THE USE OF SELECTED BIOLOGICAL MATERIALS PRODUCED BY TERTIARY WASTEWATER TREATMENT PONDS IN THE DIET OF TWO SPECIES OF FISH

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ABSTRACT

THE USE OF SELECTED BIOLOGICAL MATERIALS PRODUCED
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Fragments of aquatic plants are routinely found in the stomachs of fish not normally considered herbivores. Consequently, a series of laboratory feeding trials were conducted at Michigan State University using a filamentous algae, *Oedogonium*, in the diet of steelhead trout (*Salmo gairdneri*), and varying levels of a 50:50 mixture of fresh poultry waste and *Elodea canadensis* in the rations of Israeli carp (*Cyprinus carpio*). A preliminary feeding trial demonstrated growth of steelhead fed a diet containing 34.5% *Oedogonium* was comparable to the growth of control fish on a nutritionally complete commercial trout ration. *Oedogonium*, when coarsely chopped and combined with other ingredients, formed a floating pellet readily consumed.

Poultry waste and *E. canadensis* were fed to Israeli carp in order to evaluate the inclusion of non-protein-nitrogen (NPN) in the diet of a stomachless fish. Growth rate and feed conversion were found to be inversely related to the amount of poultry waste in the regimen.

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This thesis is dedicated to all the people who have had faith in me: my Mother and Father, my senior advisor, and above all, my lady.

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TABLE OF CONTENTS

Page

LIST OF TABLES vi

INTRODUCTION 1

Cultural Eutrophication 1
Resource Loss 2
Michigan State University Water Quality Management Project 3

PART I

FILAMENTOUS ALGAE (<u>Oedogonium</u>) IN THE DIET OF A SALMONID . 6

METHODS 8

Diet Formulation 8
Diet Production 10
Feeding 12
Experimental Animals 12
Environmental Conditions 13
Data Collection and Analysis 13

RESULTS AND DISCUSSION 15

Floating Feed 17
Submerged Aquatic Plants 17
Pigments 18

PART II

POULTRY WASTES IN THE DIET OF ISRAELI CARP 20

MATERIALS AND METHODS 22

Diet Formulation 22
Diet Production 24
Feeding 29
Experimental Animals 29
Environmental Conditions 30
Data Collection and Anaysis 31

	Page
RESULTS AND DISCUSSION	32
CONCLUSION	38
APPENDIX	39
BIBLIOGRAPHY	41

LIST OF TABLES

Table | Page

1. Proximate Analyses of Daphnia magna in % Dry Weight, Average of Two Values 9
2. Amino Acid Content of Oedogonium as % Dry Weight . . 9
3. Formulas of Test Diets 11
4. Summary of Results of Steelhead Feeding Trial #2 . . 16
5. Composition of Carp Diets as % Dry Weight 23
6. Composition of Poultry Waste Expressed as % Dry Weight 25
7. Amino Acid Content of Dehydrated Poultry Wastes . . 26
8. Chemical Composition of Elodea canadensis, as % Dry Weight, Average of Two Tests 27
9. Summary of Results of Israeli Carp Fed Varying Amounts of Poultry Waste and Elodea canadensis 33
10. Summary of Statistical Analyses 34
11. Final Proximal Analysis of Israeli Carp as % Dry Weight (n-9); Vicerated, Nubbed 35

INTRODUCTION

Before a large quantity of domestic wastewater is discharged into a receiving water that is meso- or eutrophic, tertiary wastewater treatment is desirable. Nutrient removal in the main reverses cultural eutrophication and lessens the loss of an agriculturally quite significant non-renewal natural resource, phosphorus.

Cultural Eutrophication

Relatively few lakes in North America have been studied in great detail before and after modification by artificial enrichment (20, p. 129). None the less, it may be said that during the first centenary of the United States most surface waters acted as nutrient sinks, that is, were able to ecologically process any increases in nutrient loads without significant changes in species diversity. The lakes were in trophic equilibrium, and most of their hypolimnions remained aerobic.

With the concomitant demographic transition and rapid human and domestic animal population growth of the last of the 18th and early 19th centuries, certain lacustrine systems became culturally eutrophic. Their finite capacity to sequester and cycle nutrients had been exceeded; they became transport systems instead of sinks. Anoxic conditions oftentimes develop in the hypolimnion of a eutrophic lake; all economically significant fish native to North America require relatively high dissolved oxygen levels. The fish must move to the shallower, often warmer, water. Consequently, there can occur a shift in species composition to the warm water tolerant organisms; commercially important fish species decline. Nuisance growths of aquatic plants occur; rapid geological aging occurs (58, pp. 330-332).

During the second centenary domestic water use increased at an exponential rate (r^2 = 0.991, calculated from data from Metcalf & Eddy). By the end of the second centenary, practically every major water body, including most estuaries, in the United States had shown signs of nutrient overloading. The oceans have even been adversely effected. The area off New York City, the "Dead Sea" is quite definitely no longer a sink. That Southern California coastal waters have lost much of their bull kelp (Laminaria) beds and that Florida waters have periodic blooms of often toxic dinoflagellates (Gonyaulax polyedra) should not go unnoticed.

While precise data on the amount of phosphorus discharged are not available for all cities, an estimate may be made (Appendix). If all the phosphorus in domestic wastewater were to be absorbed by aquatic plants assuming a density of 500 g/m^2, some 19.65 x 10^6 ha or 98.26 x 10^6 mt (dry weight) of aquatic plants would be produced yearly (Appendix).

Resource Loss

Cultural eutrophication is a rather salient effect of discharging too much phosphorus; however, the most significant aspect of the practice may well be the loss, for the geological present, of this most important resource. Phosphorus and potassium unlike the other plant macro-nutrients, carbon, nitrogen, and water, do not recycle back into the atmosphere. Cathcart and Gulbrandsen (18) say of United States phosphate deposits:

> These reserves can supply domestic demands for scores or hundreds of years, but political, economic, and ecological demands may radically alter the traditional supply patterns in the U.S., and at sometime in the future the supply of phosphate rock to the eastern seaboard may possibly come from foreign sources.

A rather innocuous statement until one considers that 83% of the United States production comes from Florida and North Carolina and so would have to be imported, and that the United States has the world's largest supply of identified reserves (18). The U.S.S.R. and North Africa have most of the remaining world's mineable reserves.

Michigan State University Water Quality Management Project

The Michigan State University Water Quality Management Project is a research facility that is designed to receive two million gallons per day of secondarily treated domestic wastewater into a series of four ponds. The ponds have an aggregate area of 40 acres and are located on a 320-acre site on the Michigan State University campus. The project's main goal is to evaluate and demonstrate the potential for tertiary treatment of domestic wastewater by biologically concentrating the nutrients into useful products within aquatic and terrestrial ecosystems. It is felt that the use of biological materials produced by tertiary wastewater treatment ponds in animal rations can reduce cultural eutrophication of aquatic environments, increase efficiency of resource utilization, and augment protein supplies.

Three items were used in two separate laboratory-scale biological feeding trials: the zooplankter, Daphnia magna harvested from pond 1; a common filimentous green algae, Oedogonium from pond 3; and a submerged aquatic vascular plant, Elodea canadensis, drawn from pond 4. Section I of this work presents the results of the first feeding trial performed, a 12-week test with steelhead trout (Salmo gairdneri) fed a mixture of Oedogonium, Daphnia, soybean meal, and fish meal. Section II describes a feed assessment trial using Israeli carp (Cyprinus carpio) fed varying levels of a 50:50 mixture of poultry waste and Elodea.

PART I

FILAMENTOUS ALGAE (*Oedogonium*) IN THE DIET OF A SALMONID

The use of aquatic plants for feedstuffs has lagged behind that of terrestrial plants. Tracts of aquatic vascular plants and filamentous algae can grow so dense that other beneficial uses of the water body are interfered with (8). Supply is not the problem.

Aquatic plants sequester and biologically complex large amounts of nutrients. Culley and Epps (19) estimate that a duckweed, *Spirodela oligorrhiza*, can uptake 185 kg of nitrogen/month/ha (165 lb/ month/acre) and 60 kg of phosphorus/month/ha (53 lbs/month/acre). Boyd (10) calculates that water hyacinth (*Eichhornia crassipes*) can remove 1980 kg of nitrogen/year/ha and 322 kg of phosphorus/ year/ha.

Several researchers have been actively engaged in seeking uses for aquatic plants (8,9,19,30,31,38,41,56). There is a limited use of water weeds in animal rations (11,31,38).

Many species of fish consume aquatic plants; fragments of aquatic plants are found in the gut of fish normally not considered herbivorous, such as trout (*Salmo gairdneri*), bluegills (*Lepomis macrochirus*), and channel catfish (*Ictaluris punctatus*) (28,35). Other fish, especially as adults, reported to be almost exclusively plant feeders, e.g., grass carp (*Ctenopharyngodon idella*), can control a variety of aquatic weeds (2). Many fish species including *Cyprinus carpio*, *Tilapia heudeloti*, *T. mossambica*, *T. nilotica*, and *T. zilli* consume specific plant species (2,33).

A few feeding trials have been conducted using aquatic plants in the diet of fish. Liang and Lovell (30) using channel catfish conclude:

> The most feasible nutritional contribution of water hyacinth appears to be when the plant meal is fed as a low percentage in vitamin-poor diets as a source of growth factors.

When fed to fingerlings, water willow (*Justicia americana*) was found to have good palatability, water hyacinth (*Eichlornia crassipes*) fair to good, and alligatorweed (*Alternanthera philoxeroides*) poor acceptability. Kitchell and Windell (28) found that bluegills do gain some nutritional value from *Chara* but cannot obtain sufficient dietary calories from the algae alone.

The purpose of this section of the thesis is to present the results of a preliminary laboratory biological feeding trial conducted at the Fisheries Research facility of Michigan State University from August, 1975, through February, 1976. Two sequential experiments were conducted in order to evaluate the inclusion of high percentages of *Oedogonium* in the diet of steelhead trout (*Salmo gairdneri*).

METHODS

Diet Formulation

Two diets meeting the essential amino acid and protein requirements (40) of chinook salmon (Oncorhynchus tschawytscha) were separately computer formulated. Formulation was performed using Michigan State University's CDC 6500 computer; the software employed was the Agriculture Economics Linear Program Package, Version 2 (27). The control diet was Purina Trout Chow, an extruded, nutritionally complete ration containing 40% crude protein (cp).

Ringrose (48) working with brook trout (Salvelinus fontinalis) has found that a calorie/protein ratio of 75 gives approximately optimum growth and feed conversion efficiency. Therefore, metabolizable energy was forced into the formulation at 2976 kcal/kg (1350 kcal/lb). The essential amino acid and metabolizable energy content of the diet ingredients were calculated using National Academy of Science (NAS) values (39,40). A proximate analysis was run on the Daphnia (Table 1); an amino acid analysis was performed on the Oedogonium (Table 2). The metabolizable energy values were estimated using 8.0 kcal/g of lipid, 3.9 kcal/g of protein, and 1.6 kcal/g of starch again as per NAS (40); a value of 888 kcal/kg was used for Oedogonium and 1024 kcal/kg for Daphnia. Amino acid values for Daphnia were taken from the literature (44).

TABLE 1.--Proximate Analyses of Daphnia magna in % Dry Weight, Average of Two Values.

	Sample Number		
	1	2	3
Crude protein	42.8	53.7	53.7
Ether Extract	3.46	6.00	6.29
Ash	40.82	23.00	23.01
Cell Walls	15.84	N.A.	N.A.

TABLE 2.--Amino Acid Content of Oedogonium as % Dry Weight.

Amino Acid	Value
Thr	1.582
Ser	1.538
Glu	3.786
Gly	1.703
Qla	1.895
Val	1.103
Cys	---
Met	1.892
Iso	0.975
Leu	2.173
Tyr	0.668
Phe	1.386
Lys	2.218
His	0.478
Arg	1.381
Sum of Amino Acids	22.778

The possible ingredients were Oedogonium, Daphnia magna, blood meal, fish meal, soybean meal, dried whey, brewer's yeast, fish and corn oil, and methionine; kelp meal, salt w/trace minerals, and vitamins were forced in (Table 3).

Diet Production

The Oedogonium was harvested during August from pond 3 of the Michigan State University Water Quality Management Project (W.Q.M.P.). a wastewater recycling research facility. The plants were hand-wrung and forced-air dried at 51 C (124 F) for 12 hours. A W-W 5-hp compost grinder was used to coarsely chop the algae before freezer storage. Before inclusion into diet 1, the plants were finely ground using a Clifford laboratory cutting mill with a screen equivalent to a 20-mesh Tyler (0.833 mm, 0.0328 inch). The Oedogonium used in diet 2 was not finely ground.

The Daphnia were seined from pond 1 of the W.Q.M.P. during July and August, 1976. The zooplankton used in diet 1 were air dried to a moisture content of approximately 50% and then frozen; the D. magna used in diet 2 were quick frozen on dry ice in 1-liter plastic bags.

The dry dietary components were mixed in a large bag by hand tumbling beginning with the ingredient smallest in mass and proceeding sequentially. After mixing they were finely ground in the laboratory mill. The oil was added just before pelletizing.

Final mixing and pelleting was accomplished by using a 1.5-hp Hobart food grinder. Sufficient water was added to bring the mixture

TABLE 3.--Formulas of Test Diets.

Ingredient	% Dry Weight
Diet 1, 10/11/75-11/7/75	
Oedogonium	29.2
Daphnia	44.2
Corn Oil	10.7
Blood Meal	1.0
Soy Bean Meal (49% cp)	11.4
Salt w/trace Minerals[a]	1.5
Kelp Meal	0.5
Vitamins[b]	1.5
	100.0
Diet 2, 11/25/75-2/17/76	
Oedogonium	34.50
Daphnia	8.97
Fish Meal (Menhaden)	46.78
Fish Oil (Cod Liver)	5.00
Kelp Meal	2.00
Salt w/trace Minerals[a]	1.25
Vitamins[b]	1.50
	100.00

[a]Trace minerals supplied per kg of diet: Manganese Sulfate-55.0 mg; Magnesium Oxide-500.0 mg; Iron-80.0 mg; Copper-4.0 mg; Zinc-80.0 mg; Selenium-0.1 mg; MSU Poultry Mineral Mix #2.

[b]Vitamin content supplied per kg of diet: A-33,33 IU; D_3-3.333 IU; E-33 IU; MSBC-6.6 mg; Thiamine-10.0 mg; Riboflavin-33.0 mg; Pantothenic acid-50.0 mg; niacin-333 mg; Pyridxin-20 mg; Biotin-500 mcg; Folacin 10 mg; B_{12}=33 mcg; MSU chick starter #2.

to a moisture content of 45%; the feed was passed through the grinder four times. Pellet diameter was 8 mm (3/16"); all longer-than-average pellets were hand broken. Diet 1 was forced air dried at 51 C (124 F) for 12 hours to a moisture content of approximately 7%; diet 2, which was prepared at a later date was air dried for 10 hours to a moisture level of 33.63%. The pellets were stored frozen under a nitrogen atmosphere in glass containers.

Feeding

Brett (13) has found with sockeye salmon (*Oncorhynchus nerka*) the greatest increase in appetite occurs between 7 and 11 hours of fasting. Therefore, the fish were fed all they would consume once every 8 hours. The fish were fed on a 7-day per week basis. A modified version of the electric clock-based automatic feeder described by McCauley (34) was used; control was by a 2E026 Dayton timer.

Experimental Animals

The test fish were selected for uniform size from fish hatched on the premises. The eggs were supplied by the Michigan Department of Natural Resources' Platte River Hatchery, Honor, Michigan. The steelhead were assigned growth tanks using a random number generator.

Three replications of 15 to 17 fish were used for the two treatments. At the conclusion of the first test all fish were randomly reassigned. The main phase of the two tests were of 4 and 10 weeks duration, respectively.

During the 2-week acclimation period preceeding the tests, the fish were fed at 1.8% of body weight. At the beginning of the second acclimation period, the second Oedogonium diet was mixed with the control diet in 1:7, 1:3; and 1:1 ratios; each mixture, starting with the former, was fed the test fish for three days. Thereafter, the Oedogonium-based test diet was fed.

Environmental Conditions

The fish were kept in 150-liter oval, fiberglass tanks covered with screens. The center stand pipe was adjusted to allow a volume of 120 liters. The fish density was very low, 7.413 kg/m^3 maximum. At the start of the first test, cleaning was performed daily, but was found to unduly stress the fish. So the frequency was reduced to biweekly. During the second test, weekly cleaning was deemed sufficient. All tanks were, however, scrubbed during the biweekly weighing.

The filtered well water had a temperature of 12.6 $\pm$ 0.5 C (54.5 $\pm$ 0.9 F). In earlier tests, the water hardness as $CaCO_3$ was found to be 329 mg/liter, alkalinity as $CaCO_3$ - 332 mg/liter, and the pH ranged from 7.0 to 7.6. The water flow rate was 2 liters/min. Dissolved oxygen ranged from 6.1 to 6.7 mg/liter.

The fish were kept under constant artificial light.

Data Collection and Analysis

At the beginning and end of the experiments the fish were anesthetized with a 25-ppm MS-222 solution and individually displacement weighed to the nearest one-tenth of a gram on a 1200-g Mettler balance. During the experiments the fish were displacement weighed biweekly to the nearest gram by lot on a 10-kg Mettler balance.

The plant materials were washed with distilled water before analysis; an endeavor was made to remove all animal biomass present, although some of the smaller organisms undoubtedly did remain. A Virtis freeze dryer was used to desiccate materials to be analyzed. Proximate and amino acid analyses were conducted using A.O.A.C. standard procedures by the Botany and Aminal Science departments of Michigan State University.

Statistical analysis of the test results consisted of one-way analysis of variance.

RESULTS AND DISCUSSION

The first ration tested was very poorly accepted by the steelhead. The pellets were dense and rapidly sank; the fish consumed very little food. The pellets that were eaten appeared to pass through the fish's digestive tract almost intact. Trout typically do not masticate their prey before swallowing (51) although some trituration does occur in the gut after exposure to strong gastric fluids.

Little growth resulted from the test diet, while the control fish grew satisfactorily. Consequently, after 4 weeks the trial was ceased. An excessively fine grind of the *Oedogonium* was thought to be the principal cause of the problem. A test for trimethylamine performed by the Food Science Department revealed little oxidative rancidity of the lipid fraction of the ration.

Coarsely chopped *Oedogonium* was used in the second diet. The pellets floated well and were readily consumed by the fish. The growth rate of the test fish slightly exceeded that of the control fish (Table 4), but the difference was not statistically significant, $P < 0.05$ level. The test diet was a preliminary formulation; the development of a practical, that is, low in fish meal, production ration was beyond the scope of this experiment.

TABLE 4.--Summary of Results of Steelhead Feeding Trial #2.

Item	Units	Test Diet	Control Diet
Average Initial Weight	grams	20.4	19.2
Standard Deviation		8.42	9.37
Skew		0.76	0.91
Average Final Weight	grams	55.6	51.6
Standard Deviation		25.28	22.92
Skew		0.75	0.60
Total Food Fed	grams	3,055.4	3,050.4
Total Weight Gain	grams	1,387.3	1,281.9
Feed Conversion		2.20	2.38
Weight Gain	%	273	269
Weight Gain as a % of Control	%	101.5	100.0
Daily Growth Increment	%	1.20	1.18
Mortalities		0	0
Cost of Feed per kg	$	0.241 (Estimated)	0.430[a]
per (lb)	$	(0.109)	(0.195)
Cost of Feed per kg of Weight Gain	$	(0.443)	(0.809)
per (lb)	$	(0.201)	(0.367)

[a]June, 1976 prices.

Floating Feed

The use of a floating pellet in a salmonid regimen is highly desirable. Artificial foods are not readily consumed off the bottom; trout feed almost exclusively in the water column and at the air-water interface. A 30-minute test of a grab sample of 45 pellets was conducted. After 1 minute, 39 (87%) remained afloat; at the end of the test, 21 (47%) were still buoyant.

Extrusion cooking is the main process employed to produce commercial floating pellets. Unfortunately, the extrusion process is very capital and energy intensive (50); floating rations consequently cost more. Furthermore, extruded foods must include a high starch base; starch, even after being subjected to high temperatures, has been demonstrated to be incompletely utilized by salmonids (40), at 1.4 kcal/g vice 4.0 kcal/g for mammals.

The most highly biologically ordered, and expensive, component of a trout ration is protein. High temperatures, > 80 C, can result in significant protein loss because of the Maillard reaction (non-enzymatic browning), unless the feedstuff has a moisture content of less than 10% and is rapidly cooled (26). Extrusion cooking may involve temperatures of "150-175°C (300-350°F)" according to Sanderude (50).

Submerged Aquatic Plants

Dense and diverse animal communities are often found in and among submerged aquatic plants (16,28,43,45). Protozoa, rotifers, annelids, crustaceans, insects, and molluscs have been reported to make up 17.5% of the dry weight of submerged aquatic plants (45). The production lot of Oedogonium used contained large numbers of Cladocera, Coleoptera and Hyalella; the sample tested which was well-rinsed had a sum of amino acids of 22.78% (Table 2). Boyd and Scarsbrook (12) report crude protein values of from 4.5% to 31.3% and phosphorus concentrations of 0.05% to 0.56% on a dry weight basis in filamentous algae. The fauna associated with certain aquatic plants may make a further contribution to the plant's amino acid profile.

Pigments

Aquatic plants contain relatively large amounts of photoreactive carotenoid pigments. Nelson and Palmer (41) reported that Vallisneria spiralis had a carotenoid content of 100mg/100g. The red crab (Pleuronodes planipes), a species noted for its carotenoid content, has from 56.9% μg/100g to 85.7 μg/100g (53). Visual examination of the flesh of fish sacrificed at the termination of the experiment showed no difference between dietary treatments; the muscle tissue of either lot of fish was not highly pigmented. The largest fish weighed about 125g; a larger size or greater age may have to be reached before flesh pigmentation occurs. If the carotenoids in aquatic plants are biochemically available to fish, a desirable flesh pigmentation may occur.

PART II

POULTRY WASTES IN THE DIET OF ISRAELI CARP

Calvert (17) reports that in the United States, 28.8 million kg (63.5 million lbs.) of nitrogen is excreted by animals as waste each day. Substantial interest has been shown in converting the presently underutilized non-protein-nitrogen (NPN) in animal wastes into animal biomass (5,6,17,22,23,25,32,42,47,52,55).

NPN in the form of animal wastes has been demonstrated to be sparing of protein-nitrogen in the diet of ruminant animals (5,42,55). The mineralized nitrogen is incorporated biologically into microbial biomass in the rumen. However, with a non-ruminant animal, broiler-chicks, Flegal and Zindel (23) report "feed efficiency was inversely related to the level of DPW (Dried Poultry Wastes) in the diet."

The inclusion of poultry wastes and other forms of NPN into the diet of fish has produced ambiguous results. Shiloh et al. (52) found the inclusion of DPW at a 10% and 20% level was deleterious to the growth of carp (*Cyprinus carpio*); but the addition of 4% and 5% soybean oil resulted in an improvement in growth of 3.2% and 3.5% respectively over that of the control diet. Leray (29) states somewhat cryptically that with mullet (Mugilidae), "one-half of the protein-nitrogen could be replaced by urea-nitrogen without modifying the growth curve." Lu and Kevern (32) found the growth of goldfish (*Carassius auratus*) fed a 30% DPW ration was superior to that of the control group fed Ewos F49, a salmon feed. However, with channel catfish (Ictalurus punctatus) growth of the control fish, again Ewos-fed, was significantly better than fish fed 30:70, 70:30, and 100:0 ratios of DPW:Ewos ($P < 0.05$). Fish fed 100% DPW showed little or no growth. Fowler reports that channel catfish fed a ration of 25% DPW grew better than the control group (25).

The purpose of this section of the thesis is to present the results of a laboratory-scale biological feeding trial conducted at the Fisheries Research facility of Michigan State University from January through April, 1976. The experiment was conducted to evaluate the inclusion of NPN in the form of poultry waste in the diet of Israeli carp.

MATERIALS AND METHODS

Diet Formulation

Four diets equal in metabolizable energy and essential amino acids were formulated on the MSU CDC 6500 computer. The software employed was the Agriculture Economics Linear Program Package, Version 2 (27). The control diet selected was a production ration extensively used in Israel (52,57). Three diets utilizing varying levels of a 50:50 mixture of fresh poultry waste and *Elodea canadensis*, a source of fiber, along with other ingredients were tested (Table 5). The essential amino acid and metabolizable energy content of the control diet was calculated using National Academy of Science values (39,40). Proximate and amino acid analyses were run on the *Elodea*. Metabolizable energy value for *Elodea* was estimated using 8.0 kcal/g of lipid and 3.9 kcal/g of protein (40). A value of 4.0 kcal/g of carbohydrate, the physiolgical fuel value for mammals, was used instead of the value commonly used for salmonids and ictalurids, 1.6 kcal/g (40). This substitution was felt justified because of the carp's high polysaccharide digestion coefficient (7). Poultry waste amino acid and energy values came from the literature (22,47).

When formulating diets using ingredients of low nutrient concentration, it is often difficult to get enough of the ingredients into the ration. Poultry waste and the particular batch of *Elodea*

TABLE 5.--Composition of Carp Diets as % Dry Weight.

Item	Diet Number 1	2	3	4 (Control)
FORMULA				
Elodea canadensis	12.50	20.00	27.23	
Poultry Wastes	12.50	20.00	27.23	
Wheat	42.28	20.77		70.00
Soybean meal (49% cp)	38.47	41.06	43.55	15.00
Fish meal (menhaden)				15.00
Fish oil (cod liver)[a]	3.18	7.15	10.98	
Vit.[b,c]	1.0	1.0	1.0	
Methionine	0.07	0.03		
Feeding rate as % of control	109.00	110.01	109.99	100.00
PROXIMATE ANALYSIS				
Crude protein	30.80	33.75	35.18	28.04
Cell walls	27.52	26.52	24.06	27.12
Ether extract	1.85	5.78	9.13	2.42
Ash	9.88	13.44	16.98	5.94

[a]Stabilized with 200 ppm Lenox 26 (F.D.A. Standard for human consumption).

[b]MSU chick starter vit. mix #2.

[c]Vitamin Content: A-33,333 IU; D_3-3,333 ICU; E-33 IU; MSBC-6.6 mg; Thiamine-10.0 mg; Riboflavin 33.0 mg; Pantothenic acid-50.0 mg; naicin-333 mg; Pyridxin-20 mg; Biotin-500 mcg; Folacin 10 mg; B_{12}-33 mcg per kg of diet; MSU chick starter vitamin mix #2.

used have a rather low food value (Tables 6, 7, and 8). Consequently, test diets 1, 2, and 3 were allowed to fluctuate in quantity up to 110% of the control (Table 5). When the quantity of the test diets to be fed was held to be equal to that of the control, the computer formulation always included fish meal. But at the 110% quantity level, no fish meal was needed. The latter option was chosen because it was felt that the inclusion of fish meal would obscure the experimental objective.

Diet 3 was computer formulated on a least-cost basis to be equal to the control diet in the ten essential amino acids and metabolizable energy. The possible ingredients were: a 50:50 mixture of poultry waste and Elodea, soybean meal, fish meal, wheat, fish oil, methionine, and vitamins. Vitamins were forced in at a 1% level, metabolizable energy was forced in at 2965 kcal/kg (1345 kcal/lb). Diets 1 and 2 were formulated as per diet 3 except lower levels of poultry waste and Elodea were forced in (Table 5).

Diet Production

The Elodea was harvested in July from pond 4 of the Michigan State University Water Quality Management Project, a wastewater recycling research facility. The plants were air dried for four hours to a moisture content of about 50% and then coarsely chopped using a W-W 5-hp compost grinder. Forced air drying at a temperature of 51 C (124 F) for 12 hours reduced the moisture content to 6.03%. The Elodea was stored in doubled 150-liter (40 gal) plastic trash bags. Before inclusion into the diets the plants were finely ground with a

TABLE 6.--Composition of Poultry Waste Expressed as % Dry Weight.

Item	Value	Remarks	Source
Crude Protein	33.92	Fresh n = 23	Benne (4)
Moisture	75.00	Fresh n = 29	Benne (4)
Total Nitrogen	6.73	CP = 42.06%	White et al. (59)
% of Total N	70.2 urinary	61.2% uric acid 9.0% NH_4 Salts	
	29.8 Fecal	Unutilized Protein from food	Flegal (24)
		Sloughed off gut	
		Microorganisms	
		Feathers	
P_2O_5	4.54		White et al. (59)
K_2O	2.03		
Ash	20.41		
Organic Matter	66.29		

TABLE 7.--Amino Acid Content of Dehydrated Poultry Wastes.[a]

Essential Amino Acids	gm/100 gm Crude Protein	% dry wt as Tested
Arg.	1.93	0.65
HIS	0.82	0.28
ISO	2.05	0.70
LEU	3.32	1.13
LYS	2.01	0.68
MET + CYS	4.88	1.65
PHE + TYR	2.91	0.99
THR	2.05	0.70
TRY	---	---
VAL	2.58	0.88
	22.55	7.67

[a]Flegal & Zindell (22).

TABLE 8.--Chemical Composition of *Elodea canadensis*, as % Dry Weight, Average of Two Tests.

Item	Amount
Proximate analysis	
Crude protein	9.9
Crude fiber	14.74
Ether extract	0.84
N-free Extract	48.66
Essential Amino Acids	
ARG	0.533
HIS	0.204
ISO	0.254
LEU	0.574
LYS	0.454
MET + CYS	0.081
PHE + TYR	0.718
THR	0.373
TRY	--
VAL	0.393

Clifford laboratory cutting mill with a screen equivalent to a 20-mesh Tyler (0.833 mm, 0.0328 in).

Benne (4) has demonstrated a marked nitrogen loss in poultry waste during the drying process. Therefore, fresh poultry waste, $\leq$ 24 hours old, was collected from the Michigan State University Poultry Barns from a production lot of Leghorn-type layers and was immediately frozen. Just before using, the frozen poultry waste was ground with a 6 mm (1/4") screen on the compost grinder. The Elodea and poultry waste were combined and thorough mixing was insured by passing the material through the compost grinder three times.

All other dry ingredients were mixed by tumbling the combined ingredients in a plastic container for 1 min. The two ingredients smallest in mass were mixed first; the next larger in mass was then added and tumbled. After mixing, all dry ingredients except the wheat were finely ground in the laboratory mill. The wheat could not be passed through the fine screen in the Clifford mill as the screen would quickly plug. Instead, the wheat was repeatedly passed through the next coarser screen until a fairly fine grind resulted.

Final mixing and pelleting was accomplished using a 1.5-hp Hobart food grinder. Sufficient distilled water was added to each diet to bring the moisture content to 45%. All diets were passed through the grinder three times. Shaking the fresh pellets in a plastic bag served to break most of the pellets. The diets were air dried at 18 C (64.6 F) for 12 hours to a moisture content of 9%.

Feeding

Cyprinids have a stomachless digestive system and therefore feed at shorter intervals than do many other fish. Rozin and Mayer (49) found that goldfish, when allowed free access to a self-feeder distributed their feeding responses fairly evenly over time. Therefore, the test fish were fed once every 2 hours on a 24-hour basis. A modified verion of the electric clock-based automatic feeder described by McCauley (34) was used; control was by a 2E026 Dayton timer.

The feeding rate was 4% of body weight per day for the control fish (7,52,57); diets 1, 2, and 3 were fed at 4.36%, 4.4%, and 4.4% body weight, respectively, which provided equal metabolizabel energy and essential amino acids. The fish were fed on a 7-day per week basis with pellets 5 mm (1/8") in diameter for the first two months and 8 mm (3/16") in diameter thereafter. Any longer than average pellets were hand broken before feeding.

Experimental Animals

The test fish were selected from a group of 350 full sibs that originated from Auburn University, Auburn, Alabama. The fish were maintained without mortality in 12.5 C filtered wellwater in three 150-liter fiberglass tanks until ready for use. A 45% crude protein (Purina No. 4) trout ration was fed at 1% of body weight. The fish increased from 11g to 18g mean weight during the 9 months they were held prior to the experiment.

For the test, the 240 closest to median sized fish were selected; all malformed individuals were excluded. The fish were assigned to growth tanks using a random number generator. Three replications of 20 fish each were used for each of the four diet treatments. Two acclimations periods were allowed. The first period was just after tank assignment and lasted four weeks during which water temperature was increased in daily increments of 1 C until the test temperature was reached. The fish were fed on the control ration at 2% of body weight daily. A further two-week acclimation period was allowed on the respective test diets again at 2% of body weight. The main phase of the test ran for ten weeks.

Environmental Conditions

The fish were kept in twelve 80-liter plastic barrels, round in cross section. The water volume was held at 20 liters (5.3 gal) by using a center stand pipe. The resulting fish density was considered medium by comparison with Israeli cage culture (52) at 75 kg/m^3.

In earlier tests, the water hardness as $CaCO_3$ was found to be 329 mg/liter, alkalinity as $CaCo_3$, 332 mg/liter, and the pH ranged from 7.0 to 7.6. Water temperature was controlled by a Powers 11A regulator @ 22.5 $\pm$ 0.5 C (72.5 $\pm$ 0.9 F). The water flow was 1 liter/ min. The influent water was directed at a slight angle from the vertical in order to power a slow, radial current. The tanks were self-cleaning. Because of the water current, physical action of the fish, and high water flows little aufwuchs developed, consequently no additional cleaning was performed.

Dissolved oxygen (D.O.) fluctuated over the course of the experiment. Winkler determinations taken during the first four weeks averaged 5.1 mg O_2/liter. At the beginning of the sixth week D.O. fell to less than 2 mg/liter in one of the control tanks; the average of the other four was 4.4 mg/liter. Air stones delivering an estimated 60 inches^3/min were immediately added to each tank and an aspirator was added to the facility's main water supply increasing D.O. from 64% saturation to 86% saturation. For the rest of the experiment, D.O. averaged 7 mg/liter.

The fish were kept under natural photoperiod during a time of increasing day length.

Data Collection and Analysis

At the beginning and end of the experiment the fish were anesthetized with a 25-ppm MS-222 solution and individually displacement weighed to the nearest one-hundredth of a gram on a 1200g Mettler balance. During the experiment the fish were displacement weighed biweekly to the nearest gram by lot on a 10-kg Mettler.

Proximate analysis of the fish at the conclusion of the experiment was obtained from a grab sample of three individuals netted from each tank. The trunk region, vicera removed, from each of the 36 fish was used in the analysis. Proximate and amino acid analyses were conducted using A.O.A.C. standard procedures by the Biochemistry, Botany, and Animal Science departments on the Michigan State University campus.

Statistical analysis consisted of one-way analysis of variance, Duncan's new multiple range, and linear regression tests (54).

RESULTS AND DISCUSSION

The growth rate of the test fish was inversely related to the amount of poultry waste in the diet (Table 9, $r^2 = 0.975$). The growth rate of the control fish was significantly different from the growth rate of the test fish ($P > 0.005$, Tables 9 and 10). Feed conversion efficiency was also inversely related to the level of poultry waste in the diet; the most expensive diet, the control, produced the most economical weight gain and protein production. The reduced weight gain of the fish fed the test diets indicates poor utilization of poultry waste; the amino acids found in the poultry waste were not efficiently incorporated into fish biomass.

Despite the diets being isocalorically formulated, the test fish had lower tissue lipid levels (Table 11). It would appear that the inclusion of NPN in the diet of Israeli carp has a high metabolic cost for the fish.

The tissue ash content of the test fish varied directly with the level of poultry waste in the diet ($r^2 = 0.962$). The amount of dietary mineral retained has been found to be proprotional to the quantity ingested (40). Dry matter varied inversely with level of poultry wastes fed ($r^2 = 0.947$).

Poultry waste contains high levels of calcium, phosphorus, potassium, and other minerals (Table 6); however, direct ingestion of

TABLE 9.--Summary of Results of Israeli Carp Fed Varying Amounts of Poultry Waste and *Elodea canadensis*.

Item	Units	Diet Number			
		1	2	3	4 Control
Average initial wt.	grams	22.95	23.61	22.28	21.35
Standard Deviation		5.35	4.72	4.74	3.97
Skew		0.55	0.19	0.41	0.72
Average final wt.	grams	56.26	54.20	45.25	75.09
Standard Deviation		23.16	22.42	15.48	26.37
Skew		1.29	0.92	2.36	0.84
Total feed fed	grams	5,888	5,853	5,409	6,425
Total weight gained	grams	1,998.6	1,835.4	1,378.2	3,224.4
Food conversion	g food/g wt gain	2.95	3.19	3.92	1.99
Weight gain	%	145.14	129.56	103.10	251.71
Weight gain	% of control	57.66	51.47	40.96	100.00
Daily Growth Increment	% initial wt	1.31	1.28	1.04	1.90
Mortalities		0	0	0	0
Cost of feed per kg[a]	dollars	0.143	0.136	0.130	0.160
lb	dollars	.065	.062	.059	.073
Cost per kg weight gain	dollars	0.4228	0.4354	0.5099	0.3183
lb	dollars	0.1918	0.1975	0.2313	0.1444
Cost per kg protein produced	dollars	2.20	2.45	2.90	1.91
lb	dollars	1.00	1.11	1.31	0.87

[a]Ingredient prices from (21).

TABLE 10.--Summary of Statistical Analyses.

One-Way Analysis of Variance

Within dietary treatments--No significant difference in growth rates between repetitions, $P < 0.10$.

Between dietary treatments--Significant difference in growth rates between treatments, $P > 0.005$.

Duncan's New Multiple Range Test

	Diet Number				
	3	2	1	4 (Control)	
Final Mean Weight	45.25	54.20	56.26	75.09	
		________	________		$P > 0.01$
	________	________			
		________	________		$P > 0.05$

Any two means underscored by the same line are not significantly different.

Growth Equations[a]

Diet 1	$Y = 21.41 + 3.36X$
Diet 2	$Y = 21.51 + 3.23X$
Diet 3	$Y = 21.43 + 2.45X$
Diet 4	$Y = 17.35 + 5.61X$

[a]Where Y = weight in grams
X = week of feeding trial

TABLE 11.--Final Proximal Analysis of Israeli Carp as % Dry Weight (n=9); Vicerated, Nubbed.

Item	Diet Number			
	1	2	3	4
Ash	8.59	9.69	11.86	5.47
Ether extract	30.44	18.86	16.43	38.64
Crude protein	71.56	73.13	75.69	56.56
Nitrogen-free extract	3.30	0.28	---	0.51
Dry matter (wet weight)	26.89	24.29	23.25	29.48

Ca,P, Co, and Cl is not necessary for fish. Fish absorb minerals directly from their environment (40) across their gill membranes.

Flegal and Zindel (22) found dehydrated poultry waste to contain an average of 26.1% crude protein. About 44.7% of the crude protein is protein-nitrogen consisting of unutilized ingested feed, microorganisms, sloughed-off gut, and feathers (24) as indicated in Table 6. The remaining nitrogen fraction is non-proteinaceous and consists of uric acid, urea, and ammonia salts. White et al. (59) found 70.2% of the nitrogen in poultry wastes to be urinary in origin.

In a ruminant, NPN is absorbed by mircoorganisms in the rumen and biologically complexed along with fiber, as an energy source, to form the essential amino acids required by the host animal. Digestion and absorption of the microbial protein occurs in the abomasum and intestine.

In order for fish to use NPN and fiber an analogous microbial process would have to occur. Salmonids and ictalurids do, at times, support high populations of intestinal microflora. However, bacteria are not usually found in the intestines of fasting fish (36). The presence of microorganisms depends upon the ingestion of microbially contaminated food and the presence of food as a substrate. The bacteria present are not regarded as commensal (36), although the presence of bacteria may have some beneficial side effects. It is, however, doubtful that bacteria in the intestine of fish with stomachs contribute significant amounts of protein to the fish.

Based on his own work and that of others, Beauvalet (3) as reported by Al-Hussaini (1) states that "when fishes have a stomach the secretions of their intestines do not effect proteins to any appreciable degree."

Carp along with other cyprinids do not have stomachs. The pH in their digestive systems is neutral to alkaline. Protein digestion occurs mainly in the 3rd (final) limb of the intestine and in the rectum. Carp have strong carbohydrate digesting enzymes but rather weak proteinases (1). Carp were selected to be the test fish as they are widely cultured, have a high fiber diet, and it was felt had the best chance of profitably using NPN in their diet. However, they do not appear to do so economically.

All fish fed actively during the experiment; little uneaten food was noted. The daily growth increment of the control fish was 1.90%, higher albeit not strictly comparable to that reported by other researchers with larger carp fed the same diet, 1.09% (52).

Boyd (11) has found that aquatic plants in differing states of maturity show a marked variation in crude protein content. Elodea has been demonstrated to contain 26.81% crude protein by Nelson and Palmer (41). The Elodea used in this experiment had a crude protein content of 9.9% (Table 8). Therefore, this experiment could not evaluate the plant nutritionally; it was included mainly as a source of fiber.

CONCLUSION

NPN in the form of poultry waste does not appear to be suitable for inclusion into the diet of small Israeli carp. Israeli carp do not appear to be able to satisfy dietary protein requirements via the digestion of microbes produced in their gut. Growth of non-ruminants such as fish on diets containing NPN is probably not because of, but rather in spite of, the dietary inclusion of items such as poultry manure.

APPENDIX

APPENDIX

POTENTIAL OF PHOSPHORUS IN WASTEWATER FOR AQUATIC PLANT PRODUCTION

Assumptions: U. S. Population 1976 - 223 x 10^6 persons[a]

Phosphorus in Wastewater - 10 mg/l[b]

Wastewater production - 378.541 liter/day/capita

Aquatic plant standing crop - 500 g/m^2 (5 mt/ha)

Percent P in aquatic plants - 0.3138%[c]

223 x 10^6 x 378.541 liter/day/capita x 0.01 g P/liter =

844 mt P/day x 365.25 days/year = 308,324 mt P/year

308,324 mt ÷ 0.003138 = 98.255 x 10^6 mt aquatic plants/year

÷ 5 mt/ha = 19.651 x 10^6 ha/year

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[a]Trend analysis using Bureau of the Census data (15).

[b]Metcalf & Eddy (37), p. 231.

[c]Boyd (12) average of 79 species of aquatic plants.

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