2.01 (0.01)
CG500H	74.60 (0.65)	15.11 (0.19)	8.26 (0.51)	2.01 (0.03)
CG1000H	74.69 (0.69)	15.00 (0.27)	8.10 (0.47)	2.02 (0.07)
CG1500H	74.83 (0.75)	14.81 (0.03)	8.09 (0.28)	2.12 (0.08)
CG2000H	74.63 (0.52)	15.01 (0.26)	7.93 (0.27)	2.09 (0.05)
CG0	74.96 (0.31)	14.96 (0.08)	7.89 (0.28)	2.05 (0.03)
CG500	74.91 (0.93)	14.87 (0.35)	7.83 (0.61)	2.19 (0.02)
CG1000	75.67 (0.48)	14.95 (0.16)	7.61 (0.49)	2.10 (0.03)
CG1500	75.57 (0.06)	14.48 (0.07)	7.88 (0.35)	2.11 (0.07)
CG2000	75.56 (0.05)	14.96 (0.32)	7.71 (0.08)	2.18 (0.09)
LT0H	76.05 (0.04)	14.40 (0.02)	7.39 (0.05)	2.22 (0.08)
LT500H	76.08 (0.21)	14.26 (0.04)	7.56 (0.15)	2.15 (0.07)
LT1000H	75.93 (0.24)	14.28 (0.21)	7.95 (0.01)	2.15 (0.04)
LT1500H	75.71 (0.15)	14.43 (0.24)	8.00 (0.22)	2.19 (0.05)
LT2000H	76.28 (0.80)	14.25 (0.16)	7.51 (0.75)	2.27 (0.11)
LT0	76.32 (0.10)	14.65 (0.18)	7.11 (0.04)	2.12 (0.12)
LT500	75.96 (0.01)	14.38 (0.25)	7.70 (0.01)	2.24 (0.01)
LT1000	75.56 (0.45)	14.39 (0.05)	8.27 (0.38)	2.09 (0.01)
LT1500	76.28 (0.34)	14.39 (0.07)	7.51 (0.45)	2.26 (0.04)
LT2000	75.93 (0.04)	14.50 (0.04)	7.51 (0.12)	2.22 (0.02)

^a Proximate composition of the fish at the start of the trial was: moisture, 76.21%; protein, 14.47%; Lipid, 6.29%; and ash, 2.87%. Analysis was conducted on a pooled sample of fish common to all dietary treatment groups. At trial termination, analyses were conducted on samples from each replicate: means (\pm SD) for two replicates are shown. Duplicate determinations of protein, lipid, and ash were made on each dried sample.

Table 8.13. Whole body composition (% wet weight^a) of rainbow trout fed fish meal based diets to satiation for 4 weeks in the palatability study.

Diet	Moisture	Protein	Lipid	Ash
LT0H	74.20 (0.25)	14.73 (0.13)	8.88 (0.26)	2.15 (0.06)
LT2000H	74.57 (0.15)	14.65 (0.08)	9.05 (0.75)	2.16 (0.04)
LT0	74.36 (0.19)	14.83 (0.13)	8.94 (0.13)	2.15 (0.06)
LT2000H	74.78 (0.26)	14.60 (0.15)	8.57 (0.08)	2.22 (0.02)

^a Proximate composition of the fish at the start of the trial was: Moisture, 76.21%; protein, 14.47%; Lipid, 6.29%; and ash, 2.87%. Analysis was conducted on a pooled sample of fish common to all dietary treatment groups. At trial termination, analyses were conducted on samples from each replicate: means (\pm SD) for two replicates are shown. Duplicate determinations of protein, lipid, and ash were made on each dried sample.

Chapter 9

General Discussion, Contributions of Research, and Suggestions for Future Research

9.1 INTRODUCTION

Avian black vomit disease, a condition characterized by gizzard erosion, ulceration, and cellular necrosis, has been associated with the feeding of fish meal manufactured from degraded raw materials high in histamine and subjected to thermal abuse during drying. Under those conditions, histamine and lysine combine to form gizzerosine, a compound that has been shown to induce gizzard erosion in broiler chickens fed at levels as low as 0.6 mg/kg diet, with mortality noted at levels of 0.8 mg/kg diet or greater (Sugahara et al., 1988). Research has shown that other free amines often found in high concentrations in degraded fish, including histamine and the diamines putrescine and cadaverine, also produce clinical changes in the gastric morphology of chickens (Stuart et al., 1986). These data indicate that toxicity to chickens is most likely related to complex interactions among many chemical compounds found in degraded fish meals.

While the role of histamine and its derivative, gizzerosine, in the development of gastric abnormalities in chickens has been extensively studied, few experimental data exist concerning the effects of these compounds on most other animals, including aquatic species. Two recent reports (Watanabe et al., 1987; Romero and Castro, 1989) suggested that these and perhaps other biogenic amines present in fish meal toxic to chickens may be detrimental to at least two species of farm-reared salmonids. Watanabe et al. (1987) found that fish meal supplemented with histamine induced gross and cellular abnormalities in the gastric tissue of rainbow trout, similar to those associated with feeding toxic fish meal to

chickens. Romero and Castro (1989) found that coho salmon fed practical diets made with commercially produced fish meal high in histamine and shown to induce gizzard erosion in chickens developed stomach abnormalities, including thinning of the mucosa and hemorrhage, and grew poorly, compared to those fed the nontoxic control diet.

Few data presently exist in the scientific literature concerning the effects of histamine on the nutritive value of fish meal protein. In net protein utilization assays with rats, very slight protein hydrolysis due to microbial decomposition seemed to improve the nutritional value of protein from dolphin, but the effects may have been offset by endogenous histamine (Barankowski and Brust, 1984). In contrast, Watanabe et al. (1987) found that supplemental histamine appeared to improve the NPU of fish meal protein by rainbow trout, but no effect on growth was observed. The interactive effects of histamine level and heat were not determined in either study, and no cause-and-effect relationship could be established.

Gross morphological changes in the proventriculi and gizzards of chickens have been used as reliable indicators of fish meal toxicity. Chickens exhibit discernible lesions or ulcerations when fed toxic fish meal for 5 to 10 d, and in severe cases, the birds may succumb to black vomit disease (Johnson and Pinedo, 1971). The relationship between degree of toxicity and severity of the resultant pathology has been well defined, permitting development of the standardized chicken bioassays now utilized for the evaluation of fish meal produced in Chile that is sold in the international market. These assays have proven reliable for the purpose of evaluating fish meal intended for use in poultry feeds. However, the application of the chicken bioassay has been extended to testing fish meal intended for use in the diets of salmonids, even though few data exist to justify this practice.

In summary, at least two amino acid derivatives often found in fish meal, histamine and gizzerosine, have been shown to affect important physiologic functions in animals, including chickens, rats, and two species of fish. Whether or not the presence of these compounds represented sensitive and accurate indicators of poor nutritive value of fish meal protein to aquatic species was unknown. Furthermore, available data were insufficient to definitively establish the relationship between histamine, or other compounds causative of gizzard erosion in chickens, and morphological changes in the digestive tissues of fish. A series of experiments were conducted to address the following specific objectives:

1. To determine the relationship between the results of fish meal toxicity bioassays with broiler chickens and the palatability and nutritive value of fish meal to rainbow trout.
2. To establish the effects of thermal abuse and histamine content on the palatability and nutritive value of casein and fish meal to rainbow trout.
3. To determine the effects of these factors on the digestive tract morphology of rainbow trout.

9.2 DISCUSSION

9.2.1 Factors affecting diet palatability to rainbow trout

Feeding studies were conducted with rainbow trout to: (1) assess the relationship between the results of toxicity assays with chickens and the palatability of fish meal, and (2) measure the direct and interactive effects of dietary histamine level and thermal abuse of various protein sources on diet palatability. Fish were fed to satiation using a timed-response feeding protocol, since this method allowed direct evaluation of feed palatability from both intake and feeding behavior observations.

Two feeding studies were conducted to compare the palatability of diets containing fish meals that were found to be either acutely toxic or nontoxic to chickens in a standard bioassay. In the preliminary study (Chapter 3), diets containing nontoxic and toxic fish meals obtained from Chilean sources were voluntarily consumed in equal quantities by juvenile rainbow trout, whereas Norwegian LT fish meal was less palatable than either of the Chilean fish meals ($P < 0.05$; Table 3.5). In the second study (Chapter 5), the palatability of diets containing each of four fish meals of Chilean origin (two toxic and two nontoxic to chickens) or casein as the major source of dietary protein were evaluated with juvenile rainbow trout. In that trial, the diet containing the toxic fish meal (C-8-T) was consumed in the greatest quantity ($P < 0.001$; Table 5.7) and was more readily accepted by the fish than any other protein source tested. On a dry matter basis, all diets except that containing casein and the C-8-T fish meal were consumed in statistically equivalent quantities ($P > 0.05$). Casein was relatively unpalatable to juvenile rainbow trout, and was consumed in smaller quantities and with considerably less enthusiasm than diets containing fish meal. Data obtained in the trials do not support a correlation between the results of toxicity bioassays with chickens and the palatability of fish meal to juvenile rainbow trout.

Histamine added to the diet depressed feed intake and weight gain in chickens (Shifrine et al., 1959; Harry and Tucker, 1976). Gizzerosine, a derivative which is formed by the heat-mediated interaction of histamine with lysine, had a similar effect in chickens (Sugahara et al., 1988). Chapter 8 reports the results of a study conducted to evaluate the direct and interactive effects of supplemental histamine (0 vs. 2,000 mg/kg dry diet) and heat treatment (not heated vs. heated for 9 h at 60°C, followed by 3 h at 130°C) on voluntary intake of an LT fish meal-based diet and feeding behavior by juvenile rainbow trout. Voluntary consumption of the diet was greatly affected by the addition of histamine, and the effect was dependent on whether or not the protein source had been heated (histamine x heat interaction, $P < 0.01$; Table 8.10). Of the unheated fish meal treatments, average consumption of the diet containing 2,000 mg histamine/kg was about 7.7% lower (dry matter basis) than the unsupplemented diet. Conversely, in comparing the heated-treated fish meal treatments, average consumption of the diet containing 2,000 mg histamine/kg was about 15% higher than the unsupplemented diet. Observations on feeding behavior revealed that differences in feed acceptability developed early and persisted throughout the trial (Figure 8.1).

Behavioral differences that affect feeding activity, and presumably feed intake, by the various strains of rainbow trout used in the feeding trials have been observed by UW hatchery technicians. To determine the validity of comparing feed palatability measurements obtained with different strains of rainbow trout, two strains were fed the same standard test diet, and feeding activity and feed intake measured. Pure UW strain rainbow trout consistently exhibited a higher level of feeding activity, consumed about 50% more dry matter, and grew in weight at a rate nearly 63% higher than the UW x Nisqually interstrain hybrid. The differences observed seemed related to ability of the two strains to adapt to the rearing system used in the trial, rather than intrinsic differences in growth

potential. Therefore, caution must be used when making broad generalizations concerning feed ingredient palatability from data collected in different feeding trials, or when different strains of fish are used.

Selection of food items by animals is mediated by two separate factors: the chemical constituents of a food item and the sensitivity of the animal to those constituents (Johnsen and Adams, 1986). Lindstedt (1971) classified chemical effectors of feeding behavior by animals into those that exert their effect at a distance (attractants, arrestants, and repellents) and those that require contact (incitants and suppressants, stimulants and deterrents). In a generalized feeding response, an animal approaches a food item, guided by an attractant. An arrestant causes the animal to closely examine the food, and an incitant initiates biting or tasting. A stimulant evokes the ingestion of the food and continued feeding. The equivalent negative effectors are, respectively, repellents, suppressants, and deterrents. In fish and other animals, food may be detected at a distance either visually or chemically, but stimulation of taste receptors in the mouth and pharynx by chemical compounds in the food determine whether the item is swallowed or rejected.

Numerous compounds derived from animal tissues stimulate gustation by carnivorous fish. Carr (1982) summarized the characteristics of these compounds as (1) low molecular weight (<1,000) (2) non-volatile, (3) nitrogenous, and (4) amphoteric. The specific compound or mixtures of compounds that stimulate feeding is species-specific, and appears to be related to the composition of natural prey items consumed by the fish in the wild (Mackie and Mitchell, 1985). For example, feeding by species consuming vertebrates is effectively stimulated by nucleotides and mixtures of L-amino acids. In contrast, feeding by species consuming invertebrates is, in general, most effectively stimulated by glycine-betaine and mixtures of L-amino acids. In many cases,

individual compounds may be moderately effective, ineffective, or deterrent. Combinations of compounds often have enhanced or synergistic effects on feeding.

To date, studies have not been conducted to evaluate the relationship between the degree of fish meal toxicity and its palatability to chickens. However, low molecular weight nitrogenous compounds apparently are not deterrent to chickens, since toxic fish meals containing high levels of free amino acids, amines, and amine derivatives are consumed readily by these animals (E. Castro, personal communication). Similarly, compounds in fish meal that are toxic to chickens do not appear to affect gustatory activity in rainbow trout. Data collected in three studies using fish meal of Chilean origin did not support a correlation between the results of toxicity bioassays in chickens and behavioral bioassays of palatability in rainbow trout.

Rainbow trout are highly responsive to free amino acids, which have been found in numerous studies to be effective stimulators of gustatory activity. Adron and Mackie (1978) found that feeding of rainbow trout trained to operate a demand feeder was stimulated by a synthetic mixture of L-amino acids, profiled similar to an extract of squid. Sequential elimination of individual components from this mixture revealed that feeding was stimulated by combinations of either tyrosine, phenylalanine, and lysine, or tyrosine, phenylalanine, and histidine, but that no other combination of these amino acids was effective. Jones (1989) found that gustation was stimulated by individual amino acids, including leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glutamate, and arginine. Eleven amino acids or amino acid derivatives (glutamine, methionine, leucine, homoserine, asparagine, alanine, cystine, cysteine, glycine, serine, histidine) stimulated olfactory responses in rainbow trout (Hara, 1973). Interestingly, aliphatic acids, alcohols, and amines that are effective stimulants for terrestrial

vertebrates elicit no olfactory response in rainbow trout (Hara, 1973). The effectiveness of these compounds as gustatory stimulants or repellents has not been tested with this species of fish.

Development and testing of model protein sources in the present research focused on the combined effects of histamine and heat, rather than on the independent effects of each factor. As a result, the effects of histamine, with and without heating, on the palatability of fish meal to rainbow trout were compared only once (Chapter 8). The results of that study strongly suggested that free histamine may have had a slight repellent effect, unless the protein source was heated, in which case the effect was stimulatory (Table 8.10). In contrast, the combined effects of histamine and heat treatment on casein and two other sources of fish meal were minimal, although it appeared that this treatment improved the acceptability, but not the overall intake, of those protein sources (Table 4.4, Figure 4.2). Whole fish meal is generally expected to contain relatively high levels of free amino acids, peptides, and other soluble compounds, since these materials are separated, concentrated, and recombined with the insoluble fraction during the manufacturing process. Since at least some amino acids are effective stimulants of gustation in rainbow trout, it seems logical to suggest that the presence of these materials in fish meal may, at least in part, mask the effects of compounds repellent to these animals. It must be assumed that heat treatment also affected the properties of endogenous compounds in the fish meal, so the independent effects of combined histamine and heat treatment could not be definitively assessed.

9.2.2 Factors affecting the nutritional value of dietary protein to rainbow trout

Feeding studies were conducted with rainbow trout to: (1) assess the relationship between the results of toxicity assays with

chickens and the nutritional value of fish meal protein, and (2) determine the direct and interactive effects of dietary histamine concentration and thermal abuse on the nutritional value of protein from fish meal and casein. In the preliminary study, fish were fed to satiation using a timed-response feeding protocol. Assay sensitivity was improved in a subsequent trial by pair-feeding the fish equal quantities of dietary protein from isonitrogenous diets containing the test ingredients. Dietary protein quality was evaluated using growth data, protein efficiency ratios (PER), and apparent rates of protein retention (PPV).

Two feeding studies were conducted with juvenile rainbow trout to evaluate the nutritional value of protein from fish meals that were found to be either nontoxic or acutely toxic to chickens in a standard bioassay. In the preliminary study (Chapter 3), toxic Chilean fish meal, nontoxic Chilean fish meal, or Norwegian LT fish meal were equivalent ($P>0.05$; Table 3.5) in protein quality (PER) and produced equal ($P>0.05$; Table 3.5) rates of growth in weight and length in rainbow trout fed the experimental diets for 6 weeks. In the second study (Chapter 5), the nutritional value of diets containing each of four fish meals of Chilean origin (two toxic and two nontoxic to chickens) or casein as the major source of dietary protein were evaluated with juvenile rainbow trout. Both toxic fish meals were rated by the supplier as unsuitable for use in salmonid diets, while the two nontoxic fish meals were rated either of "average" or "good" quality. The "average" fish meal and one toxic fish meal produced equivalent growth, and both were superior ($P<0.001$) to the "good" fish meal and the remaining toxic fish meal. All fish meals supported greater growth than casein. Results of the nutritive value trial showed that the degree of toxicity to chickens was not correlated with the capacity of fish meal to support growth (Table 5.6). All fish meals were superior to casein in nutritional value, whether assessed by the PER or PPV ($P<0.001$; Table 5.8) method. Rates of protein retention by fish fed one of the toxic fish

meals and the "average" quality nontoxic fish meal were not significantly different, but both were superior to the remaining toxic fish meal and the "good" quality nontoxic fish meal. Rainbow trout retained between 31 and 35% of the protein consumed in diets containing fish meal, but only about 27% of the protein consumed in diets containing casein as the major source of dietary protein. The data indicate that indices of fish meal toxicity based on chicken bioassays are not reliable indicators of nutritional quality of fish meal for use in the diets of salmonids.

Chapter 8 reports the results of a study conducted with juvenile rainbow trout to evaluate the direct and interactive effects of dietary histamine and heat treatment on the nutritive value of fish meal and casein. The two protein sources were supplemented with histamine, then either heat treated or used in the experimental diets without heat treatment. Dietary levels (mg histamine/kg dry diet) were: 0, 500, 1,000, 1,500, or 2,000. Formulated dietary histamine did not significantly affect PER or PPV, irrespective of protein source or heat treatment ($P>0.05$; Table 8.7). PER appeared to improve with increasing histamine concentration (Table 8.8), but direct determination of protein retention by carcass analysis, expressed as PPV, provided definitive evidence that histamine concentration did not affect protein retention by rainbow trout.

Heat treatment of the dietary protein sources significantly affected their nutritional value to juvenile rainbow trout, but the nature of the effects differed for casein and fish meal (Table 8.7). Heat treatment had almost no effect on weight gain by fish fed the fish meal-based diet, while feeding the diet containing heat-treated casein dramatically improved growth. PPV data (Table 8.11) showed the positive effect of heat treatment on the nutritive value of casein. Mean retention of protein from the diet containing unheated casein was 26.2%, compared to 34.5% for the heated material. Heat treatment had virtually no effect on the retention of protein from

the diet containing fish meal (unheated, 28.1% retention; heated 26.5% retention).

Digestibility and bioavailability of food proteins may be affected by many factors, including protein conformation, intermolecular bonding, and modification of amino acids by heat processing (Kennedy et al., 1989). In contrast to fish meal, which is produced from raw materials that have been cooked then dried at relatively high temperatures, casein is produced by low temperature spray-drying. The further heat treatment of casein in this study may have resulted in partial protein denaturation which increased protein digestibility, an effect not expected for high-temperature processed fish meal. This hypothesis was not tested, however.

In recent years, it has become increasingly common to use the results of toxicity bioassays with chickens as a criterion for determining the suitability of various fish meals for use in feeds intended for aquatic species, even though very few data exist justifying this practice. Certainly, if fish meals acutely toxic to chickens were found acutely toxic to fish, then the issue of nutritional value would be largely irrelevant. However, the study by Romero and Castro (1990) with coho salmon indicated that overt pathology developed only after chronic exposure to fish meals found acutely toxic to chickens. In the present research, rainbow trout were fed toxic fish meal for about 70 d before signs of stomach abnormalities were noted, further indicating that the effects were chronic rather than acute in fish. Long term (98 d) feeding of toxic fish meal did not appear to affect growth or feed efficiency, compared to nontoxic fish meal obtained from the same source. Additionally, it was found that the toxic compounds did not elicit cellular abnormalities in the gastric tissues, and apparently did not affect gastric function. Further evaluation of toxic and nontoxic fish meals (Chapter 5) clearly showed that indices of fish meal toxicity

to chickens were not reliable indicators of nutritional quality of fish meal for use in the diets of rainbow trout.

While there are few published studies regarding the effects of raw material freshness on the nutritive value of fish meal protein to aquatic species, a growing body of evidence suggests that this factor may be a pivotal determinant, irrespective of source of raw material or processing method used (Pike et al., 1990; Pike, 1991). Depending on post-capture storage conditions, fish tissues may be degraded by endogenous enzyme activity (autolysis) or by bacteria. As deterioration progresses, intact protein is degraded to peptides, free amino acids, amines, and ammonia. Whole fish meal is expected to contain relatively high levels of free amino acids, peptides and other soluble compounds, since these materials are separated, concentrated, and recombined in varying proportions with the insoluble fraction to obtain a final product with the desired proximate composition.

Studies with fish silage and liquified fish have shown that the nutritional value of fish protein is highly correlated to the extent to which the protein and polypeptides are hydrolyzed to free amino acids. In studies with carp (*Cyprinus carpio*), Murai et al. (1982) found that dietary free amino acids were rapidly absorbed, resulting in plasma essential amino acid balances different from those observed when the amino acids were liberated by gastric and intestinal digestion from intact proteins. In their study, weight gains, feed efficiencies, and protein efficiency ratios were correlated with plasma essential amino acid balance: intact protein or casein-coated amino acids were better utilized than free amino acids. Rainbow trout had higher weight gains and utilized protein more efficiently when fed silages in which autolysis was terminated after 3-7 d or restricted by temperature or pH, than when autolysis was permitted to continue until high levels of free amino acids appeared in the silage (Hardy et al., 1983; Hardy et al.,

1984; Stone and Hardy, 1986). The profile of plasma amino acids observed in rainbow trout fed ensiled fish protein suggested that essential amino acids not utilized for growth entered the TCA cycle and were metabolized for energy (Stone and Hardy, 1988). Therefore the balance of amino acids in the protein, peptide, and free amino acid fractions, as well as the extent of hydrolysis, must be considered as important factors affecting relative value of fish meal protein for growth in fish.

Results of the present research have clearly shown that dietary histamine does not affect the nutritional value of dietary protein to rainbow trout. In the preliminary study described in Chapter 4, the apparent nutritional value of casein, LT fish meal, and fish meal containing high levels of endogenous amines toxic to chickens were not adversely affected by supplementation with histamine, followed by heat treatment, despite the fact that amine treatment of the protein sources was associated with gross morphological changes in the stomachs of the fish. The interactive effects of histamine level and heat treatment on the nutritive value of protein from fish meal and casein were evaluated by treating the protein sources using a protocol designed to model commercial processing conditions. The experimental protocol modeled conditions in which high quality raw material was combined with concentrate of various quality, ranging from fresh (low in amines) to decomposed (high in amines). Supplemental histamine concentrations ranging from 0 to 2,000 mg/kg dry diet had no significant effect ($P>0.05$) on PER or PPV, irrespective of whether or not the histamine-treated protein source was subjected to heat treatment. Although it must be recognized that the model system did not precisely duplicate the conditions found in commercial fish meal processing, the data nevertheless refutes the results of Watanabe et al. (1987) which suggested that histamine directly affects the nutritive value of fish meal protein to rainbow trout.

9.2.3 Effects of biogenic amines on gastric secretory activity and the development of stomach abnormalities in rainbow trout

Feeding studies were conducted with rainbow trout to (1) assess the effects of chronic exposure to fish meals acutely toxic to chickens, and (2) evaluate the effects of chronic exposure to dietary histamine, putrescine, and cadaverine on gastric tissue morphology of rainbow trout. Fish were fed the experimental diets to satiation using a timed-response feeding protocol for periods of time ranging from 42 to 111 d. The effects of the various dietary treatments were determined by *in situ* inspection of the digestive organs and by histological examination of the stomach, liver, and spleen.

Two 42 d feeding studies (Chapters 3 and 5) were conducted with juvenile rainbow trout to evaluate the acute effects of feeding fish meals of Chilean origin that were found to be either nontoxic or acutely toxic to chickens in a standard bioassay. In the studies, a total of three nontoxic and three toxic Chilean fish meals, one nontoxic Norwegian fish meal, and casein were tested. *In situ* inspection of the visceral mass revealed no obvious inflammation or ulceration of the gastric or intestinal tissues, and no lesions or other abnormalities in the spleen, liver, and kidney. In most fish fed Chilean fish meals, but in none fed the Norwegian fish meal or casein, the visceral mass was light grey to slightly pink in color, but was not associated with any infectious or noninfectious disease. Small quantities of ingesta were observed in the intestines of most fish, and the gall bladder of all fish examined was engorged with bile, a finding consistent with fish accustomed to regular feeding. Moderate quantities of visceral fat were observed in all fish, but the pyloric caecae were visible and distinct.

Following *in situ* examination, the stomach of each fish was excised and partially everted for visual evaluation of mucosal tissue morphology. In no case was inflammation or ulceration of the mucosa or muscularis noted. However, the general appearance of the

internal structure of the stomach differed among treatment groups. In fish fed the diets containing the nontoxic Norwegian fish meal or casein, the longitudinal folding of the stomach lining was distinct, and the musculature contracted forcefully when stimulated with a sharpened probe. In contrast, a few fish fed either the toxic or nontoxic fish meals of Chilean origin were found to have somewhat flaccid stomachs, and the distinct folding of the stomach lining noted in the control fish was greatly reduced, giving the mucosa a smooth appearance. Often, the stomachs of affected fish were filled with clear, viscous mucous-like liquid, but as mentioned above, no inflammation or lesions were observed. Histological evaluation of the cardiac stomach revealed no changes in the cellular structure of the gastric glands, mucosa, or underlying musculature. Liver tissues were histologically normal. Mortality was very low in the studies, and was not associated with dietary treatment.

Chapter 4 reports the results of a 111 d study conducted with juvenile rainbow trout to document the effects of fish meal acutely toxic to chickens on gastric tissue morphology, and to determine whether similar effects could be induced by feeding various protein sources that had been treated with histamine alone or in combination with two possible potentiators of histamine activity, putrescine and cadaverine. The protein sources were heated following supplementation with the amines, since heat-treatment has been shown in previous studies (Umemura et al., 1981; Watanabe et al., 1987) to enhance the formation of compounds toxic to chickens. Five amine-treated protein sources were tested: casein supplemented with histamine; casein supplemented with histamine, putrescine, and cadaverine; Norwegian LT fish meal supplemented with histamine; Norwegian LT fish meal supplemented with histamine, putrescine, and cadaverine; and toxic Chilean fish meal supplemented with histamine, putrescine, and cadaverine. Formulated dietary concentrations of the supplemental histamine, putrescine, and cadaverine were, respectively, 2,000, 500, and 500

mg/kg dry diet. Four unsupplemented protein sources, including casein, nontoxic Norwegian fish meal, nontoxic Chilean fish meal, and toxic Chilean fish meal were used in the control diets.

Rainbow trout fed diets containing casein or nontoxic Norwegian fish meal that had been supplemented either with histamine or a mixture of histamine, putrescine, and cadaverine, had mild to severe distention of the stomach and, in some cases, distention of the intestines and pyloric caecae. The condition developed gradually, and was first observed after various periods of exposure, depending on the dietary protein source. The effects of amine supplementation were first observed on day 42 in fish fed either the casein or Norwegian fish meal-based diets supplemented with histamine. On day 70, the remaining amine-treated fish meal-based diets were affected. The condition was more severe and involved a higher proportion of the fish in groups fed the casein-based diets than in those fed fish meal-based diets. Dietary putrescine and cadaverine neither accelerated the development of the observed abnormalities, nor increased the proportion of fish affected. Importantly, supplementation of the toxic Chilean fish meal did not increase its toxicity to rainbow trout, relative to the unsupplemented toxic fish meal. Visual inspection revealed no obvious inflammation or ulceration of the gastric tissues, and no lesions or other abnormalities in the spleen, liver, and kidney were observed. All tissues were found to be histologically normal after 98 d exposure to the experimental treatments, indicating that the observed distention of the stomach did not result from changes in cellular morphology. Overall survival was 95-100% in all treatment groups. Mortality did not appear related to dietary treatment.

Gizzard erosion and the resultant black vomit disease of chickens has long been associated with feeding fish meal produced from decomposed raw materials that have been overheated during the reduction process. Considerable experimental evidence now

exists indicating that two compounds found in toxic fish meal, histamine and its derivative, gizzerosine, induce gizzard erosion by stimulating gastric acid secretion through their action on the H-2 receptor (Masumura et al., 1985; Miyazaki and Umemura et al., 1987). Apparently, persistent reduction in gastric pH results in the formation of lesions and ulcerations associated with black vomit disease in chickens. Romero and Castro (1989) reported that coho salmon reared in marine net-pens and fed a practical diet containing fish meal of Chilean origin that was highly toxic to chickens for 16 weeks exhibited gastric tissue abnormalities similar to those observed in chickens. Watanabe et al. (1987) induced gastric erosion in rainbow trout by feeding diets containing fish meal supplemented with histamine and subjected to heat treatment, and from their data inferred that the mechanisms of gastric lesion formation in teleosts were the same as those in chickens. Since gastric acid secretion can be induced by exogenous histamine and prevented by H-2 receptor antagonists in fish (Holstein, 1975a; Holstein, 1976) and chickens (Miyazaki and Umemura, 1987), it seemed reasonable to suggest that toxic amines found in fish meal, including histamine and gizzerosine, could have similar effects on both species. Contrary to expectations, the present research failed to verify the results of either Romero and Castro (1989) or Watanabe (1987). That is, rainbow trout fed a variety of protein sources treated with histamine and heat using the methods of Watanabe (1987) or fish meal found acutely toxic to chickens using a standard bioassay did not exhibit gastric lesions, ulcerations, or cellular changes consistent with impaired gastric function. Instead, the experimentally prepared protein sources and the toxic fish meal induced mild to severe stomach distention, an effect not previously reported in the scientific literature.

It is generally agreed that chickens fed diets containing histamine or gizzerosine form gastric ulcerations or lesions as a direct consequence of the hyperstimulatory effects of the active

compounds on gastric acid secretion. Human parietal cells secrete essentially pure HCl, with the pH being as low as 0.87 (Ganong, 1989). The pH of the water film present on the gastric mucosa of starved Atlantic cod was found to be about 2.1 (Holstein, 1979). Gastric HCl is concentrated enough to cause tissue damage, but animals possess protective mechanisms that prevent irritation or autodigestion of the gastric mucosa.

In fish and other animals, the gastric mucosa contains many specialized glands which secrete gastric acid, pepsins, and mucous (Noaillac-Depeyre and Gas, 1978). Mucous, which is secreted by the neck and surface mucous cells, is comprised of glycoproteins which form a flexible gel that coats the mucosa. In humans, and presumably fish and other animals, the gastric mucosa also secretes bicarbonate, which mixes with the mucous and forms an unstirred layer that has a pH of about 7.0 (Ganong, 1989). The mucosal bicarbonate barrier protects the mucosal cells from damage by gastric acid. If these mechanisms are disrupted, the mucosa may be exposed to gastric acid, leading to irritation and ultimately, ulceration.

Another mechanism by which gastric erosion in chickens may be initiated is by the effect of the ulcerogenic compounds on cellular morphology and function of the structural and secretory tissues of the proventriculus. In their study of malabsorption syndrome in broiler chickens, Stuart et al. (1986) found that dietary administration of histamine alone, or in conjunction with putrescine, and cadaverine induced marked dilation of the proventriculus and gizzard. Feeding of diets containing 2,640 mg histamine/kg resulted in proventricular dilation in 100% of the birds tested after 8 weeks feeding. The wall of the enlarged proventriculus was thickened, and mild to marked hypertrophy and hyperplasia of the deep glandular epithelium were observed. Minimal to mild inflammatory infiltrate and fibrosis focally separated

lobules of some glands. Gizzard enlargement was accompanied by proventricular dilation. The gizzard wall was flaccid, and the contents were fluid. However, lesions were only occasionally observed. The data therefore indicate that damage to subepithelial tissues evoked by the ulcerogenic compounds may precede formation of gastric lesions associated with increased gastric acid secretion.

In the present study, feeding of toxic fish meals or amine treated protein sources was associated with flaccid, distended stomachs in rainbow trout. The fact that cellular changes were not found does not preclude the possibility that the observed condition is a precursor to development of gastric erosion. Transient precursor conditions such as stomach distention may not be observed unless the animals are subjected to periodic visual evaluation of the visceral mass. Apparently, Watanabe et al. (1987) or Romero and Castro (1989) only examined fish in their studies at trial termination.

In seawater, fish lose water and gain ions by diffusion, and in order to maintain osmotic homostasis must replenish body water by drinking. Seawater is alkaline, and thus water consumed by the fish would be expected to buffer gastric acidity. In rainbow trout maintained in seawater, Smith (1989) found that the mucous layer and a clear fluid in the pyloric portion of the stomach below the food had a pH of about 5. In contrast, fish in fresh water are hyperosmotic to their external environment, so water diffuses inward across the gills. These fish drink little or no water, and must produce copious dilute urine to offset the diffusional inflow. It may be predicted that gastric pH of fish in fresh water would be lower and more uniform than that of fish in marine environments, since small quantities of fresh water ingested by the fish would have comparatively little effect on gastric pH. Therefore, it seems likely that fish in fresh water would be more sensitive to the effects of compounds that increase gastric acid secretion than fish in sea

water, but the data of Watanabe et al. (1987) and Romero and Castro (1989) clearly show that gastric erosion can occur, irrespective of external environment. Given these data, it is reasonable to suggest that one or more factors in addition to increased gastric acid secretion had a role in the development of gastric lesions observed by other researchers in rainbow trout and coho salmon, the two species of fish tested to date.

The study described in Chapter 4 was modeled after the procedure described by Watanabe et al. (1987), since they were able to produce gastric erosion in rainbow trout which they attributed to histamine toxicity similar to that observed in chickens. It was assumed that the toxic effects of the supplemental histamine and heat-treatment would be independent of the source of dietary protein, but the results of the research suggest that endogenous compounds in the protein source may have had important effects on the genesis of compounds toxic to fish. Watanabe et al. (1987) used sardine meal and white fish meal as dietary protein sources. While fish fed the unheated sardine meal for 8 weeks did not develop gastric abnormalities, 30% of the fish fed heated sardine meal developed stomach lesions. Supplementation of the sardine meal with histamine (2,000 mg/kg diet) before heat treatment induced gastric erosion in 40% of the fish. Since the effect was enhanced by supplementation, it is apparent that an interaction between the endogenous and supplemental compounds occurred. In contrast, supplementation of white fish meal at a level of 5,000 mg histamine/kg diet was required to evoke gastric erosion in rainbow trout after 4 weeks feeding, but no stomach abnormalities were noted when the diet was supplemented at a level of 1,000 mg histamine/kg. The data indicate that the sardine meal may have contained endogenous toxic compounds not present in the white fish meal, since different levels of histamine supplementation were required to elicit similar effects. Protein sources used in the present study were supplemented with 2,000 mg histamine/kg diet before

heat treatment, but considering the results of the study by Watanabe et al. (1987), it is likely that a higher concentration of histamine may have been required to evoke gastric ulceration in the test fish.

Few studies have measured phylogenetic differences in the sensitivity of various animal species to ulcerogenic compounds, but the available data suggest that this factor may be important to consider when determining threshold concentrations of the various compounds. For example, studies with isolated rabbit parietal cells, (Sugano et al., 1989) showed that gizzerosine is a relatively weak H-2 receptor agonist, compared to histamine. In their study, which indirectly assessed gastric acid secretory rates by the ^{14}C -aminopyrine uptake method, half-maximal response was attained by administration of histamine at a concentration of 6×10^{-7} M. The dose of gizzerosine required to attain half-maximal response was 50 times higher (3×10^{-5} M). In contrast, gastric acid secretion by isolated chicken parietal cells was maximized by equimolar concentrations (10^{-5} M) of either histamine or gizzerosine (Hino et al., 1987). Comparative studies have not been conducted with fish, but the available data indicate that species-specific differences should be considered in future research.

9.2.4 Biological assays for the measurement of protein quality

One of the oldest, perhaps most widely used measures of protein quality is the protein efficiency ratio (PER) developed by Osborne et al. (1919). In determining the PER of a protein source, individually housed male weanling rats, 21-28 d of age at the start of the trial, are fed a standard diet in which the protein content is limiting (9.09%) for 28 d. The PER of the protein is the ratio of weight gain to weight of protein consumed during the trial. The officially recognized method for the evaluation of protein quality is the PER method described by AOAC (1984). The standard for the reference group is Animal Nutrition Research Council (ANRC)

reference casein; the data are normalized so that the reference group has a PER of 2.5.

The nutritional value of dietary proteins can also be estimated from nitrogen retention data. Thomas (1909, cited by Evans and Witty, 1978), using human subjects, developed the first quantitative biological method for evaluating proteins. The biological value (BV) was expressed by Thomas (1909) as the percentage of absorbed nitrogen that is retained. Mitchell (1923-1924) adapted the BV method to growing and adult rats, and included in his calculations estimates of metabolic and endogenous nitrogen losses obtained when the animals were fed a nitrogen-free diet. Biological value of protein sources is determined with animals close to nitrogen balance, and is an important tool in predicting the ability of a protein source to meet the demands of animals under a variety of physiological conditions, including pregnancy, lactation, and growth (Evans and Witty, 1978).

Net protein utilization (NPU; Bender and Miller, 1953), which gives results similar to the Thomas-Mitchell nitrogen balance method, quantifies the proportion of total feed nitrogen that is retained by direct carcass analysis of animals that have been fed either the test protein, reference casein, or a protein-free diet. Rats are fed diets similar in composition to those described for PER (AOAC, 1984) on a *ad libitum* basis for 10 d, at which time they are sacrificed and carcass nitrogen determined. NPU is calculated as:

$$\text{NPU} = 100 * (\text{body N of test group} - \text{body N of protein free group}) / \text{N intake}$$

If a correction for endogenous losses is not made, the value is termed apparent NPU. Apparent NPU and protein productive value (PPV; Pfeffer, 1982) are equivalent terms.

All protein quality assays with animals are derived from the relationship between growth, or some correlate of growth, and

protein intake. Many factors, including age, sex, strain of experimental animal, and environmental conditions have been shown to affect the accuracy and reproducibility of bioassays with rats (Miller and Lachance, 1977). Consequently, considerable work has been conducted to standardize assay conditions used in studies with terrestrial homeothermic animals, and the validity of the procedures established through extensive collaborative assays. Methods for assessing protein quality with terrestrial animals have been adapted for use with fish, but few studies focused on defining appropriate bioassay conditions and criteria of biological response have been conducted (March et al., 1985).

Two assays, PER and PPV, are commonly used for evaluating dietary protein sources with fish. The results of these assays with rats (Samonds and Hegsted, 1977) and rainbow trout (Nose, 1971; Steffens, 1981) depend to a large extent on total protein intake, whether a function of feeding level or dietary protein content, relative to the optimum requirement for growth or nitrogen retention. While the effects of feeding level on the growth and efficiency of feed utilization by fish have been well documented in the scientific literature (Chapter 7), diet formulations and feeding levels have not been standardized, and consequently, published data were insufficient to permit the selection of appropriate levels of protein intake for growth and protein retention assays in rainbow trout under the experimental conditions in the University of Washington laboratory facilities. Chapter 7 reports the results of a feeding study conducted with juvenile rainbow trout to: (1) determine how rate of protein intake from graded levels of a single standard diet affected dietary protein utilization by rainbow trout and (2) evaluate the sensitivity of the assay to changes in the nutritional value of fish meal protein resulting from thermal abuse. The data were used to identify the range of feeding levels used in the study described in Chapter 8.

In the feeding study described in Chapter 7, juvenile rainbow trout were pair-fed equal quantities of protein from diets containing either herring meal (control diet) or herring meal that had been thermally abused by autoclaving (test diet). Groups of fish were pair-fed the experimental diets at graded levels of protein intake ranging from unfed to satiation for 4 weeks. PER and PPV for fish in each treatment group were calculated from data on weight gain, protein intake, and nitrogen retention.

In this study, groups of fish that were not fed, or pair-fed 1.3 or 3.0 g protein/kg body weight/d lost weight, while those pair-fed 6.0, 11.0, or 16.0 g protein/kg body weight/d or to satiation (31-38 g protein/kg body weight/d) were in positive nitrogen balance and measurable protein accretion occurred. As predicted, efficiency of protein retention (PPV; Tables 7.6, 7.7) was affected by rate of protein intake, and the rate that resulted in the highest retention differed between the two protein sources. Protein retention from the unheated protein source was maximized ($P < 0.05$) when the fish were fed 11.0 g protein/kg body weight/d. Higher intake levels, including satiation did not affect PPV. In contrast, there was no significant difference ($P > 0.05$) in protein retention by fish fed the heated protein source, irrespective of the level of protein intake, although retention seemed to increase in proportion to intake up to the highest controlled level of feeding, and to decline at the satiation level. When only the three highest levels of intake were considered, the PPV of the unheated fish meal was about 30% better than the heated protein source.

The dose-dependent effects of level of protein intake on the PER of the two protein sources were clearly shown in this study (Tables 7.6, 7.7). Protein efficiency ratios for the diet containing unheated fish meal increased significantly ($P < 0.001$) as the rate of protein intake increased from 6.0 to 16.0 g/kg body weight/d. Satiation feeding (about 31 g protein/kg body weight/d) did not

affect PER, compared to the highest controlled level of intake (16.0 g protein/kg body weight/d). In contrast, protein efficiency ratios for the diet containing the heated fish meal were statistically equivalent ($P>0.05$) at levels of protein intake ranging from 6.0 g/kg body weight/d to satiation (about 38 g/kg body weight/d). However, there was a trend toward increased PER with increasing protein intake up to a rate of 11.0 g/kg body weight/d, and toward a decline with increased intake. Overall, protein efficiency ratios were about 15% higher for groups fed the unheated fish meal than those fed the heated protein source.

From the experimental data, it was apparent that rates of protein intake below 11.0 g/kg body weight/d would be unacceptable for assessing protein quality in the subsequent experiments with juvenile rainbow trout, since the apparent rate of protein retention was, in the case of the unheated (control) fish meal, unrealistically low. It was also found that the PER and PPV were dose-dependent and that the level of protein intake that resulted in maximum protein retention differed for the two protein sources. It was concluded that: (1) valid comparisons among various protein sources can be obtained when the fish are fed equal quantities of protein, provided the level selected for the control diet lies in the linear portion of the protein retention dose-response curve, and (2) feeding trials conducted using only one level of intake cannot simultaneously determine the true nutritional values of different protein sources, since the optimum level of intake that results in maximum protein retention may differ among the various sources. Despite these limitations, the results of the study indicated that in future experiments, valid within-trial comparisons of the relative nutritional quality of various protein sources could be made over a fairly wide range of controlled protein intake levels, assuming that environmental conditions, sizes of fish, and husbandry practices would be nearly identical to those used in this experiment.

The validity of growth and protein retention assays for the measurement of protein quality has been questioned by many authors for several reasons. Protein quality values obtained from growth assays with rats (Sammonds and Hegsted, 1977) and rainbow trout (Nose, 1971; Steffens, 1981) have been shown to be dose-dependent. PER increases as protein intake increases, plateauing as growth rate reaches a maximum, then falling at higher intakes (Sammonds and Hegsted, 1977). Since the results are positively correlated with food (protein) intake (Hegsted and Chang, 1965), PER values may be affected by feed palatability (Hopkins and Steinke, 1976), or by experimental procedures that arbitrarily restrict intake to a preselected level. Furthermore, the dose-dependent relationship is not linear at low levels of intake, but instead curvilinear, depending on the profile of essential amino acids (Sammonds and Hegsted, 1977). Since weight gain is correlated with protein accretion, similar trends are observed when protein quality is assessed using the PPV method.

One of the major criticisms of techniques that evaluate protein quality over a limited range of protein intakes is that the results do not represent the actual dose-response relationship and that the results can only be used to rank the relative quality of the protein sources. In the present research, the effects of the various treatments was assessed by their effects on nutritional value, relative to a control protein. Assay sensitivity to changes in protein quality was more important than determination of absolute protein quality values. By feeding all groups of fish equal quantities of protein from the experimental diets, and by using feeding rates found to maximize protein retention from the control diet, the rankings could be unambiguously attributed to changes in protein quality, rather than feeding rate. However, animal feeding trials in which a single level of protein intake is used are of little value for estimating how well a protein source will function for productive purposes at different levels of intake. Therefore, general application

of protein quality data for formulating practical diets or establishing appropriate feeding practices requires that multiple levels of protein intake be tested and the actual dose response relationship derived. The slope-ratio assay (Hegsted and Chang, 1965), in which the effect of protein intake on weight gain by different groups of experimental animals was determined, has been adapted for protein quality bioassays with rainbow trout (March et al., 1985), with good results. However, the slope-ratio method has not yet found widespread usage for the evaluation of feed proteins for the diets of aquatic species.

The effects of feeding level on the growth and efficiency of protein utilization by fish has been well documented in the scientific literature (Chapter 7). However, most data have been obtained under widely varying and poorly defined environmental conditions, and few broad generalizations can be made concerning the relative quality of various sources of dietary protein. Many environmental conditions are known to directly or indirectly influence physiological functions that alter the growth rate of fish (Brett, 1977). Also, specific environmental and internal factors act as cues for the initiation of complex behavioral, biochemical, and neuroendocrine changes that may affect the fates of ingested nutrients. Therefore, efforts to standardize experimental techniques for protein quality evaluation must also consider many factors related to environment and husbandry if reproducible and accurate results are to be obtained. Among these factors are daylength (photoperiod), water temperature, meal timing, and feeding frequency.

Photoperiod plays an important role in the seasonal and daily adjustment of behavioral and physiological patterns in fish as well as in other animals. The relationships between photoperiod and hormonal secretion by fish have been extensively studied in recent years, particularly in regard to the mechanisms of broodstock

maturation. Light signals received via the retina of the eye, midbrain surface, or pineal organ are used by the fish to set and coordinate internal rhythms with the external environment. These rhythms stimulate brain-pituitary responses which in turn govern endocrine function and the release of various hormones, including growth hormone and anabolic steroids. Research indicates that the most important factors associated with photoperiodic effects on internal rhythms are periodicity of the entraining cycle, the ratio of light time to dark time, and the rate of transition from light to dark (twilight).

Water temperature and day length may interact in controlling biological rhythms in fish. However, numerous studies have shown that temperature acts primarily by governing the rate of metabolism and is not itself a seasonal cue. Swift (1955) observed seasonally variable rates of growth among groups of hatchery reared brown trout (*Salmo trutta*) that were not correlated with water temperature. Swift (1959, 1961) identified increasing photoperiod as the primary factor stimulating endocrine activity, and that growth rate was enhanced by increasing water temperature as the season progressed. Hogman (1968) found a close correlation between growth rate of lake whitefish (*Coregonus clupeaformis*) and day length independent of water temperature effects.

Diel variations in feeding activity have been observed by many researchers. Feeding activity by juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) varied with time of day (Hoar, 1942). Hoar (1942) showed that the feeding activity of young trout and salmon was lowest during hours of darkness, and that water temperature reduction was not correlated with the observed decrease in feeding activity. Peak feeding activity of rainbow trout reared in seawater varied with season (Landless, 1976). Timing of feeding peaks was significantly correlated with time of sunset, but not with sunrise. During some months, a high level of feeding

occurred overnight, with up to 40% of the feed being delivered between midnight and 0600.

Meal timing in relation to daily light:dark schedule has been found to significantly affect weight gain of several species of fish. Catfish (*Heteropneustes fossilis*) subjected to constant photoperiod of 12 h of light alternating with 12 h of darkness and allowed access to feed at different times during the circadian cycle exhibited different rates of weight gain (Sundararaj et al., 1982). In their studies, fish fed during the middle of the second half of the daily period of darkness exhibited greater weight gain than those fed during either early dark, early light, or late light periods. The authors concluded that the timing of food intake is critical for optimum utilization of ingested calories.

Studies involving goldfish (*Carassius aratus*) and common carp (*Cyprinus carpio*) fed at different times relative to the light:dark cycle suggested that the effects of meal timing may be species dependent. Goldfish maintained on a 12 L:12 D photoperiod at 14°C exhibited differential growth depending on feeding time relative to the onset of the light cycle: fish fed 18 h after light onset (8 h into darkness) exhibited the greatest weight gain over the 3 week experimental period. Conversely, weight gain by common carp maintained on a 16L:8D photoperiod at 15°C and fed at a rate of 2% body weight/d at one of six feeding times for 5 months was not significantly different among treatments (Noeski and Spieler, 1984).

It is evident that feeding time may have a substantial impact on the efficiency with which feed is utilized by some species of fish. This factor, as well as differences in feeding level used by various researchers may account for some of the discrepancies among studies on optimal feeding frequency. For example, in three studies described by Andrews and Page (1975) on the effects of feeding frequency on channel catfish (*Ictalurus punctatus*), optimal weight gain and feed efficiency was obtained from fish fed to

satiation two times per day (0800 and 1630). Weight gain was reduced by feeding only once per day and was not improved by feeding four times per day (0800, 1100, 1400, and 1630). In a study with automatic feeders, fish fed 24 times/d (hourly) had significantly poorer gains and feed efficiencies than those fed either four (once every 6 h) or eight times/d (once every 4 h). In contrast, Greenland and Gill (1979) reported that feed efficiency and growth by channel catfish was improved by increasing feeding frequency from one to four times/d when they were fed at a fixed ration level that was substantially below satiation (2% body weight/d). The possible interactive effects of feeding level and timing on efficiency of feed utilization clearly merit further scrutiny.

Many studies with higher vertebrates have shown that method of feeding (meal feeding versus continuous or *ad libitum* feeding) and meal timing influence many physiological variables, including rate of growth and degree of fattening. Channel catfish maintained under a light:dark regimen of 12 h each (onset of light at 0600) and fed a fixed daily ration (2.5% of body weight/d) in a single meal at 0730 gained more weight, utilized feed more efficiently, and exhibited a lower percentage of abdominal fat than fish fed a single meal at 1600, or those given half rations at each feeding time (Noeske-Hallin et al., 1985). Feeding frequency and meal timing may be an important consideration in feed protein evaluation, since factors that affect caloric efficiency, as well as the metabolic fate of dietary protein into either depot lipid, muscle tissue, or energy, impacts the resultant protein quality value.

Data presented above indicate that maximum potential growth rate of fish is directly linked to seasonal changes in day length. Water temperature interacts with photoperiod by modifying metabolic rate, and ultimately growth. Meal timing and feeding frequency affect metabolic efficiency, growth rate, and the

partitioning of feed components to calories, protein synthesis, or fat deposition. Substantial gaps remain in our understanding of the complex environmental, behavioral, and physiological interactions that affect growth and nutrient partitioning in fish. Considerable research will be required to identify the conditions of environment and husbandry most appropriate for use in experiments specifically intended to evaluate the nutritional value of various dietary protein sources to aquatic species. The available data underscore the need to develop, validate, and implement standard experimental techniques, so that valid between-trial comparisons can be made.

9.3 CONCLUSIONS

From the results of the research, it can be concluded that:

1. The nutritional value and palatability of fish meal are not correlated with the results of toxicity bioassays with chickens.
2. The nutritional value of fish meal to rainbow trout is not directly affected by the presence of compounds acutely toxic to chickens. However, chronic exposure can result in severe distention of the stomach, which may represent a transient precursor condition for the development of gastric erosion. The duration and level of exposure required to induce detrimental changes that impair digestive and adsorptive function are not known.
3. Rainbow trout fed diets containing either fish meal or casein treated with supplemental histamine followed by heating exhibit severe stomach distention, as do rainbow trout fed Chilean fish meal toxic to chickens. However, none of the experimental treatments produced gastric ulceration, and additional research will be required to determine whether the experimental protein treatments with supplemental histamine adequately model the activity of endogenous amines in toxic fish meal.
4. Endogenous histamine level in commercially produced fish meal is not a sensitive and reliable indicator of fish meal protein quality to rainbow trout. Studies with model protein systems (fish meal low in endogenous histamine and casein were treated with histamine at levels ranging from 0 to 2,000 mg/kg dry diet) revealed no direct positive or negative effects of histamine on growth or efficiency of diet or protein

utilization by rainbow trout, irrespective of protein source or heat treatment.

5. Histamine and heat may interact to produce palatability enhancing compounds in fish meal. Studies with a model protein system (fish meal low in endogenous histamine treated with histamine at two levels, 0 and 2,000 mg/kg dry diet, and used with or without heat treatment) showed that voluntary feed consumption was greatly affected by the addition of histamine, and the effect was dependent on whether or not the fish meal had been heated. Free histamine was slightly repellent, unless the protein was heated, in which case the effect was stimulatory.
6. Palatability assays may be affected by interstrain differences in the ability of fish to adapt to a rearing system. Consideration should also be given to the possible effects of interstrain differences in protein utilization.

9.4 SUGGESTIONS FOR FUTURE RESEARCH

1. Additional research is required to determine the effects on fish of chronic exposure to compounds toxic to chickens. Development of a suitable bioassay conducted under conditions that evoke an acute response in fish will be required, as will identification of suitable model protein sources, and development of methods for determining the metabolic activities of the suspected toxicant *in vivo*. Initially, research should focus on the uptake, distribution, and metabolism of histamine administered by intramuscular or oral routes.
2. The results of the present research emphasize the need for the development of a simple, inexpensive, and reproducible bioassay method with fish for the measurement of protein quality. Future research should focus on development of standardized methodology for conducting protein quality bioassays with fish. Critical areas of research include: (1) identification of a suitable reference standard protein against which test proteins can be tested, (2) development of standard test diet formulation guidelines, including sources and levels of diet ingredients, (3) identification of appropriate feeding techniques and levels of intake that emphasize measurement of the actual potencies of dietary protein sources to produce a predetermined biological response (e.g.; synthesis of body protein and maintenance of body tissue and function), and (4) assessment of the validity of using the results of nutritive value bioassays for the formulation of practical diets for fish.
3. Reliable assays of feed ingredient palatability based on the ability of fish to voluntarily differentiate between protein sources must be developed and validated. Assays should include criteria for growth performance, since economic analysis would be enhanced by knowledge of palatability effects on intake and resultant expected rates of growth.

Literature Cited

- Andrews, J. W., and J. W. Page. 1975. The effects of frequency of feeding on culture of catfish. *Transactions of the American Fisheries Society* 104(2):317-321.
- Andron, J.W., and A.M. Mackie. 1978. Studies on the chemical nature of feeding stimulants for rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* 12:303-310.
- AOAC. 1984. Official Methods of Analysis, 14th edition. Association of Official Analytical Chemists, Inc., Arlington, Va., USA.
- Baker, D.H. 1984. Equalized versus ad libitum feeding. *Nutrition Reviews* 42(8):269-273.
- Barankowski, J.D., and P.A. Brust. 1984. Nutritional value of protein from fresh and spoiled mahi mahi (*Coryphaena hippurus*). *Nutrition Reports International* 30(6):1337-1342.
- Barankowski, J.P., F.A. Hilmer, P.A. Brust, M. Chongsiriwatana, and R.J. Premaratne. 1990. Decomposition and histamine content in mahi mahi (*Coryphaena hippurus*). *Journal of Food Protection* 53(3):217-222.
- Barlow, S.M., and M.L. Windsor. 1984. Fishery by-products. International Association of Fish Meal Manufacturers (IAFMM) Technical Bulletin Number 19 (September 1984), 22 pp.
- Bender, A.E., and D.S. Miller. 1953. A new brief method of estimating net protein value. *Biochemical Journal* 53(vii).
- Brett, J.R. 1977. Environmental factors and growth. Pages 599-675 in W.S. Hoar, D.J. Randall, and J.R. Brett, editors. *Fish Physiology*, Volume 8. Academic Press, New York, New York.

- Bryant, M., A. Buchan, M. Gregor, M. Chatel, J. Polak, and S. Bloom. 1982. Development of intestinal regulatory peptides in the human fetus. *Gastroenterology* 83:47-54.
- Carr, W. 1982. Chemical stimulation of feeding behavior. Pages 259-273 *in* *Chemoreception in Fishes*. T.J. Hara, editor. Elsevier Scientific Publishing Company., New York.
- Castro, E. 1987. Erosiones a la molleja y vomito negro aviar: su prevencion a traves del control de calidad a las harinas de pescado. *Avicultura Profesional* 5(2):55-56.
- Douglas, W.W. 1980. Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. Pages 609-646 *in* *The Pharmacological Basis of Therapeutics* (6th edition). Gilman, A.G., Goodman, L.S., and Gilman, A., editors. Mac Millan Publishing Co., New York, New York.
- Evans, E., and R. Witty. 1978. An assessment of methods used to determine protein quality. *World Review of Nutrition and Dietetics* 32:1-26.
- Ganong, W.F. 1989. *Review of medical physiology*. Appleton and Lange, East Norwalk, Connecticut, USA. 673 pp.
- Gespach, C., S. Emami, and E. Chastre. 1988. Membrane receptors in the gastrointestinal tract. *Bioscience Reports* 8(3):199-235.
- Grayton, B.D., and F.W.H. Beamish. 1977. Effects of feeding frequency on food intake, growth, and body composition of rainbow trout (*Salmo gairdneri*). *Aquaculture* 11:159-172.
- Greenland, D. C., and R. L. Gill. 1979. Multiple daily feedings with automatic feeders improve growth and feed conversion rates of channel catfish. *Progressive Fish-Culturist* 41(3):151-153.

- Hara, T.J. 1973. Olfactory responses to amino acids in rainbow trout, *Salmo gairdneri*. *Comparative Biochemistry and Physiology* 44A:407-416.
- Hardy, R.W., K.D. Shearer, F.E. Stone, and D. Weig. 1983. Fish silage in aquaculture diets. *Journal of the World Mariculture Society* 14:695-703.
- 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.
- Harry, E.G., J.F. Tucker, and A.P. Laursen-Jones. 1975. The role of histamine and fish meal in the incidence of gizzard erosion and proventriculus abnormalities in the fowl. *British Poultry Science* 16:69-78.
- Hegsted, D.M., and Y. Chang. 1965. Protein utilization in growing rats. I. Relative growth index as a bioassay procedure. *Journal of Nutrition* 85:159-168.
- Heitkemper, M., and S. Marotta. 1983. Development of neurotransmitter enzyme activity in the rat gastrointestinal tract. *American Journal of Physiology* 244:G58-64.
- Hesterberg, R., J. Sattler, W. Lorenz, C.D. Stahlknecht, W. Barth, M. Crombach, and D. Webber. 1984. Histamine content, diamine oxidase activity and histamine methyltransferase activity in human tissues; fact or fiction. *Agents and Actions* 14(3/4):325-334.
- Hino, Y., T. Noguchi, and H. Naito. 1987. Effect of gizzerosine by isolated mucosal cells of the chicken proventriculus. *Poultry Science* 66:548-551.

- Hoar, W. S. 1942. Diurnal variations in feeding activity of young salmon and trout. *Journal of the Fisheries Research Board of Canada* 6(1):90-101.
- Hogman, W. J. 1968. Annulus formation on scales of four species of coregonids reared under artificial conditions. *Journal of the Fisheries Research Board of Canada* 25:2111-2112.
- Holstein, B. 1975a. gastric acid secretion in a teleostean fish: a method for the continuous collection of gastric effluence from a swimming fish and its response to histamine and pentagastrin. *Acta Physiologica Scandinavica* 95:417-423.
- Holstein, B. 1975b. Intestinal diamine oxidase of some teleostean fishes. *Comparative Biochemistry and Physiology* 50B:291-297.
- Holstein, B. 1976. Effect of the H-2 receptor antagonist metiamide on carbachol and histamine-induced gastric acid secretion in the Atlantic cod, *Gadus morhua*. *Acta Physiologica Scandinavica* 97:189-195.
- Holstein, B. 1979. Gastric acid secretion and water balance in the marine teleost *Gadus morhua*. *Acta Physiologica Scandinavica* 105:93-107.
- Hopkins, D.T., and F.H. Steinke. 1976. Effects of water of hydration on the measurement of protein efficiency ratio of casein and soybean proteins with rats. *Journal of Nutrition* 106:1438-1446.
- Hui, J.Y., and S.L. Taylor. 1985. Inhibition of *in vivo* histamine metabolism by foodborne inhibitors of diamine oxidase, histamine-N-methyltransferase, and monamine oxidase. *Toxicology and Applied Pharmacology* 81:241-249.

- Huisman, E.A. 1976. Food conversion efficiencies at maintenance and production levels for carp, *Cyprinus carpio* L, and rainbow trout, *Salmo gairdneri* Richardson. *Aquaculture* 9:259-273.
- Humason, G.L. 1967. *Animal Tissue Techniques*, 2nd edition. W.H. Freeman and Company, San Francisco, CA. 569 pp.
- Hungerford, J.M., K.D. Walker, M.M. Wekell, J.E. LaRose, and H.R. Thom. 1990. Selective determination of histamine by flow injection analysis. *Analytical Chemistry* 62:1971-1976.
- Ito, Y., T. Noguchi, and H. Naito. 1985. Fluorometric determination of gizzerosine, a histamine H-2 receptor agonist discovered in feedstuffs, employing high-performance liquid chromatography. *Analytical Biochemistry* 151:28-31.
- Ito, Y., H. Terao, T. Noguchi, and H. Naito. 1988. Gizzerosine raises the intracellular cyclic adenosine-3',5'-monophosphate level in isolated chicken proventriculus. *Poultry Science* 67:1290-1294.
- Johnsen, P.B., and M.A. Adams. 1986. chemical feeding stimulants for the herbivorous fish *Tilapia zilli*. *Comparative Physiology and Biochemistry* 83A(1):109-112.
- Johnson, D.C., and C. Pinedo D. 1971. Gizzard erosion and ulceration in Peru broilers. *Avian Diseases* 15:835-837.
- Jones, K.A. 1989. The palatability of amino acids and related compounds to rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* 34:149-160.
- Kennedy, J.F., R.J. Noy, J.A. Stead, and C.A. White. 1989. A new rapid enzyme digestion method for predicting *in vitro* protein quality (PDD Index). *Food Chemistry* 32:277-295.

- Kindschi, G.A., C.E. Smith, and R.F. Koby, Jr. 1991. Performance of two strains of rainbow trout reared at four densities with supplemental oxygen. *The Progressive Fish Culturist* 53(4):203-209.
- Landless, P. J. 1976. Demand-feeding behavior of rainbow trout. *Aquaculture* 7:11-25.
- Larsson, L.I., and J.F. Rehfeld. 1977. Evidence for a common evolutionary origin of gastrin and cholecystokinin. *Nature (London)* 269:335-338.
- Lindstedt, K.J. 1971. Chemical control of feeding behaviour. *Comparative Biochemistry and Physiology* 39A:553-581.
- Little, T.M., and F.J. Hills. 1978. *Agricultural experimentation: design and analysis*. John Wiley and Sons, Inc., New York, New York, U.S.A.
- Lorenze, W., E. Matejka, A. Schmal, W. Seidel, H-J. Reimann, R. Uhlig, and G. Mann. 1973. A phylogenetic study on the occurrence and distribution of histamine in the gastro-intestinal tract and other tissues of man and various animals. *Comparative and General Pharmacology* 4:229-250.
- Luna, L.B. 1968. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*, 3rd edition. McGraw-Hill, New York, New York. 258 pp.
- Mackie, A.M., and A.I. Mitchell. 1985. Identification of gustatory stimulants for fish applications in aquaculture. Pages 177-190 *in* *Nutrition and Feeding in Fish*. Cowey, C.B., A.M. Mackie, and J.G. Bell, *editors*. Academic Press, London, England.

- March, B.E., C. MacMillan, and F.W. Ming. 1985. Techniques for evaluation of dietary protein quality for the rainbow trout (*Salmo gairdneri*). *Aquaculture* 47:275-292.
- Masumura, T., M. Sugahara, T. Noguchi, K. Mori, and H. Naito. 1985. The effect of gizzerosine, a recently discovered compound in overheated fish meal, on the gastric acid secretion in chicken. *Poultry Science* 64:356-361.
- Miller, G.A., and P.A. Lachance. 1977. Techniques in rat bioassays. Pages 149-161 in C.E. Bodwell, editor. *Evaluation of proteins for humans*. AVI Publishing Co., Inc., Westport, Connecticut, U.S.A.
- Mitchell, H.H. 1923-1924. A method for determining the biological value of protein. *Journal of Biological Chemistry* 58:873-903.
- Miyazaki, S., and Y. Umemura. 1987. Effects of histamine antagonists, an anticholinergic agent and antacid on gizzard erosions in broiler chicks. *British Poultry Science* 28:39-45.
- Mori, K., T. Okazaki, T. Noguchi, and H. Naito. 1983. Synthesis of (\pm)-gizzerosine, and inducer of gizzard erosion in broiler chicks. *Agricultural and Biological Chemistry* 47(9):2131-2132.
- Mori, K., T. Sugai, Y. Maeda, T. Okazaki, T. Noguchi, and H. Naito. 1985. Synthesis of the racemic and optically active forms of gizzerosine, the inducer of gizzard erosion in chicks. *Tetrahedron* 41(22):5307-5211.
- Morrow, J.D., G.R. Margolies, J. Rowland, and L.J. Roberts. 1991. Evidence that histamine is the causative toxin of scombroid-fish poisoning. *The New England Journal of Medicine* 324(11):716-720.

- Motil, K.J., and N.S. Scrimshaw. 1979. The role of histamine in scombroid poisoning. *Toxicology Letters* 3:219-223.
- Murai, T., T. Akiyama, H. Ogata, Y. Hirasawa, and T. Nose. 1982. Effect of coating amino acids with casein supplemented to gelatin diet on plasma free amino acids in carp. *Bulletin of the Japanese Society of Scientific Fisheries* 48(5):703-710.
- Noaillac-Depeyre, J., and N. Gas. 1978. Ultrastructural and cytochemical study of the gastric epithelium in a fresh water teleostean fish (*Perca fluviatilis*). *Tissue and Cell* 10(1):23-37.
- Noeske, T. A., and R. E. Spieler. 1984. Circadian feeding time affects growth of fish. *Transactions of the American Fisheries Society* 113:540-544.
- Noeske-Hallin, T. A., R. E. Spieler, N. C. Parker, and M. A. Suttle. 1985. Feeding time differentially affects fattening and growth of channel catfish. *Journal of Nutrition* 115:1228-1232.
- Nose, T. 1971. Determination of nutritive value of food protein in fish-III. Nutritive value of casein, white fish meal, and soybean meal in rainbow trout fingerlings. *Bulletin of the Freshwater Fisheries Research Laboratory* 21(1):85-98.
- Ogino, C. 1980. Protein requirements of carp and rainbow trout. *Bulletin of the Japanese Society of Scientific Fisheries* 46(3):385-388.
- Okazaki, T. T. Noguchi, K. Igasashi, Y. Sakagami, H. Seto, K. Mori, H. Naito, T. Masumura, and M. Sugahra. 1983. Gizzerosine, a new toxic substance in fish meal, causes severe gizzard erosion in chicks. *Agricultural and Biological Chemistry* 47(12):2949-2952.

- Osborne, T.B., L.B. Mendel, and E.L. Ferry. 1919. A method of expressing numerically the growth-promoting value of proteins. *Journal of Biological Chemistry* 37:223-229.
- Parker, N.C. 1984. Chronobiologic approach to aquaculture. *Transactions of the American Fisheries Society* 113:545-552.
- Pfeffer, E. 1982. Utilization of dietary protein by salmonid fish. *Comparative Biochemistry and Physiology* 73B(1):51-57.
- Pike, I.H., G. Andorsdottir, and H. Mundeim. 1990. The role of fish meal in diets for salmonids. *International Association of Fish Meal Manufacturers Technical Bulletin No. 24* (March 1990), Potters Bar, Hertfordshire, England.
- Pike, I.H. 1991. Freshness of fish for fish meal-effect on growth of salmon. *Proceedings: IV International Symposium on Fish Nutrition*. Biarritz, France, June 24-27, 1991. *In press*.
- Reite, O.B. 1969. Phylogenetical persistence of the non-mast cell histamine stores of the digestive tract: a comparison with mast cell histamine. *Experientia* 25(3):276-277.
- Romero, J.J., and E. Castro. 1989. Procedures used in Chile for the certification of the nutritional and biological quality of fish meals used in salmon production. *The Third Symposium on Feeding and Nutrition in Fish*. August 28-September 1, 1989. Toba, Japan. (unpublished).
- Salguero-Fernandez, J., and I.M. Mackie. 1987. Comparative rates of spoilage of filets and whole fish during storage of haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) as determined by the formation of non-volatile and volatile amines. *International Journal of Food Science and Technology* 22:385-390.

- Sammons, K.W., and D.M. Hegsted. 1977. Animal bioassays: A critical evaluation with specific reference to assessing nutritive value for the human. Pages 68-80 *in* C.E. Bodwell, editor. Evaluation of proteins for humans. AVI Publishing Co., Inc., Westport, Connecticut, U.S.A.
- Shearer, K.D. 1988. Dietary potassium requirement of juvenile chinook salmon. *Aquaculture* 73:119-129.
- Shifrine, M., L.E. Ousterhout, C.R. Grau, and R.H. Vaughn. 1959. Toxicity to chicks of histamine formed during microbial spoilage of tuna. *Applied Microbiology* 7:45-50.
- Smith, H.W. 1930. The absorption and excretion of water and salts by marine teleosts. *American Journal of Physiology* 43:480-505.
- Smith, L.S. 1989. Digestive functions in teleost fishes. Pages 331-421 *in* Fish Nutrition, Second Edition. J.E. Halver, editor. Academic Press, Inc., San Diego, Ca.
- Smit, H. 1967. Influence of temperature on the rate of gastric juice secretion in the brown bullhead, *Ictalurus nebulosis*. *Comparative Biochemistry and Physiology* 21:125-132.
- Steffens, W. 1981. Protein utilization by rainbow trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*): A brief review. *Aquaculture* 23:337-345.
- Stone, F.E., and R.W. Hardy. 1986. Nutritional value of acid stabilised and liquified fish protein. *Journal of Science, Food, and Agriculture* 37:797-803.

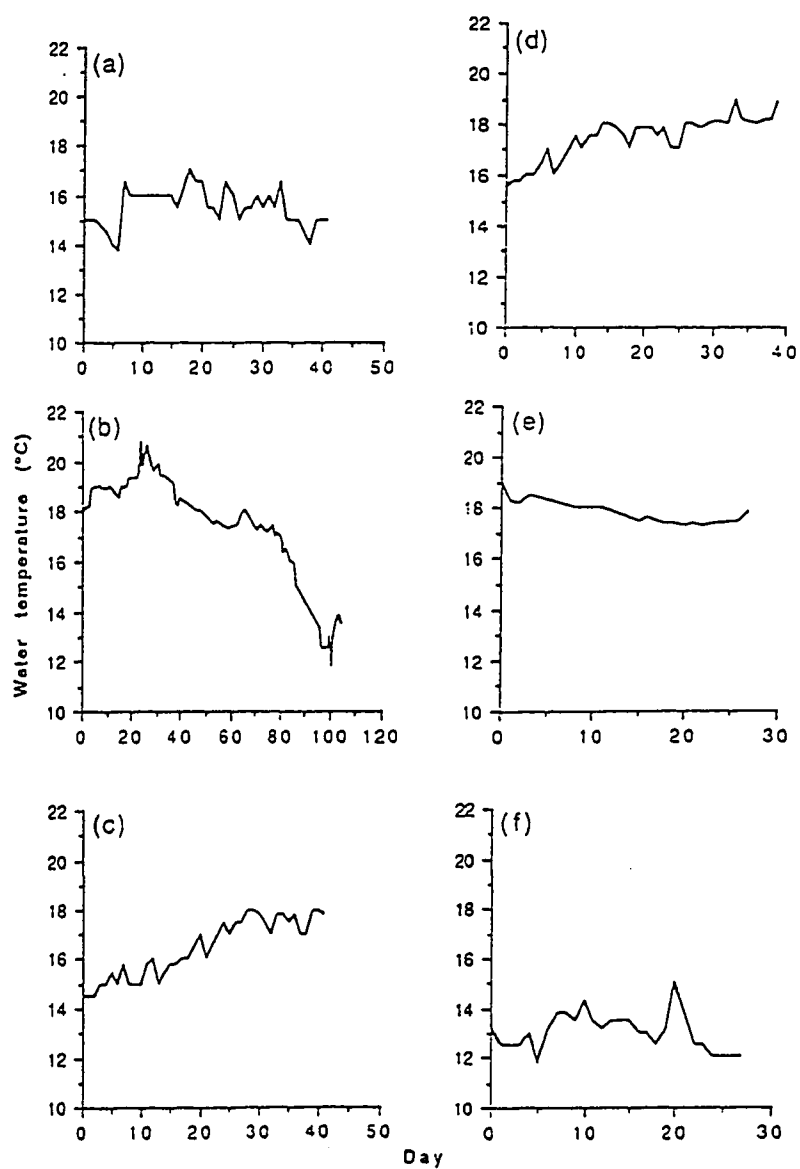
- Stone, F.E., and R.W. Hardy. 1988. Plasma amino acid changes in rainbow trout (*Salmo gairdneri*) fed freeze-dried fish silage, liquefied fish, and fish meal. Pages 419-426 in Congress Proceedings: Aquaculture International Congress and Exposition. September 6-9, 1988. Vancouver, British Columbia, Canada.
- Stuart, B.P., R.J. Cole, E.R. Waller, P.E. Vesonder. 1986. Proventricular hyperplasia (malabsorption syndrome) in broiler chicks. *Journal of Environmental Pathology, Toxicology and Oncology* 6(314):369-386.
- Sugahara, M., T. Hattori, and T. Nakajima. 1988. Effect of synthetic gizzerosine on growth, mortality, and gizzard erosion in broiler chicks. *Poultry Science* 67:1580-1584.
- Sugano, K., K. Tsuji, M. Kato, H. Natomi, E. Saito, A. Seki, and F. Takaku. 1989. Effect of gizzerosine, an ulcerogenic compound to chick gizzard, on isolated rabbit parietal cells. *Gastroenterology* 96(5/2):A494.
- Sundararaj, B. I. , P. Nath, and F. Halberg. 1982. Circadian meal timing in relation to lighting schedule optimizes catfish body weight gain. *Journal of Nutrition* 112:1085-1097.
- Swift, D. R. 1955. Seasonal variations in the growth rate, thyroid gland activity, and food reserves of growth trout (*Salmo trutta* Linn.). *Journal of Experimental Biology* 32:751-764.
- Swift, D. R. 1959. Seasonal activity of the activity of the thyroid gland of yearling brown trout.(*Salmo trutta* Linn.). *Journal of Experimental Biology* 36:120-125.
- Swift, D. R. 1961. The annual growth rate cycle of brown trout (*Salmo trutta* Linn.) and its cause. *Journal of Experimental Biology* 38:595-604.

- Taylor, S.L., L.S. Guthertz, M. Leatherwood, F. Tillman, and E.R. Lieber. 1978. Histamine production by food-borne bacterial species. *Journal of Food Safety* 1:173-187.
- Taylor, S.L. 1986. Histamine food poisoning: Toxicology and clinical aspects. *CRC Critical Reviews in Toxicology* 17(2):91-128.
- Toyama, K., M. Okuzumi, TI Yokoi, and H. Aoe. 1981. Histamine content of fish meal. *Bulletin of the Japanese Society of Scientific Fisheries* 47(3):415-419.
- Toyama, K., M. Hoshi, M. Ishiguro, and H. Aoe. 1982. Behavior of histamine during the manufacturing process of fish meal. *Bulletin of the Japanese Society of Scientific Fisheries* 48(9):1333-1339.
- Umemura, Y., S. Miyazaki, H. Yamanaka, T. Ohya, S. Homma, M. Oka, S. Sato, and T. Nakahara. 1981. Properties of gizzard erosion-inducing substance in fish meal. *National Institute of Health Quarterly (Japan)* 21:52-60.
- Watanabe, T., T. Takeuchi, S. Satoh, K. Toyama, and M. Okuzumi. 1987. Effect of dietary histadine or histamine on growth and development of stomach erosion in rainbow trout. *Nippon Suisan Gakkaishi* 53(7):1207-1214.
- Watts, D.A., and W.D. Brown. 1982. Histamine formation in abusively stored Pacific mackerel: effect of CO₂-modified atmosphere. *Journal of Food Science* 47:1386-1387.
- Wessels, J.P.H., and B.J. Post. 1989. Effect of heat treatment of fish meals, fines and the addition of lysine as related to gizzard erosion in chickens. *Journal of Science, Food, and Agriculture* 46:393-406.

- Wetterqvist, H. 1978. Histamine metabolism and excretion. Handbook of Experimental Pharmacology. Volume 18 (Part 2). Roche e Silva, M., editor. Springer-Verlag, New York.
- Wilson, R.P., and J.E. Halver. 1986. Protein and amino acid requirements of fishes. Annual Reviews of Nutrition 6:225-244.

APPENDIX 1

Water temperature profiles ($^{\circ}\text{C}$) observed during the experiments. Figure codes indicate the respective chapters: Chapter 3, (a); Chapter 4, (b); Chapter 5, (c); Chapter 6, (d); Chapter 7, (e); Chapter 8, (f).



APPENDIX 2

Methods for Proximate Analysis of Feed Ingredients, Diets, and Fish.

1.0 MOISTURE

1.1 Apparatus

Model 18 convection oven, (Precision Scientific Group, 37373 Cortland Street, Chicago, Illinois 60647).

1.2 Procedure

Collection and processing of feed ingredients, diets, and samples of experimental fish is detailed in the text of each chapter. Sample size varied from about 2 g (feed ingredients and diets) to about 100 g (experimental fish). Moisture content was determined without replication: all subsequent analysis was conducted using the dried samples.

Samples were dried to constant weight at 105°C, placed into desiccators, and cooled to room temperature under vacuum before weighing. Drying time was 6 h for feed ingredients and diets and 16 h for fish samples. Dried samples were ground to a flour-like consistency with a mortar and pestle, placed into individual airtight plastic containers (Catalog #4014, Falcon Plastics, 1950 Williams Drive, Concord, California 93030) and stored at -20°C until analyzed for protein, lipid, and ash content.

2.0 PROTEIN

2.1 Apparatus

Sample digestion was conducted using a Buchi 430 digestion unit (Brinkman Instruments Inc., Westbury, New

York). Steam distillation of the digested sample was conducted using a Buchi 322/343 distillation/control unit. Distillate was titrated using a Metrohm 655 dosimat digital titrator.

2.2. Procedure

2.2.1. Digestion

- a. Sample weight: 0.2500-0.5000 g, dry weight basis.
- b. Two determination of each sample were made.
- c. Reagents: 0.5 g CuSO_4 , 5.0 g Na_2SO_4 , 10.0 ml concentrated H_2SO_4 .
- d. Digestion time: Samples were heated on the highest setting until the reaction mixture became clear (about 1 h). Heating was continued an additional 20 min beyond this point, then the sample was removed from the heat and allowed to cool for 20-30 min before steam distillation.

2.2.2. Distillation and titration

Distillation and titration of the samples was fully automated. Specific assay conditions were as follows:

- a. Before distillation, sample was diluted with 50 ml distilled H_2O , followed by the addition of 60 ml of 25% (weight:volume) NaOH solution. Reaction time was 10 sec.
- b. Condensed distillate was collected in a beaker containing 30 ml of a 2% (weight: volume) boric acid solution, pH 4.25. Distillation was continuous, and 200 sec were allowed to elapse before titration was initiated.
- c. Distillate was titrated with 0.1N HCl to the original pH (4.25). Endpoint was accepted when the pH of the distillate was stable for 10 sec. The pH probe was calibrated at pH 4.0 and pH 7.0 daily. Assays were conducted at room temperature (24°C).

d. Two reagent blanks were analyzed at the beginning of each day. Blank values ranging from 0.1-0.2 ml were expected.

c. Data were reported as crude protein (% dry weight) = %N · 6.25.

3.0 LIPID

3.1 Apparatus

Determination of lipid in samples analyzed in Chapter 3 was conducted using a Labconco Goldfish Extraction unit Catalog number 35001 (Labconco Corporation, Kansas City, Missouri 64132). All other samples were analyzed using a Buchi 810 soxhlet extraction apparatus.

3.2 Procedure

3.2.1 Goldfish extraction

- a. Sample weight: 0.4000-0.6000 g, dry weight basis.
- b. Two determination of each sample were made.
- c. Solvent: Petroleum ether, boiling point 35-60°C, reagent grade, 60 ml per sample.
- d. Mantle temperature was adjusted until ether was observed dripping from the condenser at a rate of about 1 drop/sec. Samples were extracted for 16 h.
- e. At the end of the extraction period, residual ether was evaporated by heating the receiving beaker in a convectionless oven to 105°C for 30 min. The samples were cooled to room temperature in desiccators before weighing.
- f. Data were reported as % crude lipid, on a dry weight basis.

3.2.2 Soxhlet extraction

- a. Sample weight: 1.000-2.000 g, dry weight basis.
- b. Two determination of each sample were made.

- c. Solvent: Methylene chloride, boiling point 39.6-40.5°C, reagent grade, 100 ml per sample.
- d. Samples were heated by steam, and were extracted for 3 h.
- e. At the end of the extraction period, residual solvent was evaporated by continued steam heating the receiving beaker for 30 min. The samples were cooled to room temperature in desiccators before weighing.
- f. Data were reported as % crude lipid, on a dry weight basis.

4.0 ASH

4.1 Apparatus

Samples were preashed by infrared heating (Infra-radiator model #11-504-5, Fisher Scientific, Pittsburg, Pennsylvania). Final ashing was conducted using a Temco model F1730 muffle furnace (Thermoelectric Manufacturing Corp, Dubuque, Iowa).

4.2 Procedure

- a. Sample weight: 1.0000-1.5000 g, dry weight basis.
- b. Two determination of each sample were made.
- c. Samples were weighed into numbered, tared 20 ml borosilicate glass liquid scintillation vials (Wheaton #986568, Wheaton Scientific, Millville, New Jersey), pre-ashed for 6 h, then ashed in the muffle furnace at 550°C for 16 h. The ashed samples were allowed to cool to 150°C in the furnace, then were placed into desiccators where they were allowed to cool to room temperature before weighing.
- d. Data were reported as % ash, on a dry weight basis.

APPENDIX 3

Summary of Survival Data (% of initial number \pm SD) for
Rainbow Trout Fed the Various Experimental Diets.

Chapter 3: Preliminary studies on the palatability, nutritional value,
and toxicity of Chilean fish meals to rainbow trout.

Diet		
LT-1-N	C-1-T	C-1-N
100	100	100

Chapter 4: Effects of histamine on the development of digestive
tract abnormalities in rainbow trout.

Diet								
CG	CGH	CGHPC	LT-1-N	LT-2-N(H)	LT-3-N(HPC)	C-3-N	C-4-T	C-5-T(HPC)
98.3	98.3	96.7	100	98.3	98.3	95.0	96.7	100

Chapter 5: Comparison of toxic and nontoxic fish meals as protein
sources in the diets of rainbow trout.

Feeding trial	Diet				
	Casein	C-6-T	C-7-N	C-8-T	C-9-N
Nutritive value	98.7 (2.3)	97.3 (2.3)	97.3 (4.6)	97.3 (4.6)	93.3 (4.6)
Palatability	98.7 (2.3)	98.7 (2.3)	100	100	100

Chapter 6: Evaluation of feed intake, growth rate, and protein utilization by interstrain hybrids of rainbow trout.

Treatment	
UW x UW	UW x Nisqually
97.3 (2.3)	90.7 (6.1)

Chapter 7: Effects of protein intake on nutrient partitioning in rainbow trout.

Protein source	Rate of protein intake (mg/kg/d)						
	Not fed	1.3	3.0	6.0	11.0	16.0	Satiation
Not heated	100	100	100	100	100	100	100
Heated	100	100	97.5 (3.5)	100	100	97.5 (3.5)	100

Chapter 8: Effects of dietary histamine level and heating on the nutritive value of fishmeal and casein to rainbow trout.

Diet (pair-fed groups)

	CG0	CG500	CG1000	CG1500	CG2000
Heat	95.0 (7.1)	97.5 (3.5)	100	100	97.5 (3.5)
No heat	97.5 (3.5)	95.0 (0)	100	100	85.0 (14.1)

Diet (pair-fed groups)

	LT0	LT500	LT1000	LT1500	LT2000
Heat	97.5 (3.5)	97.5 (3.5)	92.5 (3.5)	97.5 (3.5)	95.0 (0)
No heat	87.5 (17.7)	100	95.0 (7.1)	92.5 (3.5)	100

Diet (satiation-fed groups)

	LT0	LT2000
Heat	92.5 (3.5)	95.0 (0)
No heat	95.0 (7.1)	100

Vita

William Thomas Fairgrieve, Jr.
2552 Alaska Avenue East
Port Orchard, Washington, 98366

Education

- 1992 Doctor of Philosophy. Fisheries / Food Science;
University of Washington, Seattle, Washington.
- 1983 Master of Science. Fisheries Resources / Aquaculture;
University of Idaho, Moscow, Idaho.
- 1974 Bachelor of Science. Fisheries; Humboldt State
University, Arcata, California.
- 1972 Associate of Arts. Fisheries and Game Management;
Fullerton College, Fullerton, California.

Experience

- 1986-1987 Fish Nutritionist. Domsea Farms, Inc., Bremerton,
Washington.
- 1983-1985 Fish Nutritionist. Oregon Department of Wildlife,
Clackamas, Oregon.
- 1980 Research Assistant. Douglas County Public Utilities
Department, East Wenatchee, Washington.
- 1975-1979 Aquaculture extension agent. United States Peace
Corps, Bureau of Fisheries and Aquatic Resources,
Banaue, Ifugao, Republic of the Philippines.
- 1975 Reseach Assistant. California Department of Fish and
Game, Monterey, California.

Publications

- Dong, F.M., W.T. Fairgrieve, D.I. Skonberg, and B.A. Rasco. 1992. Preparation and nutrient analyses of lactic acid bacterial ensiled salmon viscera. *In press*.
- Hardy, R. W., W.T. Fairgrieve, and T. Scott. 1991. Periodic feeding of low-phosphorus diet and phosphorus retention in rainbow trout (*Oncorhynchus mykiss*). Proceedings: IV International Symposium on Fish Nutrition. Biarritz, France, June 24-27, 1991. *In press*.
- Hardy, R. W., T. Masumoto, W.T. Fairgrieve, and R.R. Stickney. 1989. The effects of dietary lipid source on muscle and egg fatty acid composition and reproductive performance of coho salmon (*Oncorhynchus kisutch*). Proceedings of the Third International Symposium on Feeding and Nutrition in Fish. Aug. 28-Sept 1, 1989. Toba, Japan, pp. 347-355.

Personal

Born March 15, 1952 in Glendale, California, to William T. and Muriel A. Fairgrieve. Married to Rhonda J. Schmitz (1980); two children, Catherine Elizabeth and Marian Johanna Ruth.

Professional affiliation

Member of the American Fisheries Society (1972) and the World Aquaculture Society (1991).