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PILLETED DRY RATIONS VERSUS STANDARD
MEAT RATIONS FOR TROUT

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY

Edward F. Grassl
1956

Ph. D.

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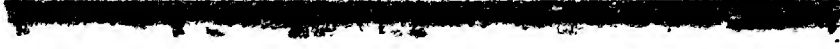
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PELLETED DRY RATIONS VERSUS STANDARD MEAT RATIONS
FOR TROUT

By

EDWARD F. GRASSL

A THESIS

Submitted to the School of Advanced Graduate Studies of
Michigan State University of Agriculture and Applied
Science in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Poultry Husbandry
and
Institute of Nutrition

1956

ABSTRACT

Diets consisting of animal and vegetable meals were pelleted to compare dry meal diets to chopped raw meat mixtures when fed to rainbow and brook trout. Evaluation of the performance of each diet was based on data gained from feeding large numbers of trout. Bimonthly weight and length measurements, daily losses, and physiological changes were used to determine the nutritional value of each diet.

Pelleted dry-meal diets were not immediately accepted as food by rainbow and brook trout. This was evidenced by a loss of weight during the first two weeks of feeding pelleted food. Lost weight was completely regained after four weeks of feeding.

All dry-meal diet ingredients in the combinations used were acceptable as trout food when supplemented with raw beef liver at least one day every three weeks. The dry diets can be fed at levels one-half to five-eighths lower than those recommended for raw-meat mixtures without reducing trout growth rates.

The difference between a diet containing torula yeast and one containing brewers yeast was statistically significant when evaluated by percent gain in weight of rainbow trout. Also, it was shown

graphically and statistically that one dry pelleted diet (Diet 2) was superior to a raw beef liver diet.

Feeding the dry diets described did not cause a reduction in numbers of red blood cells per millimeter of blood as was originally thought. Counts were as high and higher than those from rainbow and brook trout fed an all-beef liver diet. Even after feeding dry diets for twelve months without raw meat supplementation no nutritional anemia was evident.

Fish foods in pellet form offer several advantages over raw meat diets for administering medicaments to trout for the prevention and control of bacterial diseases. As demonstrated by one experiment, antibiotics appeared to relieve the severity of an attack of furunculosis in brook trout.

The adoption of dry pelleted diets as standard rations by all the state fish hatcheries in Michigan increased trout production (in pounds) by 60 percent and reduced fish food expenditures by 40 percent.

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Dr. P. J. Schaible, committee chairman, for his valuable assistance during the period this work was undertaken.

He is also deeply grateful to Professor C. Card and Dr. H. C. Zindel for allowing this work to be conducted in their department; and Drs. W. D. Baten and L. Champion for their valuable help in conducting statistical work.

Grateful acknowledgment is also due to Drs. P. I. Tack, C. Hoppert, J. Leonard, L. M. Turk, and all the members of the Poultry Department for their helpful suggestions and assistance in one way or another.

The writer wishes to thank Dr. L. Allison who supplied some of the blood count data.

The writer deeply appreciates the financial support and the use of the hatchery facilities of the Fish Division, Michigan Department of Conservation, and the Institute for Fisheries Research, and to the following men in these departments: Mr. M. J. DeBoer, Drs. A. S. Hazzard and G. Cooper, and Mr. F. A. Westerman.

Also the author wishes to thank all the superintendents and employees of the state fish hatcheries in Michigan, especially Messrs. H. Hatt, J. Curnow, R. Robertson, L. Hoodemaker, J. Southwick, and others who helped me to gain data from experiments conducted on a production scale.

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Final Examination: May 4, 1956, 3:00 p.m.; Conference Room,
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INTRODUCTION

Trout, like other animals, depend on complex organic materials as food. In their natural habitat, small animal organisms and other fish largely supply the necessary nutrients. When man attempted to domesticate trout, one of the major problems was to provide a diet similar in composition to that found in nature. Being carnivores, raw meat products were the first investigated. Such raw animal products as beef liver, heart, lungs, spleen, and fish were found to serve as a reasonable substitute for natural food. Since they were abundant and low in cost and as long as trout production remained at a relatively low level, there was little incentive to investigate other types of food. The practice became so firmly established that even now many hatchery trout-feed formulations contain large proportions of raw meat products.

However, during recent years the greatly increased interest in fishing compelled drastic changes in trout culture and its management. One large and important change occurred in the Michigan state policy of stocking legal or near-legal length (seven inches) trout instead of fry or fingerlings. The change intensified the problem of feeding trout in several ways. Trout kept in hatcheries

for the longer time to grow them to legal size required much more food, better balanced diets, and more labor. Increased trout production in conjunction with a rapidly developing cat and dog food business as well as the increased manufacture of gland extracts for pharmaceutical purposes created a shortage of meat by-products. Prices went up as the demand grew and gradually reached a level that prohibited the use of raw meats as the sole diet ingredient for the efficient production of hatchery trout.

Besides availability and initial purchasing costs, disadvantages associated with feed preparation and storage contributed to the overall problem. Efficient utilization required expensive refrigeration units, grinders, special food mixing equipment, and much labor. During thawing, preliminary to grinding and mixing, some of the water-soluble nutrients escaped with the exuding liquids into waste lines and were lost. The remaining portion of raw meat often contained only minimum amounts of certain nutrients, allowing no margin of safety for the more demanding individuals, or necessitated feeding larger quantities per unit of time to offset the nutrient loss during food processing. In many cases, the latter function was ignored and poor growth or even increased mortality resulted.

During actual feeding operations, it was difficult to hold the diet intact when placed in water. Usually the finely ground particles

of meat floated uneaten through the pond or raceway or settled to the bottom. Frequent pond cleaning to prevent serious pollution increased labor costs. Indigestible connective tissue accumulated at the screens, which hampered circulation and reduced the rate of flow necessary for clearing the water after each feeding.

The interaction of these disadvantages created interest in vegetable and animal products in meal form. The widespread use of dry feeds in livestock rations stimulated investigators to compare the economical and nutritional value of dry feeds with raw meat supplements in trout diets. This intensified the problem of binding the fine particles of feed to prevent a large loss when placed in water.

One method which proved successful was binding ground beef and pork glands with salt and dry meals to form a gelatinous mixture. The dry meals absorbed much of the free meat juices and prevented considerable loss of both. Since vegetable and animal meals cost less than raw meat per unit of nutrient on a dry basis and since it was demonstrated that greater growth gains were evident, there was a noticeable reduction in the cost per pound of fish raised. Nevertheless, high labor costs, expensive refrigeration, grinding, and mixing units still remained.

Several years ago, a commercial fish food in the form of pellets appeared on the market. These pellets were manufactured

in the sizes $3/32$ x $1/8$ inch and $3/16$ x $1/8$ inch. Neither refrigeration nor mixing equipment was necessary for the utilization of this food. The pellets consisted of firmly packed particles of vegetable and animal meals which did not separate immediately upon contact with the water, and, if care was exercised during the feeding process, most of the pellets were consumed before they could sink to the bottom of the raceway. This concentrated food provided many dietary essentials, and yet due to unknown missing dietary factors, it was still necessary to feed some raw meat. The advantages of pelleted food over a total raw meat diet quickly created interest in fish cultural circles, and many agencies began investigating the value of pelleted dry meals as a standard trout diet.

In Michigan, investigations to determine the practicability of using pelleted dry feeds for trout diets began early in 1952. Results of the preliminary feeding tests were very encouraging and further experimentation on a wider scale was begun in January, 1953.

This study was directed toward the goals of (1) developing a practical, economical, pelleted, dry meal diet which could be used to produce strong, healthy, legal-sized trout in the shortest time possible; and (2) eliminating the need of raw meat.

Presented in various parts of this paper are detailed descriptions of seemingly insignificant experiments. In some cases, solutions

are offered without exacting supporting data. Nevertheless, the information is more than justified if only one novice scientist who, by chance, may read this thesis is led to understand that solutions must be found for many and varied minor problems before attempting to solve major problems.

REVIEW OF LITERATURE

The technical literature pertaining to nutrition of fish in hatcheries is widely dispersed--especially the phases dealing with mineral nutrition. In Europe, most information is found in technical journals of limited circulation. In the United States, scientific advances are published in the Transactions of the American Fisheries Society, Chemical Abstracts, various nutrition journals, Progressive Fish Culturist, and government mimeographed pamphlets and leaflets.

An extensive survey of other investigators in the field of trout nutrition before 1937 is presented in reviews by McCay (1937a and 1937b).

Since 1937, the increasing demand for hatchery-bred fish has become more imperative, with the result that many investigations were started to find large sources of inexpensive and nutritionally adequate food.

In order to determine the type of food eaten by fish, Raney (1941), Idyll (1942), Ide (1942), Churchill (1944), and Wells (1944) studied the stomach contents of wild fish. From their work it was shown that trout eat what food is present and available; for example, plankton, algae, crustaceans, young insects, protozoa, copepoda, and

smaller fish. However, even though this information has been made available, few investigators have used it to formulate a diet for trout consisting of substances with a definitely known composition.

Mechanics of Feeding and Associated Problems

There are many variables other than diet itself which must be controlled in determining the value of a diet fed to trout. Some of these are: (1) the amount and manner of feeding; (2) the consistency of the diet; (3) temperature; (4) pH; (5) water purity; (6) the number of fish per cubic foot of water; and (7) the size of the fish. These variables were ably pointed out by Agersborg (1934), Tunison and McCay (1935a), Stiles and Russ (1938), Tunison and McCay (1935b), and Tunison et al. (1944).

The amount of food given is determined chiefly by the size of fish and the temperature of water. Small trout feed on particles suspended in the water, and they are fed often. However, Einarsen and Royal (1929) showed that if too much food is given there is inefficient utilization of food. Hagen (1940), Gutsell (1939), Davis (1935), and Wilkinson (1938) believed that overfeeding would result in sluggish, unhealthy fish, and that it is particularly easy to overfeed fish when meat or fish meals are fed. There was some disagreement as to the proper time to begin inclusion of dry meals in the meat diet.

Some workers, one of whom was Thompson (1929), used dry meals from the time fish began to feed, whereas others, such as Davis (1929), believed that the fish should be at least two inches long before dry meals are incorporated in the diet.

Nutrients

Protein. Many investigators have attempted to determine the identity and amount of the various nutrients needed by trout. McCay (1934) found that a 14 percent level of protein in the diet was necessary for growth and that more than 25 percent protein in the diet did not increase the rate of growth. Sekine and Kakizaki (1931) showed that there was a greater utilization of protein with diets containing the lower levels of protein. The source of protein was found to affect its utilization by fish. Animal protein was better utilized than the protein from vegetable sources, as was shown by Tunison et al. (1942).

Fat. The amount of fat in a diet produced varying results. McCay (1937) pointed out that whether 7 or 25 percent of fat was added to the diet of trout, 80 to 90 percent was digested. McCay and Tunison (1935) believed that the lower-melting-point fats (salmon oil and cottonseed oil) were utilized slightly better than hydrogenated

fats such as Crisco. However, as found by Hess (1935), feeding high levels of fat resulted in the degeneration of the pancreas. The livers of fish fed diets containing 100 percent raw liver, or a high fat or high carbohydrate content, showed large deposits of fat.

Carbohydrate. Trout utilize carbohydrates to some degree, but ordinarily, when fed diets containing more than 20 percent, it was demonstrated that they developed large livers with a high percentage of glycogen. This was indicated by McLaren et al. (1947). It was thought that trout could not utilize high levels of carbohydrate because they have fewer islets of Langerhans than other animals.

Mineral. Very little work has been carried out to determine the optimum mineral content of a diet. Nevertheless, it was demonstrated by McCay et al. (1936) that fish absorb calcium from the water, and therefore the need for calcium in the diet was contingent upon the amount of calcium available in the water. Tunison et al (1942) at a later date found also that, although ferrous sulphate was toxic to anemic fish, other iron compounds did not affect them adversely. None of the iron compounds had any effect on normal fish.

Titcomb et al. (1929) showed that when roughage was included in a diet, lower mortality resulted, but the reasons for this effect

could not be established. Other tests by McCay et al. (1930) indicated that when the amount of roughage was greater than 24 percent the growth of trout decreased.

Foster et al. (1939), working with young salmonids, indicated that bulk added to a diet may lessen dangers of overfeeding and forestall the resulting degeneration of the pancreas and liver.

Vitamins. The ability of trout to utilize vitamins from different sources is not well known, and the recognition of deficiencies attributable to the lack of a specific vitamin is often difficult. A number of attempts have been made to establish definite vitamin levels required for normal growth. McCay and Phillips (1940) found that trout store and lose Vitamin A very slowly, and that beef liver was a better dietary source than cod liver oil or carotene.

Hewitt (1937) stated that B and C vitamins are necessary for fish two to three years old.

The requirement for Vitamin D has not been definitely determined.

The requirements of fish for B vitamins have been studied more extensively than the requirements for the fat-soluble vitamins. Workers at the Cortland, New York, hatchery have attempted to establish the requirements of these vitamins for trout by feeding various

amounts of the vitamins, and after varying periods of time assaying the organs of trout to determine maximum storage. It was acknowledged that these values were only approximate, and that all the vitamins tested may not be necessary for the production of healthy fish. Phillips et al. (1947), McLaren et al. (1947), and Wolf (1945) presented excellent data which showed that trout require certain B vitamins for normal growth.

Unknown Growth Factors and Anemia

In early investigations by McCay and Dilley (1927) and McCay (1939), it was found that raw fresh meat contained a substance or combination of substances the lack of which resulted in anemia and death of trout. The required substances were never isolated, but when the alcohol, acetone, and other extracts of liver had been added to diets which of themselves would not support growth, the fish had grown (Heard, 1930). The presence of the growth factors have been demonstrated in many animal tissues. It was reported present in beef, pork, and sheep liver, spleen, heart, lungs, kidneys, and melts by McCay et al. (1931), in skim milk by Tunison and McCay (1933), in the eggs, viscera, and possibly carcasses of salmon by Donaldson (1935), in fly maggots by Phillips et al. (1940), and in midge and mosquito larvae.

Pelleted Feeds

Published work on the use of pelleted feeds for trout is limited to a few scattered reports. Brockway's report (1953) was concerned with describing the potential value of pelleted trout foods, pointing out the advantages and disadvantages of this type of food in respect to the pelleting process and the problems encountered when fed to trout for the first time. Willoughby's work (1955) was designed to compare pelleted food with a raw meat and meal mixture when fed to fairly large brown trout (13.4 per pound). His work and part of the work reported in this paper were conducted at approximately the same time. The problem of introducing pellets to trout was investigated by Wolf (1953). His reported work consisted of coloring pellets with vegetable dyes of various colors. He found that when first fed trout preferred red-colored pellets to green, blue, or yellow.

The work reported in this paper is, to the best of my knowledge, some of the first conducted to show the performance of a non-commercial pelleted food when fed to rainbow and brook trout.

PRELIMINARY FEEDING TRIALS TO COMPARE A
COMMERCIALY PELLETTED FOOD WITH A
STANDARD HATCHERY DIET

The objectives of these experiments were to determine, at a very general level, the value of pelleted dry animal and vegetable meals when used as food for hatchery-reared trout. They are included here merely to show the manner in which trout nutrition investigation evolved in the state of Michigan rather than to answer specific questions pertaining to the nutritional requirements of trout.

In 1952 feeding trials were begun to compare the nutritive value of a commercially pelleted trout food with one of Michigan's standard hatchery diets. The experiments were conducted at the Oden State Fish Hatchery, Oden, Michigan. All feeding trials were carried on in outside, gravel-bottom raceways receiving a continuous flow of spring water. Extra precautionary measures were not taken to prevent predatory losses, nor was the natural incidental food controlled. Brook trout, Salvelinus fontinalis, approximately six inches long (42 to 48 grams) were used for the tests. Since large numbers were used (7,000), no attempt was made in either group to sort the fish for size uniformity. All testing was done by hatchery personnel on a production scale.

Diets 17 and 18 were tested during two separate seasons of the year. The first feeding trial extended from February, 1952, to June, 1952, a period of twenty weeks; and the second, from November, 1952, to May, 1953, a period of twenty-eight weeks. Diet 17 consisted of dry animal and vegetable meals manufactured in pellet form. Since this was a commercial product it was distributed under a closed formula. Diet 18 consisted of a raw-meat mixture composed of fifty parts pork melts and fifty parts of a commercial baby chick starter mash. The levels of both diets fed were based on those recommended for feeding raw-meat mixtures (Duel et al., 1942). When feeding pellets, meat was recommended once every two weeks as a change in diet, although it was pointed out by the feed manufacturer that meat was not necessary for normal trout growth and survival. At that time an unfortunate experience at another hatchery, where the latter feeding recommendation was followed, indicated that raw meat was a necessary supplement for this pelleted food. The details were not available, but an extremely high mortality resulted. Since no outward disease symptoms were discernible, the high losses were attributed to a dietary deficiency. Thereafter, whenever these pellets were used, meat was recommended to be fed one day per week in amounts double those of the daily allowance of pellets.

One test of the adequacy of an experiment in nutrition is the ability to duplicate results. For this reason, the second feeding trial was carried out at the same hatchery four months later and continued for twenty-eight weeks. The same diets and methods were employed as in the first feeding trial. As shown in Table 1, the only differences in organization of the experiment were in the numbers of fish used, the season of the year, and the duration of the experiment.

Table 1 shows that both diets produced poor results in both experiments. From the data presented, the rate of growth appears to be the best means available for evaluating the feedstuffs. In both cases, even though growth was slow, the pellet-fed groups showed a greater gain in weight than the meat-fed groups. The fact that pellets produced better gains is probably due to the physical rather than the nutrient quality of each diet. Since finely ground raw meat tends to disintegrate immediately upon contact with water, perhaps the trout were able to derive more nutritive benefit from the pellets which remained intact long enough to enable the fish to ingest the whole particle.

Other advantages attributed to the use of pelleted fish food, however, were observed during this period of experimentation. When a pellet was ingested by a fish it received all of the nutrients in the same proportions as when the diet was manufactured. Labor for

Table 1. Feeding trials comparing pelleted diets versus raw meat diets (average water temperature 46° F.).

Item	February, 1952 to June, 1952		November, 1952 to May, 1953	
	Diet 17	Diet 18 ^a	Diet 17 [#]	Diet 18 ^a
Number of weeks on diet . . .	20	20	28	28
Cost per 100 pounds of diet	\$12.00	\$11.00	\$13.40	\$11.80
Cost per pound of trout reared	\$ 0.14	\$ 0.30	\$ 0.56	\$ 1.15
Mortality by four-week periods (pct.)	3.5	5.5	0.006	0.006
Gain by four-week periods (pct.)	11.5	6.2	7.2	6.0
Conversion	1.13	2.69	4.16	9.99
Average percent of body weight fed per day	1.12	2.45	0.98	1.99

^a50 percent pork melts; 50 percent commercial chick starter.

food preparation was reduced because the pellets were fed as purchased. Also pellets could be stored for relatively long periods without benefit of refrigeration. Since meat was fed only periodically, labor involved in meat thawing, grinding, et cetera, was reduced. More complete consumption of food occurred; consequently the degree of pond pollution was lowered and the amount of labor allotted to pond-cleaning operations was reduced.

Considering all of the advantages of pelleted fish food over raw-meat diets, it was deemed advisable to formulate a dry diet which would lend itself well to the pelleting process and furnish the required nutrients for normal trout growth and survival.

An ideal diet would be one that produced reasonably rapid growth with the cheapest feedstuffs, and yet furnish the necessary nutrients for the production of strong, hardy fish which are able to withstand natural competition when stocked in streams and lakes.

FORMULATION OF PELLETED DIET 1

The formulation of Diet 1 was based as much on the pelleting properties of the diet ingredients as on the potential nutritive value of the finished diet. Since no data were available on the growth-producing performance of this diet when fed to trout, the first step was to determine which combination of dry meal ingredients known to have been used as raw-meat supplements would produce a functional pellet. The desired pellet was one which disintegrated slowly or not at all in water and which would furnish enough buoyancy to prevent rapid sinking to the bottom of a tank or raceway. For this reason, preliminary studies were carried out to determine which ingredient combination produced a compressed mass that performed as desired when placed in water.

Since miniature laboratory pelleting machines were not available, a hand-operated Eureka tablet machine was used. Although awkward in function and permitting the production of only one tablet at a time, it served the intended purpose.

On a production scale, steam plays an important role in compressing and extruding dry meals to form compact pellets. In the use of the tablet machine, it was impractical to use steam as

a humidifier. To circumvent this problem, a small amount of water was added to the meal mixtures and comparable results were attained.

The diet ingredients were selected from the group of animal and vegetable meals known to produce favorable results when added to a raw-meat mixture to form a mush-type food. Skim milk, buttermilk, whey, casein, cottonseed and soybean oil meals, yeasts, and various tissue and gland meals of animal origin have been used successfully for this purpose. Of these, a group of products selected for their availability in this area at nominal costs, and nutrient quality and quantity, were mixed in various combinations and tableted. Skim milk, cottonseed meal, wheat flour middlings, soybean meal, brewers and torula yeasts, and fish meals were given the most extensive investigation. After tableting, each compressed particle was subjected to a water test employing a glass tube eight feet high and two inches in diameter filled with water. Each ingredient combination, after tableting, was placed into the water at the top of the tube and allowed to sink to the bottom. The time required to sink from the top to the bottom of the tube and the condition of the tablet in respect to disintegration at various distances from the top were used as criteria in selecting the diet combination.

As shown in Table 2, twenty different diet combinations were prepared for tableting. Table 3 shows conditions applied after stock

Table 3. Rate of fall and disintegration of tablets in a tube of water eight feet high.

Code	Conditions	Time to Fall 8 Feet (sec.)	Depth Disintegration Began (feet)	Condition of Tablet at 8 Feet
1		a	0	tallow separation
2	20 pct. H ₂ O	38	2.5	complete disintegration
3	20 pct. H ₂ O	38	2.5	complete disintegration
4	20 pct. warm H ₂ O	27	0	disintegrated after 4 minutes
5	20 pct. warm H ₂ O 70° C.	27	0	no disintegration for 10 minutes
6	10 pct. warm H ₂ O 70° C.	27	0	no disintegration for 10 minutes
7	no water or heat	42	3.0	tallow separation
8	20 pct. H ₂ O	36	2.5	one-third disintegrated
9	--	32	1.5	complete disintegration
10	--	33	1.5	complete disintegration
11	--	32	2.0	complete disintegration
12	--	33	3.5	two-thirds disintegrated
13	--	32	3.5	one-half disintegrated
13a	1 pct. H ₂ O dried 18 hours at 70° C.	25	0	no disintegration for 6 minutes
14	1 pct. H ₂ O dried 18 hours at 70° C.	25	0	no disintegration for 6 minutes
15	10 pct. H ₂ O	--	b	----

Table 3 (Continued)

Code	Conditions	Time to Fall 8 Feet (sec.)	Depth Disintegration Began (feet)	Condition of Tablet at 8 feet
16	10 pct. H ₂ O dried 5 min. at 60° C.	25	0	disintegrated from 4 to 8 minutes
17	1 pct. H ₂ O dried 12 hrs.	27	0	disintegrated from 2 to 15 minutes
18	1 pct. H ₂ O air dried 12 hrs.	27	0	disintegrated from 2 to 15 minutes
19	1 pct. H ₂ O air dried 12 hrs.	28	0	disintegrated from 2 to 20 minutes
20	1 pct. H ₂ O air dried 12 hrs.	0	0	crumbled to touch

^aFloated.

^bImmediately.

material was mixed, rate of fall through a water medium in seconds, the depth at which disintegration of the tablet began, and the condition of the tablet at the various time intervals. Three sizes were tableted, corresponding to a $3/16$ inch, to a $1/8$ inch, and to a $3/32$ inch pellet. The difference in size had little effect on rate of dissociation in water except that the larger size separated into more individual particles. The smaller size did show a slightly better surface glaze.

Any one of the diet combinations shown in Table 2, except 1, 7, and 20, produced a reasonably stable pellet. The information of most value gained from this experiment rests in the facts that tablets when not sufficiently dried will disintegrate more quickly than dried tablets, and that a small amount of moisture, preferably heated moisture, aided in producing a firmer tablet when oven- or air-dried than when moisture was not added.

To begin with, it was felt that granulated gelatin was nutritionally undesirable in the diet, and since during these tests the advantages over the diets which contained no gelatin were negligible, diet combinations 2 through 8 were temporarily shelved. Diets 16 to 19, inclusive, after tableting and drying, produced particles which appeared to be too firmly associated for initial pellet feeding studies,

Diets 15 and 20 disintegrated immediately upon contact with water and therefore were unsuitable.

It will be noted that in Diets 9 to 14, as the percent of condensed fish solubles was decreased, the binding property was increased. At a 3 percent level and after drying, disintegration of tablets in water first began at the end of six minutes.

In the pelleting process used by most feed manufacturers, steam application and pellet drying are two important steps. Since combination 13a appeared to give the best laboratory results in these respects it was pelleted on a production scale in three sizes ($1/8$, $3/32$, and $3/16$ inch in diameter, and $1/4$ inch long) at a local feed-manufacturing plant. A very small amount of dusting occurred during the pelleting process. When pellets were placed in water, no immediate disintegration took place, and they remained intact for as long as ten minutes.

From the twenty combinations tested, number 13a was selected for pelleting on a larger scale for further trout nutrition studies.

This combination will be referred to hereafter as Diet 1.

PRELIMINARY FEEDING TRIALS USING DIET 1

This experiment was designed to (1) compare diet performance of an all-meat diet with an all-pellet diet when fed to rainbow trout, and (2) provide tentative initial pellet size preference data essential for full-scale hatchery-trout feeding.

Rainbow trout were obtained from the Benton Harbor station, Benton Harbor, Michigan, on February 18, 1953, and transferred to the Hastings, Michigan, station. Eighteen pounds of rainbow trout were placed in each of four concrete tanks preparatory to feeding pelleted Diet 1 and raw pork liver. Diet 1 was manufactured by a commercial feed manufacturer in Michigan¹ in three pellet sizes: 3/32, 1/8, and 3/16 inch in diameter, and 1/4 inch long. Each one of three lots was fed a different size of pellet, and the fourth, fed a raw pork liver diet, served as a control. Once a week, all fish were weighed and the data recorded. These experiments were conducted over an eight-week period. Rainbow trout averaging 4.5 inches in length and 18 grams in weight were used. These trout had previously been fed a meat diet. All fish were counted before and

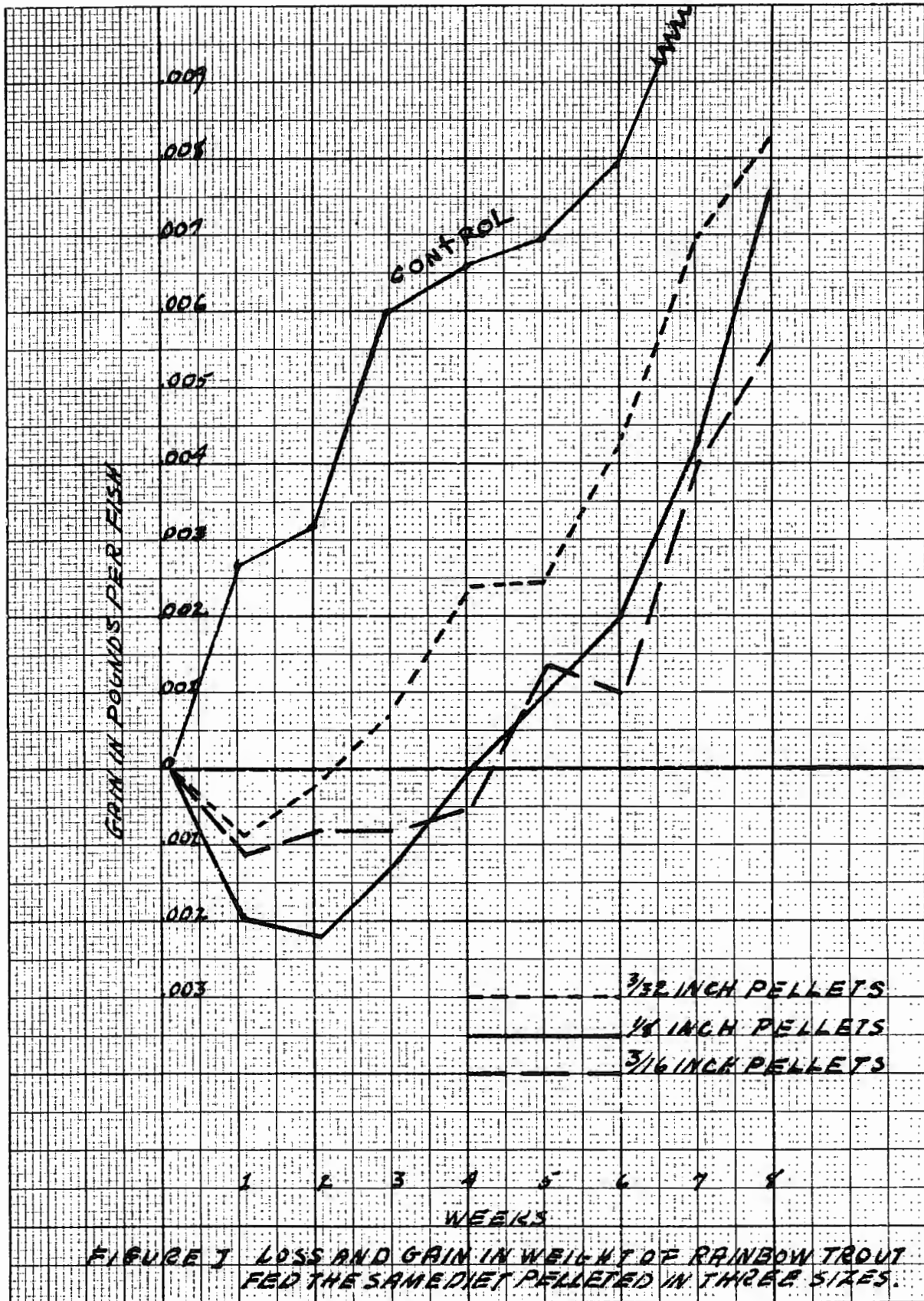
¹Valley City Milling Company, Portland, Michigan.

after the eight-week period. The length and weight of every fish that died was recorded. Diet 1 was fed throughout without meat supplementation. Raw pork liver was fed to the control group without dry-meal supplementation. All diets were fed in amounts greater than required. At no time during the experiment was all of the food consumed.

Figure 1 shows the growth rate of rainbow trout when fed the same diet pelleted in three different sizes and when fed a pork liver diet. The curves are constructed from weekly individual gains in pounds derived from dividing the total weight at the end of the period by the total number of fish present in each group.

The radical change from a soft meat diet to a hard pellet diet was reflected in loss of weight in the groups fed the pelleted diet. Figure 1 shows that it was necessary to feed the $1/8$ and $3/16$ inch pellets four weeks before lost weight was regained, whereas only two weeks was required for the group receiving $3/32$ inch pellets. Even though the $3/32$ inch group recovered more quickly than the others, the $1/8$ inch group shows a steadier gain in weight after recovery. The group fed raw pork liver did not lose weight, and gained weight steadily throughout the experiment.

In any experimental work, the investigator may be confronted with the inadequacy of the data to answer specific questions. It is



felt that the curves shown in Figure 1 are reliable but inconclusive that a particular size pellet was preferred by five-inch fish. It is shown, however, that any one of the pellet sizes used for each group will support fish life and growth for eight weeks when fed to trout averaging five inches in length. As determined by loss and gain of weight per unit of time, rainbow trout should be allowed a two- to four-week adjustment period when changing from a meat to a pellet diet.

In summary of the pellet size preference tests, it can be stated that even though rainbow and brook trout are capable of ingesting large particles of relatively soft foods, they are temporarily reluctant to accept particles of nonresilient food which are not easily crushed or indented when first moved through the buccal cavity to the pharyngeal area. In other words, teaching fish to eat pellets of any size is a very important function in changing from a meat to a dry pelleted diet.

The results of these experiments were encouraging enough to warrant further work to determine what combination of feedstuffs after pelleting would furnish adequate nutrition for trout when fed alone and in combination with raw meat. Due to frequent water stoppages and various other conditions adverse to experimental work, use of the Hastings hatchery for this purpose was discontinued. New

facilities were installed at the Wolf Lake State Fish Hatchery, and all subsequent experiments for this area were carried out at that station.

WOLF LAKE

Experimental Facilities

The following experiments were carried out at the Wolf Lake State Fish Hatchery located ten miles west of Kalamazoo, Michigan. The water supply for the hatchery comes from a spring which has a flow of approximately 1500 gallons per minute. The clear water flows into an open area which was formed by building a dam across the lower end of a small valley. The created pond measures 100 yards long, 30 yards wide, and 18 feet deep. The water from this pond flows through underground metal pipes into the hatchery building. In the hatchery, the water temperature varied from 42° to 50° F. during the colder months, and from 50° to 58° F. during the warmer months. The concentration of dissolved oxygen varied between 6.0 and 9.0 parts per million. Free carbon dioxide averaged 2.2 parts per million. The pH varied between 7.5 and 8.1, with an average pH of 7.7. Chemical analysis of the water showed a methyl orange alkalinity of 160 parts per million.

Description of Experimental Equipment

Eleven concrete tanks and sixteen small wooden troughs were constructed at the Wolf Lake State Fish Hatchery early in 1953. Each concrete tank measured 25 x 2 x 3.25 feet. Each of these tanks was divided into two individual water-tight compartments that measured 11.5 x 2 x 3.25 feet. Each compartment was constructed to contain an individual water inlet and outlet. At no time during the course of the experiments was water allowed to flow from one compartment into the other. The rate of flow of water was regulated by two 1-inch gate-valves inserted in a 2-inch pipe feeder line.

The sixteen wooden troughs were salvaged from cut-down incubation troughs, the type commonly used for hatching trout eggs. Each finished trough for experimental use measured 32 x 6.5 x 14 inches. One end was fitted with a 2-inch galvanized metal outlet tube directly in front of which was inserted a 6-mesh screen. At the opposite end water entered through a one-half inch spigot from a 2-inch feeder pipe line. A one-fourth inch mesh screen debris collector was placed directly beneath each water inlet spigot. All troughs were completely covered by a framed one-fourth inch mesh screen. Flow of water entering individual troughs was restricted to eight gallons per minute. The source of water for troughs was the

same as for the tanks. No attempt was made to filter out small organisms entering the hatchery building with the water supply.

Methods and Procedure for Applied Experiments

The application of scientific investigation to practical field use requires four phases of work. The first phase is concerned with the discovery of major facts through the use of small numbers of animals and small amounts of materials; the second utilizes the results obtained on larger numbers of animals and considerable amounts of materials; the third phase applies the results of the second to semiproduction scale; and the fourth phase applies the promising results of the third to full-scale production. In some instances, separate laboratories are maintained for each phase of the development.

The experimental work at Wolf Lake State Fish Hatchery was designed to follow this system of planning. The first phase of the experimental work was carried out in small troughs; the second, in large tanks inside the building; the third, in outside concrete and gravel-bottom raceways on a semiproduction scale at two different stations, one the most southern, Wolf Lake Hatchery, and one the most northern, Marquette; and finally the results were subjected to full-scale hatchery feeding by all the Michigan hatcheries.

Small troughs were used in each case that required testing an entirely new diet ingredient and/or combination. Three pounds of trout were fed the diet in question for a sufficient length of time to determine whether fish growth occurred. After it was determined that the diet warranted further investigation, a more extensive experiment was carried out in the larger concrete tanks using numbers of fish which compared to a semiproduction scale. For these experiments 12,000 rainbow trout, Salmo gairdneri, ranging from 2.9 to 5.9 inches in length and 4 to 20 grams in weight, were employed. These trout were seven months old at the start of the experiment. Their diet up to this time consisted of raw beef and sheep liver and pork spleen in various combinations. One thousand were placed in each of the twelve tanks previously described. The investigation included fourteen two-week periods, extending from October 1, 1953, to April 28, 1954.

Seven different dry animal and vegetable meal combinations (Table 4) were compressed into 3/32 inch pellets. Even though there was a risk of losing some of the nutrients present in each diet due to a long storage period, the same diets pelleted at the beginning of the experiment were used for the duration of the investigation. Proximate analysis of each diet is recorded in Table 5. After the fish reached a size large enough to accept 1/8 inch pellets

COMMERCIAL SOLVENTS CORPORATION

November 29, 1956

Dr. Gerald P. Cooper, Director
Institute for Fisheries Research
University Museum Annex
Ann Arbor, Michigan

Dear Dr. Cooper:

In reference to your inquiry concerning the proximate analysis of the various diets listed in my recent paper, the NFE means exactly what you thought it did, but for the records here is a more technical description of the term. Nitrogen Free Extract is made up of a more soluble part of carbohydrates. It does not include fiber. It includes starch, the sugars and the more soluble portions of pentosans and the other complex carbohydrates. Organic acids, such as lactic acid and acetic acid, which are present in silage are also included in this group. The term ether extract is merely used due to the fact that all fats are soluble in ether and certain other solvents. Chemists often use the term lipids for the entire group of fats, but in nutritional work, we generally use the term ether extract rather than fat or lipids.

I hope that this information will be of some value to you and I expect to be in Michigan shortly after the first of the year.

Sincerely,

COMMERCIAL SOLVENTS CORPORATION

E. F. Grassl

EFG:dk

C

O

P

Y

Table 5. Proximate analysis of various diets.

Diet	Pct. Moisture	Crude Protein	Ether Extract	Fiber	NFE	Ca	P	Ash
1	8	39.6	5.08	4.70	25.2	3.20	2.10	13.30
2	8	39.3	4.40	4.90	25.3	2.90	2.00	12.80
3	8	40.8	4.23	2.27	27.1	3.34	1.91	13.43
4	8	40.8	4.22	2.27	27.1	3.34	1.91	13.43
5	8	41.9	5.36	4.99	26.3	3.36	2.06	13.80
6	8	41.8	3.84	3.28	25.8	3.05	1.78	12.78
7	8	40.3	5.84	3.28	25.8	3.05	1.78	12.78
8 wet	70	20.2	3.10	0.00	6.0	0.007	0.358	1.3
8 dry	0	66.6	9.99	0.00	19.98	0.023	1.3	4.32

all diets were returned to the feed mill, crushed, and repelleted to 1/8 inch size. Then, this size pellet was used for the remaining experimental period.

As was pointed out previously, the percentages of ingredients were selected primarily for their pelleting properties. Since it had been established that Diet 1 would support trout life all other diets were based upon this combination. As shown in Table 4, the diet variables were yeast, cottonseed meal, soybean oil meal, condensed fish solubles, xanthophyll, stabilized fat, and gelatin. Diets 1, 2, 3, and 4 were fed to duplicate groups of fish. Diet 8 served as the raw meat control. As is shown, each diet contains one or more variables. Since the diet as a whole had not been tested for a sufficiently long time, the experiments were not designed to test the value of a specific diet ingredient. However, as will be pointed out later, several diet ingredients can be judged when gain in weight and mortality are used as criteria of diet performance. For this reason, emphasis was placed on duplication and on determining the performance of dry pelleted meal diets when supplemented periodically with raw beef liver.

After pelleting, part of each diet was placed in a metal can situated near the group of fish which were to be fed the respective food. The remainder of each diet was stored in a cool, relatively

dark, dry place. The amount of food fed was based on percent of body weight per day, and all groups except the meat control and the Diet 1 group were started at a 3.0 percent level. Since definite feeding levels have not been established for pelleted foods, the levels used here are to be considered tentative.

The feeding levels used are approximations, determined by calculating the dry equivalents of the levels suggested for raw meats by Duel et al. (1942). Both meat and Diet 1 groups were started at a 4.7 percent level for reasons which are to be described later. The amounts of food to be fed were determined by using a metal container marked at a level which corresponded with the desired weight of food for each feeding. This eliminated the necessity of weighing many small amounts of pellets. The amount of ground raw liver was determined in the same manner. This, no doubt, introduced some error, but the chances for feeding more than the designated weighed amount were considered to be equal to the chances of feeding less than the designated amount. Therefore, the magnitude of the error was considered very small. All fish were allowed to adjust to the pelleted food for a period of four weeks. Each morning before feeding operations began all tanks and troughs were drained to a four-inch level and the accumulated refuse swept out with water flowing down the drain. At this time dead trout were weighed,

measured, and recorded. All trout were examined to determine the cause of death, if possible. A 1,000 pound capacity, platform, Fairbanks-Morse scale was used to weigh each lot of fish at two-week intervals. All fish were weighed by means of a container sufficiently large to hold a volume of water plus the fish to be weighed. The water was weighed before adding the fish, and weight of fish was determined by difference. In every case after dipping fish from the tank excess water was allowed to drain from the net and its contents. The length of time allotted to this usually did not exceed fifteen seconds. After determining the total weight of fish present in each tank, the amount of food in percent of body weight was recalculated to conform to the gain in weight, and except for mortality the numbers of fish were held constant. The amount of food fed in percent of body weight was not increased after every weighing period. It was observed that all the food was not eaten at the beginning of the experiment. However, rather than reduce the amount fed per day, that is in cases where the amount in excess was not too great, the trout were allowed to adjust to the percent in body weight fed by growth increase. This invalidates the conversion factors but probably at this time, establishing a definite feeding level was as important as gaining accurate conversion factors. Since

the tanks used were adequate in size to compensate for increase in growth, no "thinning" was carried out.

Pellet feeding schedules were planned to include four periods per day for four days and raw beef liver for one day every week. Due to conditions beyond the control of the investigator, all food was withheld on Saturday and Sunday. The raw meat control group received raw beef liver exclusively for five days a week, and were also fed four times a day. The usual practice of adding water to ground raw beef liver before feeding was not followed, the liver being fed as it came from the meat chopper. This reduced the amount of particle separation during feeding.

Regression analysis, standard error of estimate, and "t" values according to the method of Snedecor (1946) were used to statistically analyze for differences in the percent gain in weight between groups fed pelleted diets and a raw beef liver diet and between groups fed various pelleted diets.

Experimental Results

The results of twenty-eight weeks of feeding Diet 1 are presented in detail in Table 6. All other data from Diets 2 to 8, inclusive, are presented in summary only (Table 7) and include a summary of Diet 1 performance. The results of feeding Diet 1

Table 6. Performance of pelleted Diet 1 when fed concurrently to two different groups of rainbow trout.

Item	Four-Week Period					
	1		2		3	
	Dupli- cation 11	Dupli- cation 12	Dupli- cation 11	Dupli- cation 12	Dupli- cation 11	Dupli- cation 12
Number at start .	1000	1000	999	999	997	994
Number at end ..	999	999	997	994	996	992
Weight at end (pounds)	51.1	51.5	61.6	61.5	75.7	77.0
Weight at start (pounds)	39.6	39.2	51.1	51.5	61.6	61.5
Gain in weight ..	11.5	12.3	10.5	10.0	14.2	15.5
Cost per lb. of food	8¢	8¢	8¢	8¢	8¢	8¢
Conversion	3.4	3.1	3.2	3.3	2.4	2.1
Cost per lb. of fish gain. (cents) .	27¢	25¢	26¢	26¢	19¢	17¢
Percent of fish gain	28.9	31.2	20.5	19.4	23.0	25.2
Amount of food fed:						
Pellets	31.1	30.0	28.0	27.0	28.0	27.0
Meat	7.6	7.5	5.6	5.6	5.6	5.6
Total	38.7	37.5	33.6	32.6	33.6	32.6
Percent of body weight fed daily .	4.9	4.8	2.9	2.9	2.6	2.5
Average water temperature (° F.)	52	52	49	49	45	45

Table 6 (Continued)

Four-Week Period							
4		5		6		7	
Dupli- cation 11	Dupli- cation 12	Dupli- cation 11	Dupli- cation 12	Dupli- cation 11	Dupli- cation 12	Dupli- cation 11	Dupli- cation 12
994	992	994	991	992	988	988	987
994	991	992	988	988	987	986	983
91.7	96.7	107.0	108.0	130.5	130.5	144.9	144.4
75.7	77.0	91.7	96.7	107.0	108.0	130.5	130.5
16.0	19.7	15.3	11.3	23.5	22.5	14.4	13.9
8¢	8¢	8¢	8¢	8¢	8¢	8¢	8¢
2.2	1.7	2.2	2.9	1.7	1.7	2.5	2.5
18¢	14¢	18¢	23¢	13¢	14¢	20¢	20¢
21.1	25.6	16.7	11.7	21.7	20.8	11.0	10.7
28.0	27.0	28.0	27.0	33.2	33.0	30.2	29.2
7.0	7.0	5.6	5.6	5.6	5.6	5.6	5.6
35.0	34.0	33.6	32.6	38.8	38.6	35.8	34.8
2.0	1.8	1.6	1.5	1.6	1.5	1.3	1.3
43	43	41	41	42	42	48	48

Table 7. Performance of various diets during a period of twenty-eight weeks when fed to rainbow trout.

Item	Diet			
	1	1	2	2
Number at start	1000	1000	1000	1000
Number at end	863	864	911	880
Weight at end	144.9	144.4	164.0	163.5
Weight at start	39.6	39.3	39.9	36.6
Gain in weight	105.3	105.3	124.1	126.9
Cost per pound of food	7¢	7¢	8¢	8¢
Conversion	2.4	2.4	1.4	1.4
Cost per pound of fish gain (cents)	16¢	16¢	11¢	11¢
Percent of gain	266.0	267.4	311.0	346.7
Food proportions fed:				
Meat (pct.)	17.1	16.8	25.7	23.7
Pellets (pct.)	82.9	83.2	74.3	76.3
Average water temperature . .	47		47	
Percent of recorded loss	1.4	1.7	4.3	2.5
Percent of unaccountable loss .	12.3	11.9	4.6	9.5
Percent of total mortality . . .	13.7	13.6	8.9	12.0

^aNo data.

Table 7 (Continued)

Diet							
3	3	4	4	5	6	7	8
1000	1000	1000	1000	1000	1000	1000	1000
963	922	816	934	a	823	a	948
169.0	133.5	115.0	139.0	130.5	134.0	156.0	158.5
38.1	36.5	29.1	35.5	34.5	31.3	38.5	39.5
130.9	97.0	85.9	103.5	96.0	102.7	117.5	119.0
8¢	8¢	8¢	8¢	7¢	8¢	8¢	14¢
1.3	1.6	1.6	1.5	1.6	1.5	2.0	3.0
11¢	12¢	13¢	13¢	11¢	12¢	16¢	42¢
343.5	265.8	295.2	291.5	278.3	328.1	305.1	301.2
24.4	26.9	28.8	26.3	16.2	25.9	17.1	100
75.6	73.1	71.2	73.7	83.8	74.1	82.9	a
47		47		47	47	47	47
2.6	1.9	3.0	4.5	a	4.0	a	3.2
1.1	5.9	15.1	2.1	a	13.7	a	2.0
3.7	7.8	18.4	6.6	a	17.7	a	5.2

concurrently to two different groups of rainbow trout are presented in detail to offer a sample of the methods used for determining the growth-producing value of a particular diet. Diets 1, 2, 3, and 4 were fed to duplicate groups, and the summary for each was recorded in Table 7 in duplicate. Conversion factors in Table 6 are calculated by dividing the pounds of food fed during the month by the gain in weight of fish. The conversion factors in Table 7 are calculated by dividing the total pounds of food fed for the twenty-eight week period by the total gain in weight of fish.

Percentage gain is calculated by dividing the gain in weight by the weight of fish at start of each period and multiplying the result by 100.

Food fed daily in percent of body weight is calculated by dividing the total pounds fed during the period by the number of days in the period; this is divided by the average weight of fish on hand which is obtained by averaging the weight of fish on hand at the beginning and end of the period. Other conditions noted in Tables 6 and 7 are self-explanatory.

A graphic comparison of percent gain in weight in relation to average water temperature and amount of food fed in percent of body weight per day is given in Figure 2. The curves represent the results of feeding Diet 1 concurrently to two different groups of rainbow

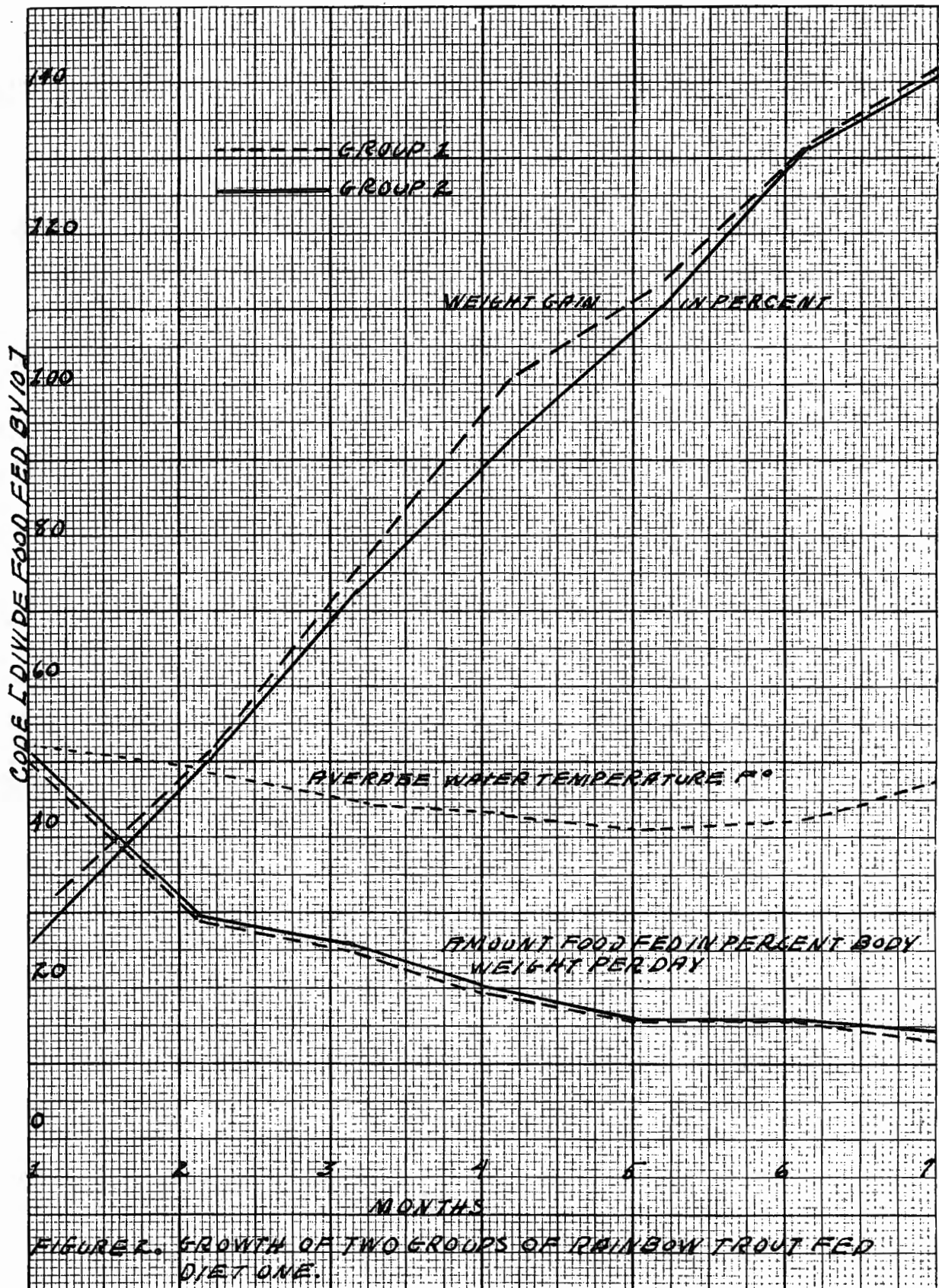


FIGURE 2. GROWTH OF TWO GROUPS OF RAINBOW TROUT FED DIET ONE.

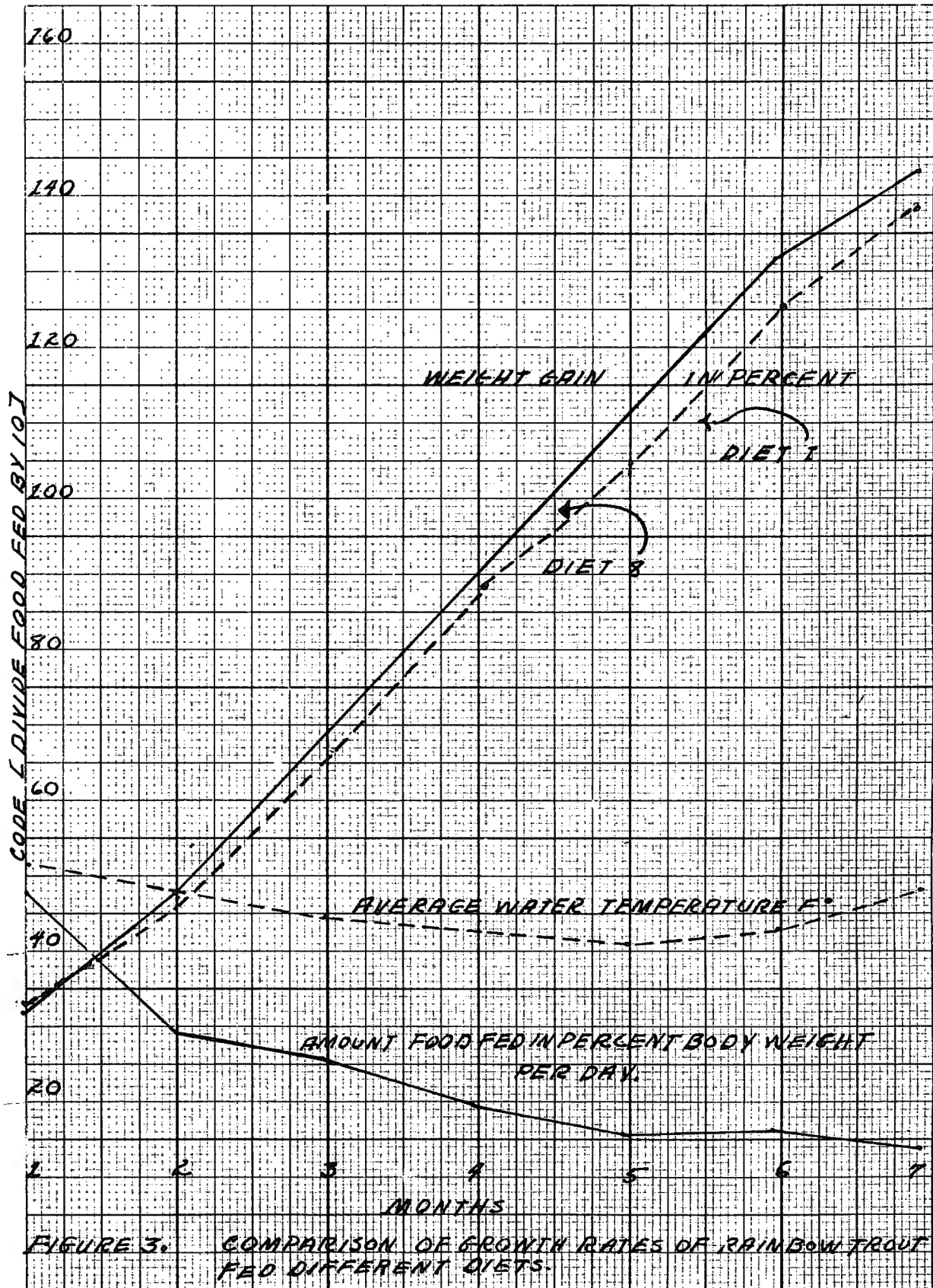
trout. The amount of food fed in percent of body weight per day included both pellets and meat. As shown, the amount of food fed at the beginning of the experiment apparently was in excess and a certain portion was wasted. This is reflected in a high conversion factor (Tables 6 and 7). Therefore, the amount of the food fed at the end of the first month was reduced approximately one-third, resulting in less wasting of food. Inasmuch as the rate of gain remained unchanged, the assumption that food was being fed in excess was correct. This amount was fed during the remainder of the experimental period in an attempt to produce a marked change in rate of gain of weight and establish a definite requirement in daily volume of food intake for a particular size fish at a definite water temperature. This appeared to occur near the end of the sixth month of feeding when the amount of food was equal to 1.5 percent of fish body weight per day. The water temperature at that time was 42° F. and rising.

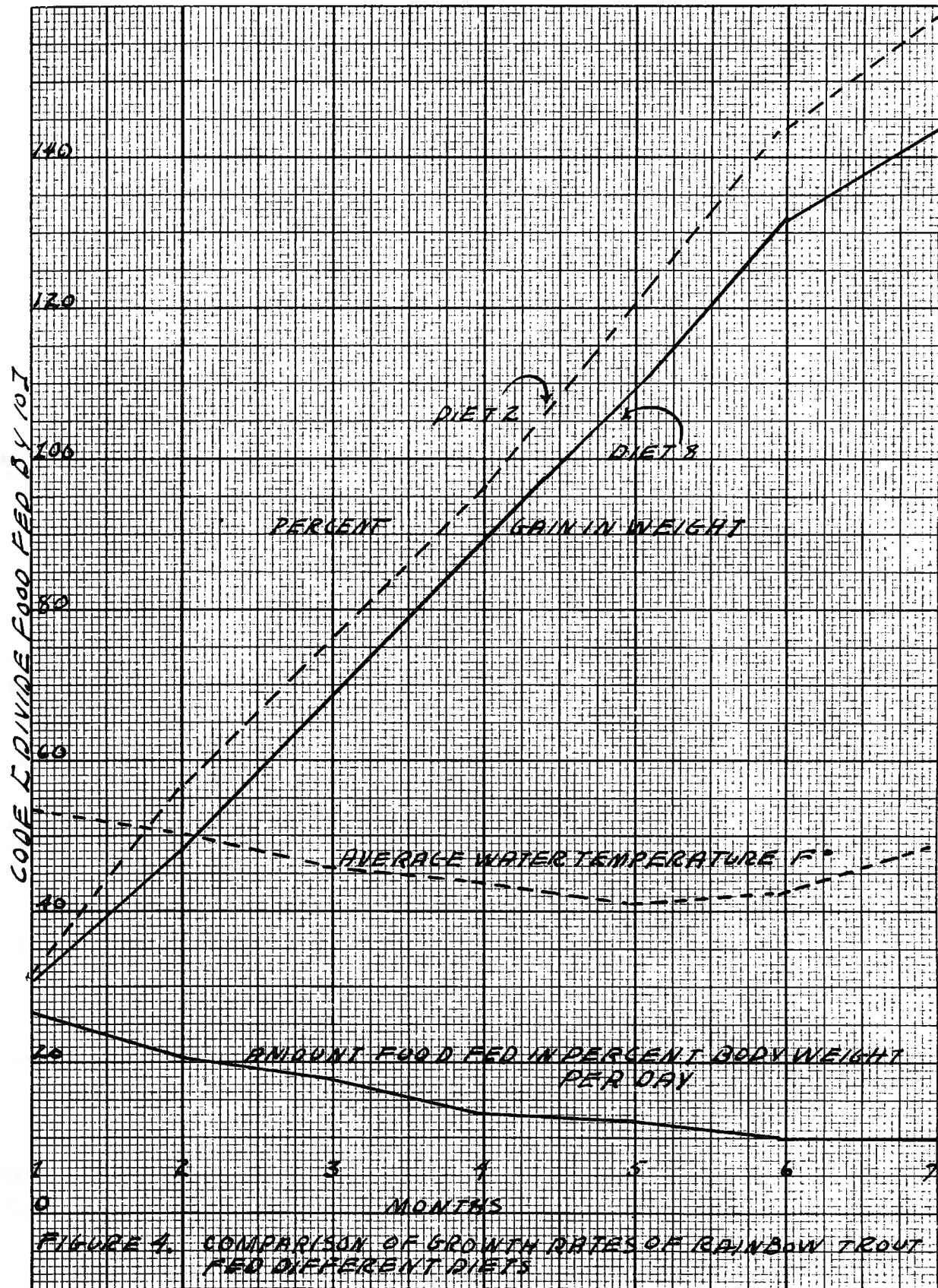
It is evident (Table 6) that reducing the amount of food fed per day from 4.8 to 2.9 percent at a water temperature of 49° F. had no effect on the rate of gain in weight of rainbow trout averaging seventeen fish per pound. From Figure 2 (also others), when the water temperature rose from 42° F. to 48° F. during the last month shown, the rate of gain in weight fell off slightly. At this

time the amount of food fed in percent of body weight was 1.3. The reduction in the rate of gain indicates that the amount of food fed was not adequate to maintain a steady growth rate at this temperature with trout averaging seven per pound. The amount of food necessary daily at these water temperatures and for this size fish (as determined by numbers per pound) appears to be between 2.9 and 1.5 percent of body weight.

The performance of Diets 2 through 7, inclusive, with respect to percent gain in weight, water temperature and amount of food fed in percent of body weight per day when compared to Diet 8 (control) is graphically presented in Figures 3 to 9, inclusive. It will be noted that Diet 2 and Diet 6 produced greater gains per unit of time than any of the other diets with Diets 3 and 4 next in that order. The average rate of growth produced by Diets 1, 5, and 7 was less than that of Diet 8.

Figure 10 compares the rate of growth in percent of body weight gained per twenty-eight day period of Diets 1 and 2; Figure 11, of Diets 3 and 4; and Figure 12, of Diets 6 and 7. It should be remembered (Table 4) that the only difference in the formulation of Diets 1 and 2 was the use of two different yeast products. Diet 1 contained torula yeast and Diet 2 contained brewers yeast.





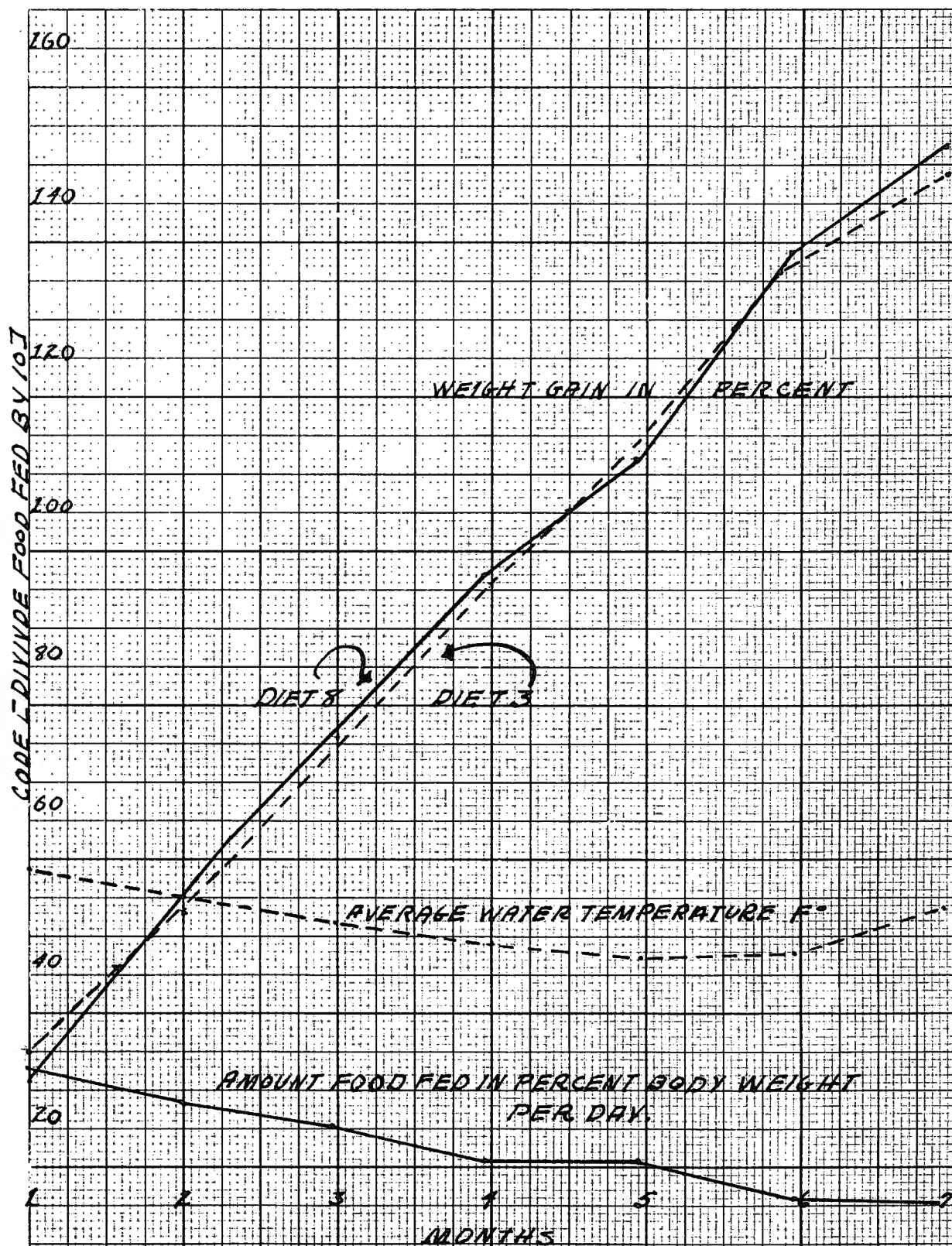
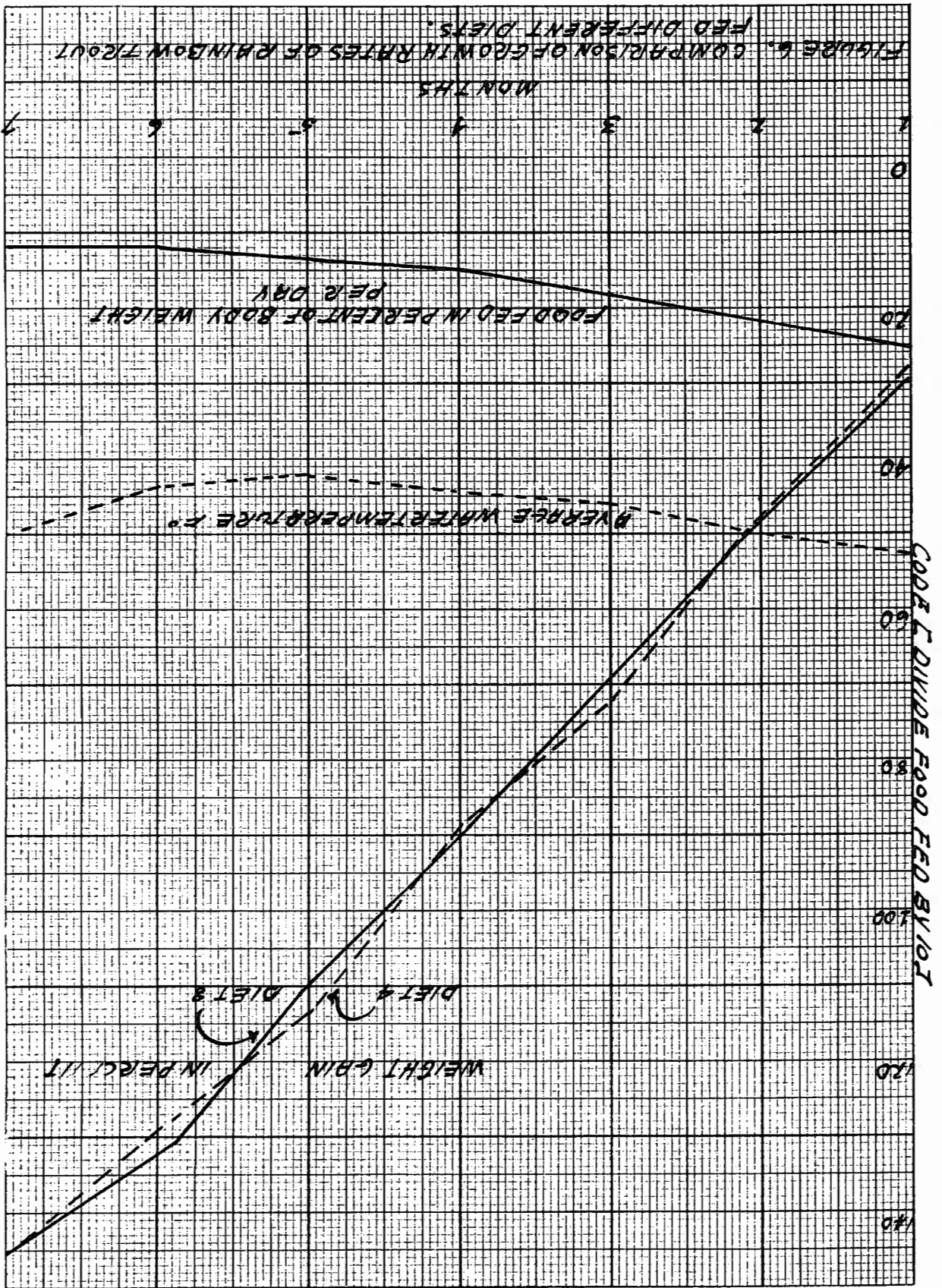


FIGURE 5 COMPARISON OF GROWTH RATES OF RAINBOW TROUT FED DIFFERENT DIETS.



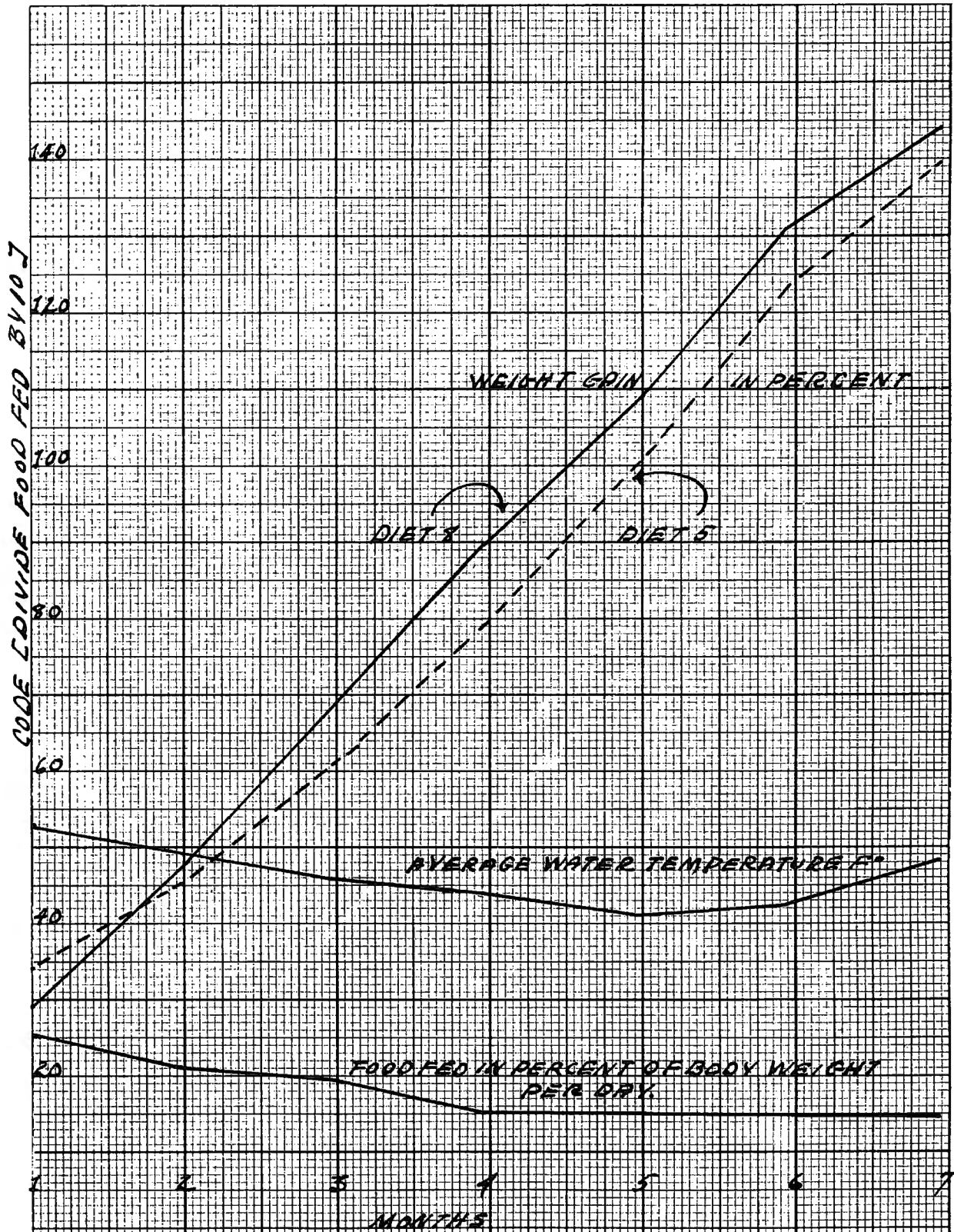


FIGURE 7. COMPARISON OF GROWTH RATES OF RAINBOW TROUT FED DIFFERENT DIETS

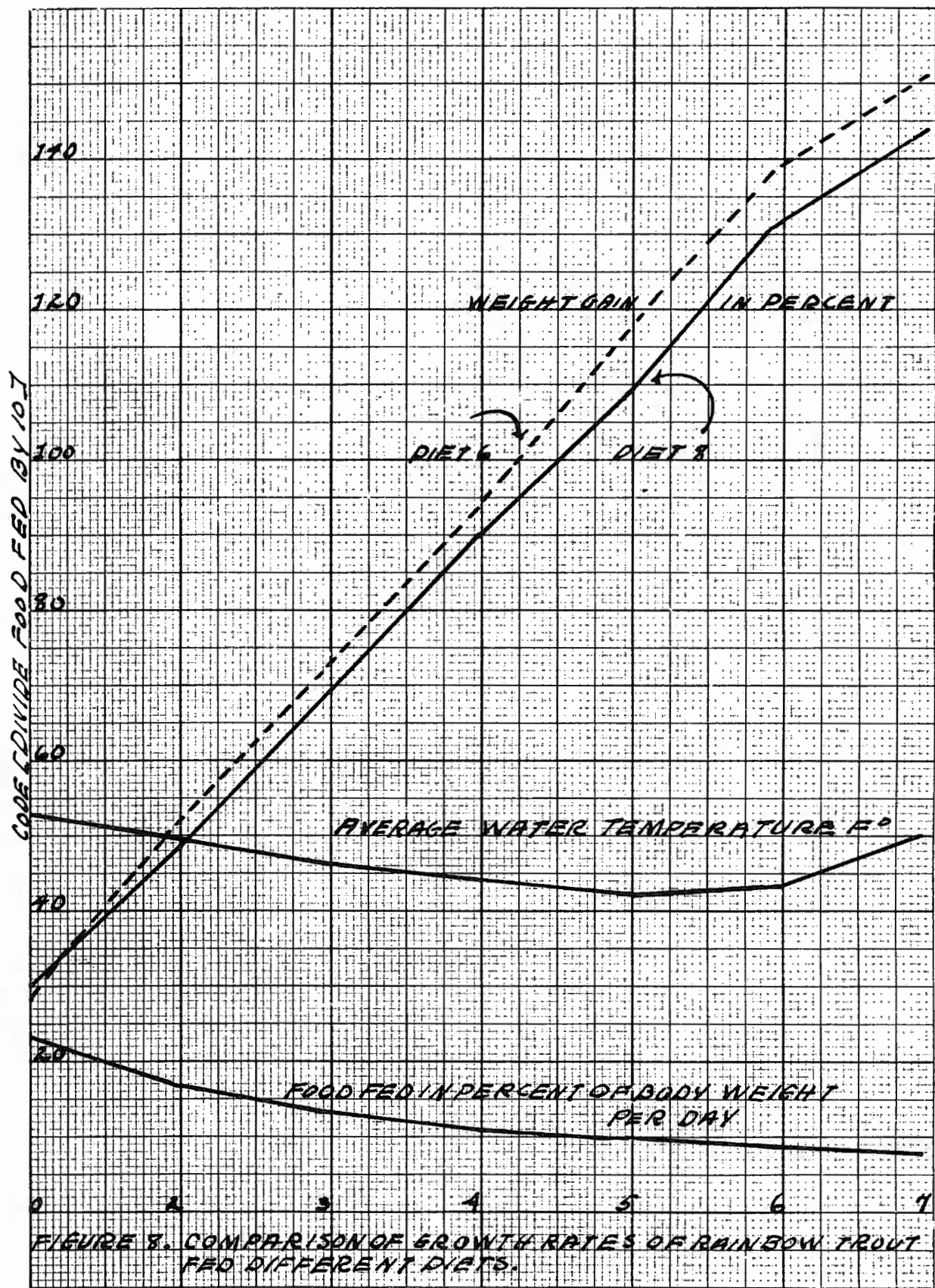


FIGURE 8. COMPARISON OF GROWTH RATES OF RAINBOW TROUT FED DIFFERENT DIETS.

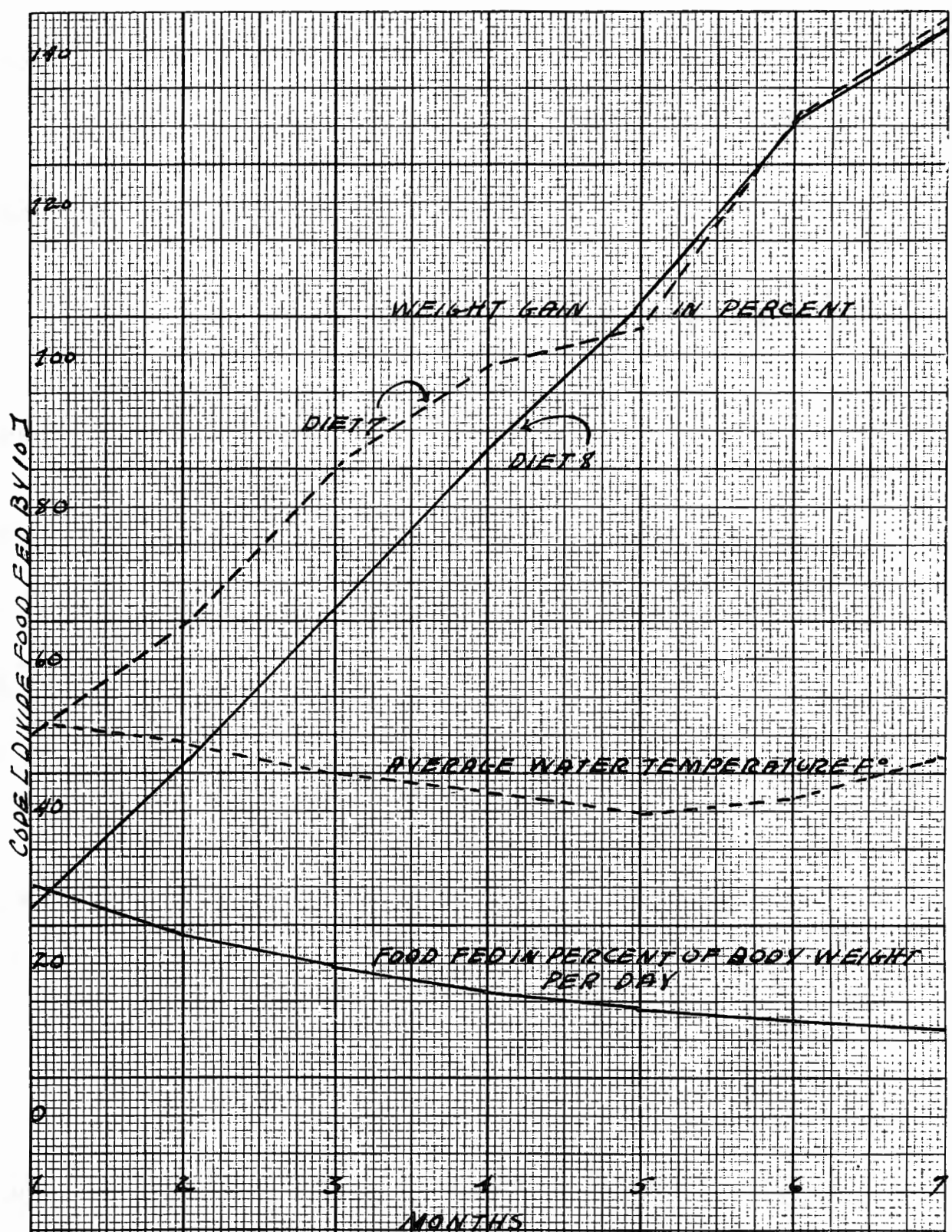
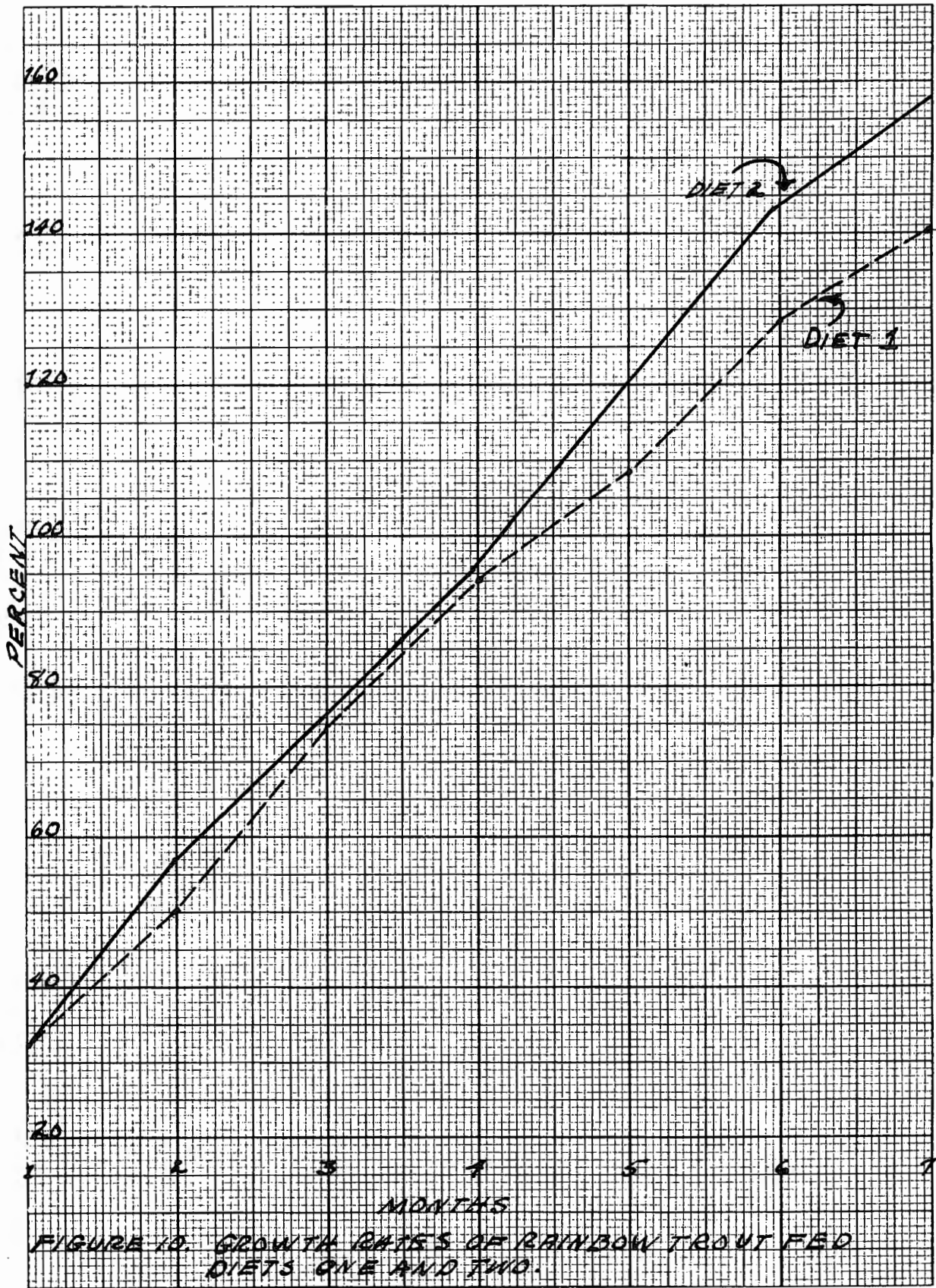
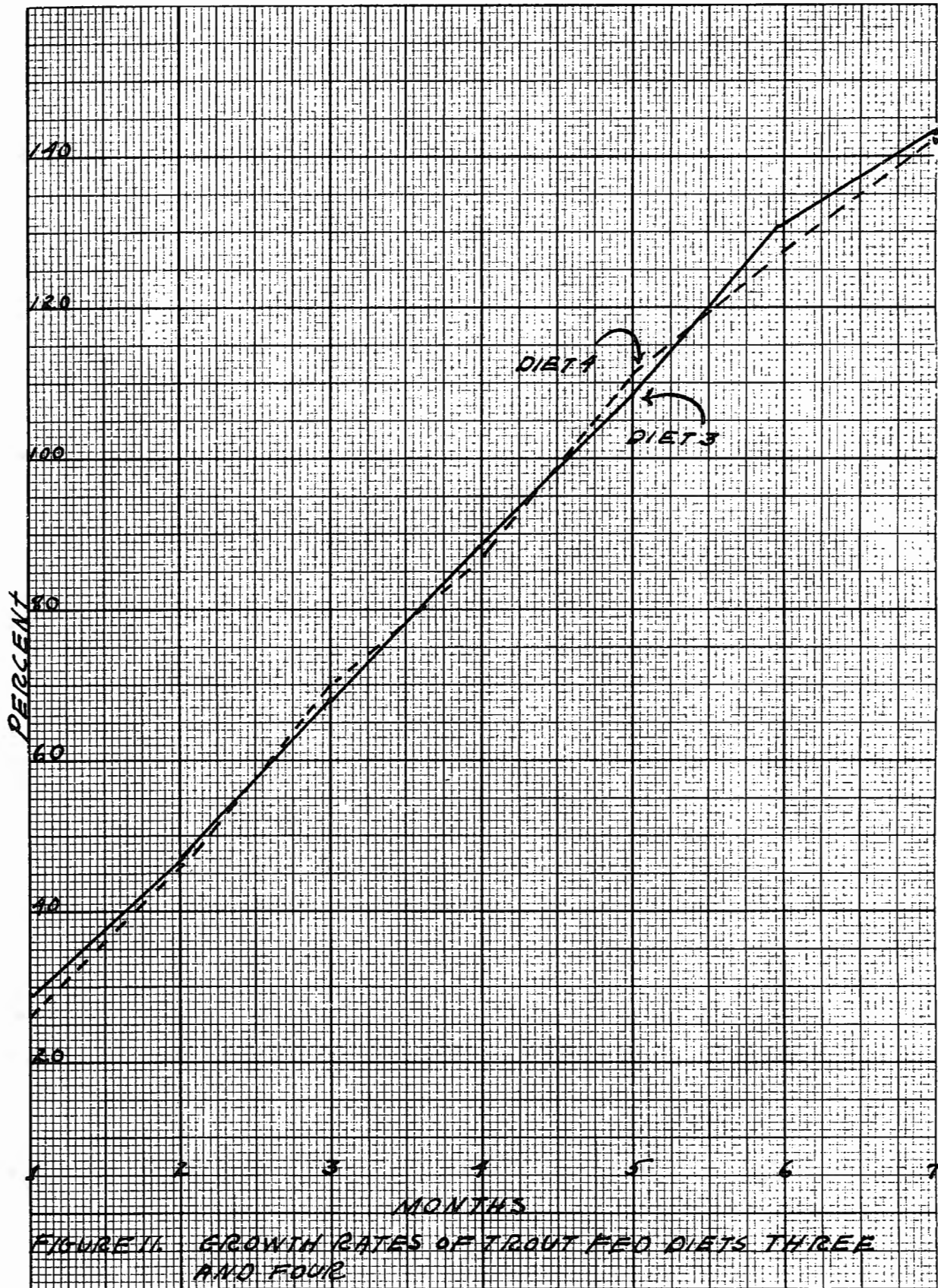
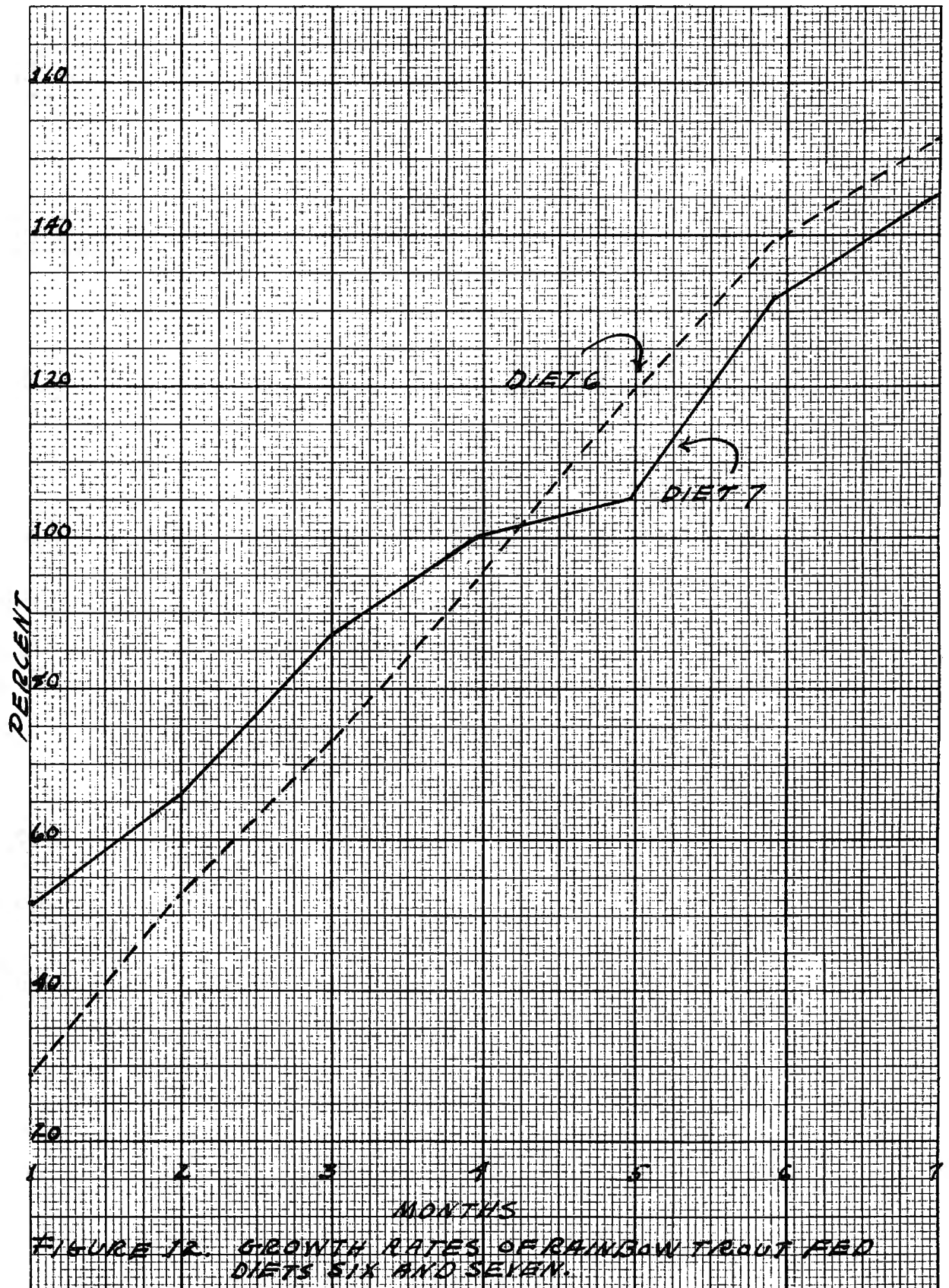


FIGURE 9. COMPARISON OF GROWTH RATES OF RAINBOW TROUT FED DIFFERENT DIETS.







As shown in Figure 10, Diet 2 produced gains considerably higher than Diet 1 during this period. Statistical analysis bears this out (Table 8). Figure 11 shows no difference, as determined by percent gain in weight, between the two yeasts when soybean oil meal was substituted for cottonseed meal. Diet 6, containing gelatin, produced fish at a steadier rate of gain in weight than Diet 7, containing a stabilized fat (Figure 12). Diet 5 showed a rate in gain of weight consistently lower than Diet 8. It is interesting to note that the growth curves for all diets declined from the fifth to the sixth month.

The results of statistical analysis for differences in percent gain in weight (based on cumulative percent gain per two-week period) between groups fed pelleted diets and a raw beef liver diet and between groups fed various pelleted diets showed a highly significant difference ($t = 2.96^{**}$) between Diets 1 and 2 and a significant difference (calculated $t = 2.01$, tabular $t = 2.056$) between Diets 2 and 8 (control; Table 8). The difference between Diets 6 and 8 approached significance. All other diets compared in Table 8 did not differ significantly. Statistical analyses support the graphic differences portrayed in Figures 3 to 12, inclusive.

In Michigan, it is the policy to rear a large portion of the total supply of trout to a legal size of seven inches before planting

Table 8. The results of a statistical analysis for differences in the percentage gains in weight between groups fed pelleted diets and a raw beef liver diet and between groups fed pelleted diets.

Diet Comparisons	Total Cumulative Pct. Gain	Pct. Loss or Gain	Calculated "t" Values
1 ^a and 8 ^b	139.4--143.3	Diet 1, - 4.3	1.27
2 ^a and 8	152.9--143.3	Diet 2, + 9.3	2.01
3 ^a and 8	151.6--143.3	Diet 3, + 8.3	1.65
4 ^a and 8	143.5--143.3	Diet 4, + 0.2	1.30
6 and 8	153.8--143.3	Diet 6, + 10.5	1.96
1 and 2	139.4--152.9	Diet 1, - 13.5	2.96**
3 and 4	151.6--143.5	Diet 3, + 7.9	1.22

^aReplicated diets.

^bControl diet.

Tabular t_{05} , 26 d.f. = 2.056* (significant).

Tabular t_{01} , 26 d.f. = 2.779** (highly significant).

them in streams and lakes. Since this is the policy, many of the experiments were terminated when the majority of fish had reached this size. Table 9 presents by group the number and total weight of legal- and sublegal-sized rainbow trout, the counted total at the end of the experiment, recorded loss, and unaccountable loss. The recorded losses consisted of fish which died from jumping out of the tank and from handling during tank cleaning and weighing operations. The data shown in Tables 9, 10, 11, and 12 were derived from the preceding experiments. Results from groups fed Diets 1, 2, 3, 4, 6, and 8 are shown in Tables 9 and 10, and from groups fed Diets 1, 2, 3, 6, and 8 in Tables 11 and 12. It was never possible to diagnose the cause of death as malnutrition. The extremely high and varied unaccountable losses between groups practically defies analysis. All experiments were conducted inside a hatchery building preventing losses by predators except man himself; therefore, it appears that due to large differences in length of fish in each group cannibalism operated as the major factor in producing the high unaccountable losses. Since special precautionary measures were taken to gain an accurate hand count before, during, and at the end of the experiment by one person verifying the count taken by another at the same time and at separate times, it is felt the numbers of fish presented in Table 9 are accurate.

Table 9. Comparison of number and weight of legal- and sublegal-sized trout in groups receiving five different pelleted diets and one meat diet.

Diet	Legal Size		Sublegal Size ^b		Hand Count (total no.)	Re-corded Loss (no.)	Unac-count-able Loss (no.)
	Wt. (lbs.)	No.	Wt. (lbs.)	No.			
1	130	552	31	311	863	14	123
1	147	713	11	151	864	17	119
2	149	728	13	183	911	43	46
2	150	722	12	158	880	25	95
3	152	723	16	240	963	26	11
3	118	533	18	389	922	19	59
4	99	512	17	303	816	30	151
4	125	631	18	303	934	45	21
6	167	705	9	118	823	40	137
8	166	805	12	143	948	32	20

^a More than 7 inches long.

^b Less than 7 inches long.

Table 10. Comparison of percentage of legal and sublegal rainbow trout fed five different pelleted diets and one meat diet.

Diet	Legal Size ^a		Sublegal Size ^b		Re- corded Loss (pct.)	Unac- count- able Loss (pct.)
	By Wt.	By No.	By Wt.	By No.		
1	80.7	64.0	19.3	36.0	1.4	12.3
1	93.0	82.4	7.0	17.6	1.7	11.9
2	92.0	79.9	8.0	21.1	4.3	4.6
2	92.6	82.0	7.4	18.0	2.5	9.5
3	90.5	75.0	9.5	25.0	2.6	1.1
3	86.8	57.8	13.2	42.2	1.9	5.9
4	85.3	62.7	14.7	37.3	3.0	15.1
4	87.4	67.6	12.6	32.4	4.5	2.1
6	94.9	85.6	5.1	14.4	4.0	13.7
8	93.2	84.9	6.8	15.1	3.2	2.0

^aMore than 7 inches long.

^bLess than 7 inches long.

Grading each lot of fish to a uniform size at the beginning of the experiment would, no doubt, directly prevent cannibalism by establishing more equal competition. However, the fact remains that in several groups some factor other than size differences could have caused a greater cannibalistic urge than in other groups. Since all groups were treated in an identical manner, it appears that a dietary factor may have operated to stimulate or depress this instinct. In one group of trout fed Diet 3 (Table 9) only eleven trout were unaccounted for. A first glance at the composition of Diet 3 suggests that low unaccountable losses could be due to a higher percentage of fish meal, the substitution of soybean oil meal for cottonseed meal, and the complete lack of condensed fish solubles. After more careful scrutiny of the composition of other diets (Table 4) and of the results of duplication (Table 9), it was seen that groups receiving diets other than Diet 3, which contained the same percentage of these ingredients, also showed high unaccountable losses. The data presented here offer inconclusive evidence that one dietary constituent operated more than another to instigate cannibalism; nevertheless, it appears probable that one or perhaps a combination of dietary factors in several diets affected the natural cannibalistic instinct. By further experimentation it is hoped to find a dietary solution for this major problem.

The most significant points shown in Table 9 rest in the considerable variation of legal- and sublegal-sized trout between groups fed various diets. Diet 6 produced a higher percentage of legal-sized fish than any of the others (Table 10). Since percent is based on a total value, it should be pointed out that Diet 8 (raw beef liver) produced a larger number of legal-sized fish but a lower percent than Diet 6. Diets 6, 8, 1, and 2, in that order, produced the highest percentage of legal-sized fish; Diets 3 and 4, the lowest. Recorded losses in groups receiving Diet 1 were the lowest, and those receiving Diet 4 were the highest. However, one group fed Diet 3 showed the lowest total loss of any group.

Table 11 shows the range in inches and the average length in inches at the start and end of the experiment. It is presented here merely to point out the extreme range in length at the end of the period when compared to the length at the start and that a part of the small three-inch group were still present at the end of the twenty-eight week period. At that time these trout were sixteen months old. However, in unsorted fish, the presence of a few three- to four-inch fish in a group of rainbow trout sixteen months old is not uncommon.

The number and average length of sorted fish by size groups at the end of a six-month experimental period is shown in Table 12.

Table 11. Average weight and length of rainbow trout fed various diets at the start and end of a six-month period.

Diet	Start			End		
	Range (in.)	Avg. Length (in.)	Avg. Wt. (g.)	Range (in.)	Avg. Length (in.)	Avg. Wt. (g.)
1	3.1--5.9	4.80	17.9	3.8--9.2	6.4	59.4
2	3.0--4.7	4.06	17.8	3.8--10.4	6.3	54.5
3	2.9--5.1	4.50	17.3	3.2--10.2	6.0	55.0
6	3.0--5.0	4.40	14.8	3.4--9.7	6.0	54.4
8	3.1--5.1	4.53	19.9	3.2--8.5	5.8	54.5

Table 12. Number and average length of sorted rainbow trout at the end of a six-month experimental period.

Diet	Length (inches)		No.	Total Wt. (lbs.)
	Range	Avg.		
<u>Small</u>				
1	3.8--5.9	5.3	54	2.6
2	3.8--5.9	4.9	64	3.0
3	3.2--5.7	4.5	194	7.4
6	3.4--5.7	4.6	237	8.8
8	3.2--6.1	4.7	70	3.3
<u>Medium</u>				
1	5.3--7.3	6.0	269	23.0
2	4.9--7.2	5.9	187	15.3
3	5.0--7.5	5.7	231	18.5
6	5.2--7.3	6.0	231	20.0
8	5.1--7.3	6.0	294	24.7
<u>Large</u>				
1	6.0--9.2	7.9	657	99.3
2	6.1--10.4	7.8	672	123.9
3	6.2--10.2	7.7	504	85.9
6	6.3--9.7	7.5	494	86.7
8	5.6--8.5	6.8	614	95.6
<u>Total Number</u>				
1			980	
2			943	
3			929	
6			962	
8			978	

When comparing Diet 8 with pelleted dry diets, the average length of large fish produced by the raw beef liver diet was approximately one inch less than those from groups receiving dry pellets. All fish in the medium size groups were similar in length to each other as were those in the small size groups. It should be noted, nevertheless, that even though the legal-sized fish in the liver-fed group were shorter one month before termination of the experiment almost the same percent of legal-sized trout were present at the end of twenty-eight weeks as there were in the groups fed pellets (Table 10). This shows that more fish from the medium size group fed liver gained in length to become part of the large size group during the last month than those fed the other diets.

Table 13. Condition factor of rainbow trout fed five different diets over a period of twenty-four weeks.

Diet	Start	End	Difference
1	0.99	1.34	0.35
2	1.63	1.76	0.13
3	1.17	1.52	0.35
6	1.05	1.52	0.47
8	1.30	1.99	0.69

Table 14. Condition factor of three size groups of rainbow trout fed various diets.

Diet	Centi- meters	Grams	A
<u>Small</u>			
1	13.5	21.8	0.89
2	12.4	21.2	1.11
3	11.6	17.3	1.11
6	11.8	16.9	1.02
8 ^a	11.9	21.4	1.27
<u>Medium</u>			
1	15.2	38.8	1.10
2	14.9	37.1	1.12
3	14.7	36.3	1.14
6	15.4	39.3	1.08
8 ^a	15.2	38.1	1.08
<u>Large</u>			
1	20.0	68.6	0.86
2	19.9	83.5	1.06
3	19.6	77.2	1.03
6	19.1	79.7	1.14
8 ^a	17.3	70.6	1.36

^aControl.

EVALUATION OF GROWTH

It is a customary practice to evaluate growth by weight alone in Michigan hatcheries. On a production scale this is probably the most practical method. However, weight alone does not give the necessary information when attempting to evaluate a diet. Length measurements, chemical analysis of various individual parts of the body, and mortality are necessary to determine the value of a diet. Since, in this study, growth in relation to gain in weight and length was used as an index of diet value, it is necessary to briefly describe some aspects of trout growth.

The rate of growth of the rat, chicken, and pig is more rapid than that of trout. Trout can live for long periods without exhibiting growth when placed on a faulty diet (Titcomb et al., 1928). Restricted feeding and extreme water temperatures have a similar effect. A regular rate is assumed as soon as an adequate diet is provided, for the power to grow is not lost. Titcomb points out that since trout growth is slower than other animal growth and therefore a curve representing trout growth has a more gradual slope, trout are excellent experimental animals for studies on growth acceleration.

One factor of outstanding importance in trout production is maximum growth rate. It is believed that in this study maximum growth rate was not attained in any of the groups. A type of unintentional restricted feeding was carried out that did not permit maximum growth; therefore, a depressed growth rate occurred throughout the experiment. This appears to be the case in most hatchery feeding practices. To be sure, an adequate amount of food was fed during any one feeding period, and in many cases not all the food was consumed, but the fact remained that trout would take food again a very short time later. Usually trout are fed six or seven times a day when very small, the number of feeding periods per day gradually being reduced as trout become older until one to three feedings are given per day. Because of these practices, even though at present necessary, the maximum growth rate of trout cannot be demonstrated. It is planned for future experiments to employ an automatic feeding device which dispenses small amounts of food at short intervals in an attempt to determine the effect of continuous feeding on the growth rate of trout. This type of feeding would resemble more closely the type used for other animals which are fed ad libitum. With this method, it is hoped to acquire a more accurate comparison of growth rate between trout and other domestic animals.

CONDITION FACTOR OF RAINBOW TROUT

The condition factor is a term used to express the relationship between the weight and length of trout (Tunison and Phillips, 1939). The formula $100y = Ax^3$ is used to calculate the condition factor where y is the weight in grams, x the standard length in centimeters, and A represents the condition factor. Well-nourished fish should have a higher condition factor than those which have been starved. Usually as fish grow older the condition factor increases. Such factors as sex, spawning period, disease, crowding, and injury can influence the condition factor.

Certain diets, no doubt, influence this factor by the difference in food efficiency due to different dietary combinations. Some combinations may deposit more fat than others and produce a fish heavier but not as long as a fish fed another diet. In Table 12, it is shown that Diet 8 (control) produced trout which were shorter in length than those produced by the other diets.

In an attempt to analyze this difference the condition factor was calculated for a group of trout fed each of five diets at the start and end of the experiment (Table 13), and for three size groups on the same diets at the end of the first six months (Table 14). The

values presented in Table 13 were obtained from the average of 100 fish from each lot. The values presented in Table 14 were obtained from the average of fifty fish in three size groups present in each of the five diet groups. It will be noted that there is an increase in the condition factor in all the groups and between all of the groups in Table 13. Gross examination during and at the end of the experimental period revealed a large deposit of mesentery fat in the liver-fed group. This might have been the result of a high fat-to-protein ratio present in the liver diet. The liver was fed "as purchased"; that is, without removing excess fat.

Size groups of the same age showed considerable variation in condition factor (Table 14). However, here again it was demonstrated that Diet 8 produced fish in each group with a condition factor equal to and greater than those fed other diets. It is clearly shown that diets of the composition used here will influence the condition factor of trout not only in the large size groups but in the medium and small as well.

BROOK TROUT FEEDING EXPERIMENT

This experiment was carried out on a production scale at Marquette, Michigan, and was designed to serve a dual purpose. It was desired to know the performance of a pelleted diet when fed to brook trout as well as when fed to rainbow trout. Also, since a meat supplement was used periodically, it was designed to determine what length of time could intervene between raw meat feedings. Four lots of 4,000 young-of-the-year brook trout were placed in gravel-bottom races sufficiently large to compensate for normal growth during the experimental period without danger of overcrowding. Group 1A received Diet 1 four days and raw beef liver one day per week. Group 1B received Diet 1 nine days and raw beef liver one day every two weeks, and Group 1C received Diet 1 fourteen days and raw beef liver one day every three weeks. A diet consisting of 8 percent torula yeast and 92 percent beef liver served as the control. (This control was used because it was the usual practice at the hatchery to use this mixture for the regular hatchery diet.) The total number of fish from each group were removed at the end of each month and weighed. The methods were similar to those previously described. The experiments were

conducted over a twenty-eight week period. Meat control group received food in amounts equal to 3.6 percent of body weight per day and the other groups received amounts equal to 1.7 percent of body weight per day.

Table 15 presents the complete data of averages of twenty-eight weeks of experimentation. These data furnish information to the effect that feeding raw meat once every five days is not always necessary. All three pellet-fed groups showed lower mortality and a higher rate of gain at a lower cost per pound of fish grown than the control group.

Table 16 shows the difference in each group in respect to conversion, average percent gain at twenty-eight day intervals, cost per pound of trout reared, and cost per hundred pounds of diet. The cost per pound of trout in the meat-fed group was almost triple that of pellet-fed fish, and a lower percent gain was evident.

Table 15. Performance of Diet I when supplemented with raw beef liver one day every week, one day every two weeks, and one day every three weeks, using brook trout as test animals (average water temperature, 40.8° F.).

Group	Meat Control ^a	Group		
		1A	1B	1C
Number at start	4,000	4,000	4,000	4,000
Number at end	3,937	3,958	3,963	3,953
Weight at end	372.5	416.5	392	392.5
Weight at start	144.5	150.0	139	141.0
Gain in weight (lbs.)	228	266.5	253	251.5
Cost per lb. of food	0.10	0.085	0.082	0.082
Conversion:				
Wet	5.0	2.7	2.5	2.4
Dry	3.11	1.6	1.8	1.9
Cost per lb. of fish gain	0.50	0.24	0.20	0.20
Percent of fish gain (total)	104.3	113.2	112.6	116.3
Total lbs. of food fed:				
Wet	1,160.0	652.0	585	568
Dry	810.0	452	473	477
Percent of body weight fed	3.6	1.8	1.6	1.6
Percent mortality	1.52	1.05	0.93	1.04

^aMeat control diet--torula yeast, 8 percent, and raw beef liver, 92 percent--five days per week.

Group 1A--Diet I fed 4 days and raw beef liver 1 day per week.

Group 1B--Diet I fed 9 days and raw beef liver 1 day every 2 weeks.

Group 1C--Diet I fed 14 days and raw beef liver 1 day every 3 weeks.

Table 16. Summary of brook trout growth experiments (average water temperature, 40.8^a F.).

Feeding Schedule	Weeks	Cost per 100 lbs. of Diet	Cost per Pound of Trout Reared	Conver- sion	Pct. Gain per 28 Days
Diet I					
4 days meat 1 day/week . . .	28	\$7.00	23¢	2.7	23.0
Diet I					
9 days meat 1 day/2 weeks.	28	7.00	20	2.5	22.5
Diet I					
14 days meat 1 day/3 weeks.	28	7.00	19	2.3	23.3
Yeast and meat control ^a	28	9.90	50	5.0	20.8

^a 8 percent torula yeast--92 percent raw beef liver 5 days per week.

RED BLOOD CELL COUNT IN BROOK AND RAINBOW TROUT FED VARIOUS DIETS

Early investigators such as McCay and Dilley (1927) found that trout fed synthetic diets for long periods died in great numbers. Wales and Moore (1938) found that a very low red blood cell count occurred in trout when fed a commercial dog food without meat for long periods.

Tunison and Phillips (1939) very astutely showed that after brook trout fingerlings were fed a synthetic diet and developed an anemia (600,000 red blood cells per cubic millimeter of blood) over a period of nine weeks, their blood count could be restored to 1,500,000 red blood cells per millimeter by adding fresh beef liver to the extent of 50 percent of the diet. The same investigators found that house-fly maggots as well as raw beef liver were rich in some factor that cured nutritional anemia developed in trout but that various liver extracts did not. Since then it has been found by Tunison et al. (1943) that many unknown factors influence nutritional anemia in trout and that several vitamins play an important role in its prevention.

Blood Studies

During the past several years, erythrocyte counts were taken periodically from fish fed the dry pelleted diets and from those fed raw beef liver diets to establish the red cell counts for these diets.

The erythrocyte or red corpuscle counts were made on blood taken from the severed caudal peduncle of trout. The blood was diluted with Hayme's solution in a pipette with a red bead by filling the capillary to the 0.5 mark with blood and to the 101 mark with the diluting fluid. The diluted blood was shaken in the pipette for a period of two minutes. After shaking, a small drop of diluted blood was placed on the counting chamber of an AO Bright-Line Haemocytometer and the number of red blood cells counted under a microscope. This value multiplied by 10,000 gave the number of erythrocytes per cubic millimeter of fish blood. Each value in Tables 17 and 18 represents an average of two counts from each of five fish fed each diet.

Rainbow and brook trout with red blood cell counts below 900,000 are considered as approaching an anemic condition. As shown in the two tables both species of trout fed dry pelleted diets for prolonged periods contained numbers of erythrocytes equal to the liver-fed groups. Neither of the groups was considered as

Table 17. Number of red blood cells per cubic millimeter of blood in brook trout.

Date	Group 1 ^a	Group 2 ^b	Group 3 ^c
March 25, 1953		763,000	
May 18, 1953	1,269,000	942,000	
July 12, 1953	1,130,000	844,000	
October 14, 1953	1,111,000	746,000	
February 4, 1955	1,386,000		
February 15, 1955	1,074,000		1,201,000
April 26, 1955	1,170,000		1,116,000
Average	1,190,000	824,000	1,158,000

^aDiet 1, 4 days and raw beef liver 1 day per week.

^bPork liver and melts, 50-50, 5 days per week.

^cRaw beef liver 5 days per week.

Table 18. Number of red blood cells per cubic millimeter of blood in rainbow trout.

Date	Group 1 ^a	Group 2 ^b
May 20, 1953	1,123,000	1,120,000
July 24, 1953	1,130,000	1,200,000
November 6, 1953	1,020,000	1,260,000
April 30, 1954	1,370,000	1,170,000
February 15, 1955	1,074,000	1,201,000
April 26, 1955	1,170,000	1,116,000
Average	1,148,000	1,177,800

^aDiet 1,4 days and raw beef liver 1 day per week.

^bRaw beef liver 5 days per week.

suffering from nutritional anemia except the brook trout fed pork liver and melt mixtures. The total average of red blood cell counts from this group was approximately 366,000 counts below the average of the other groups.

It should be noted that all groups fed pellets in the preceding experiments were fed a raw beef liver diet one day every week. In order to determine to what extent this raw meat supplementation affected the erythrocyte content of fish blood a group of rainbow and brook trout which had been fed Diet 1 four days and raw beef liver one day per week were placed on an all-pellet diet, Diet 1, and were fed for a prolonged period without raw meat of any type. Table 19 presents the red cell counts at the beginning and end of a twelve-month period of rainbow and brook trout when fed an all-pellet diet. For comparison, erythrocyte counts taken from rainbow and brook trout fed a raw beef liver diet also are presented. In all cases but one both species of trout fed Diet 1 showed higher red blood cell counts than those fed raw beef liver. Although not specifically determined, gross examination revealed that there was a large number of cell fragments present in the blood from the groups fed pellets. This may be due to a number of reasons, and no explanation will be attempted at this time.

Table 19. Number of red blood cells per cubic millimeter of blood.

Date	Rainbow Trout		Brook Trout	
	Diet 1	Liver	Diet 1	Liver
March, 1954	1,140,000	1,344,000	1,269,000	1,215,000
March, 1955	1,298,000	1,153,000	1,386,000	1,158,000

DIRECT AND INDIRECT SAVING DUE TO FEEDING
PELLETED DRY MEALS TO HATCHERY-
REARED TROUT

In 1953 the first Michigan State trout food, Diet 1, was formulated and pelleted. After several months of experimentation the results were so favorable that it was adopted by the Michigan Department of Conservation as a standard trout production diet even before experimental tests were completed. Some state fish hatcheries were slow in adopting the pellet food on a production scale, but all without exception are now feeding pellets, resulting in lowered food costs.

Table 20 presents the manner in which trout food funds were utilized and pounds of trout planted for each year starting in 1950 and ending on February 29, 1956. Pounds of trout planted are calculated on a calendar year, expenditures on a fiscal year basis. Fish food expenditures during 1955-56 have not been completely compiled. However, the trend up to this time indicates that expenditures will not exceed those of last fiscal year. Pounds of trout planted for that year are correctly shown.

It will be noted (Table 21) that, since the pellet-feeding program started in 1953, fish food expenditures decreased and number

Table 20. The financial story of fish fund in relation to trout production.

Year	Appropriation	Allotment	Expenditure	Pounds of Trout Planted
1950-51	\$190,000	\$182,715	\$197,083	299,450
1951-52	200,000	200,562	178,751	282,641
1952-53	217,000	200,000	182,344	284,628
1953-54	200,000	183,800	146,896	297,774
1954-55	165,905	165,905	132,392	390,613
1955-56	150,000	150,000	72,970 ^a	417,495
1956-57	140,000	--	--	--

^aTo February 29, 1956.

Table 21. Production costs as compared to the old and new trout feeding regime.

Year	Expenditure Actual Food Cost (pellets and meat)	Pounds Trout Planted	Cost per lb. of Trout	Calcu- lated Cost at 64¢	Differ- ence ^a
1950-51	\$197,083	299,450	65¢		
1951-52	178,751	282,641	63		
1952-53	182,344	284,628	64		
<u>Pellet Feeding Program Started</u>					
1953-54	146,896	297,774	49	\$190,575	\$ 43,679
1954-55	132,392	390,613	33	249,992	117,600
1955-56	72,970 ^b	417,495		267,196	194,226
Total, 1953-56	\$352,258	1,105,882		\$707,763	\$355,505
Other ex- penditures ^c					18,000
					\$337,505

^aCalculated cost at 64¢ minus actual food cost.

^bTo February 29, 1956.

^cSalary and expenses allotted to investigator for formulating and testing dry pellet diets during the 3-year period.

of pounds of trout planted increased. This is directly reflected in the cost per pound of fish produced.

Before the pellet-feeding program was adopted, it cost 64 cents for food to produce one pound of trout. When the cost of total weight of trout planted during the period 1953 to 1956 (the pellet-feeding period) is based on the 64-cent food cost required to raise one pound of trout before pellets were used, total food costs would have been \$707,763 to produce 1,105,822 pounds of trout. However, it cost only \$352,258 to produce this weight of trout. Therefore, it can be definitely stated that as a direct result of the research described in this paper the Michigan Department of Conservation was able to effect a saving in fish food expenditure of \$355,505 during a three-year period.

The indirect saving by use of pellets was also a major advantage. Less labor was needed to prepare and feed pellets as compared to meats and mixed foods. Ponds and pond screens were cleaner. Less refrigerated storage space was needed. Growth of trout was more rapid and more trout reached legal size for planting in the second year that formerly possible. This means fewer trout had to be carried over into the third year, making more pond space available for smaller fish.

POSSIBLE VALUE OF PELLETTED FOOD AS A
MEDIUM FOR DISPENSING MEDICATION
TO TROUT

In Michigan, and in general wherever trout are reared under hatchery conditions, a disease of bacterial origin, Aeromonas salmonicida (commonly known as furunculosis) has caused serious epidemics. It has been stated (McCraw, 1952) that no fish disease is to be feared more by the hatchery man than furunculosis. His review published by the United States Department of Interior presents information gathered from the various literature sources. The disease appears to act more severely in certain species of trout than in others. In Michigan, brown trout, Salmo trutta, are more susceptible than others, and brook trout, Salvelinus fontinalis, are next. Various investigators have determined that many other species of fish contract furunculosis but epidemic status was reached less frequently than in brown or brook trout. A method of treatment often used is to feed a mixture of sulfaguanadine and sulfamerazine with the daily food ration. Two hundred grams of a mixture of one part sulfaguanadine and two parts of sulfamerazine when mixed with 100 pounds of raw meat is fed for a certain number of days depending on the severity of disease present.

In hatcheries where furunculosis frequently reoccurs, it is often necessary to feed successively higher levels of sulfa drugs for adequate treatment or control. Because of this it might be safe to assume that the particular strain of pathogen was becoming somewhat resistant to the drug and that if the levels of drugs administered continued to be increased, the host could be adversely affected before the drug acted on the pathogen. It seems as though medicaments of mild chemical and physiological nature, adapted to continuous feeding, could offer a means for controlling this disease indirectly; that is, by reducing the numbers of organisms of low pathogenicity in the intestinal tract. In other words, "cleaning up" the intestinal tract may be all the pharmacotherapy needed to prevent a serious outbreak of furunculosis.

In order to test this hypothesis, it was necessary first to select a drug or group of drugs that could be applied to continuous feeding without adversely affecting the animal in general.

In investigations conducted with warm-blooded animals it was found that a group of drugs, antibiotics, could be continuously fed effectively, and that benefit was derived from this practice. They were found to: (1) be different in structure and biochemical activity, (2) have considerable variance in spectra, (3) be compatible to other diet ingredients when mixed together, and (4) be essentially

nontoxic even in excess quantities. Besides suppression of organisms causing subclinical diseases, antibiotics work to suppress intestinal organisms which compete with an animal for vitamins in the food it eats and thins the walls of the intestine permitting better absorption of vitamins and nutrients in the body. This group of drugs appeared to provide the physical and chemical qualifications necessary to test the hypothesis previously described.

Recently a new type of trout food was adopted by the state of Michigan. It consists of animal and vegetable meals pelleted in various sizes. Trout food in this form offers several advantages, for the introduction of medicaments to trout. Medicated diets in pellet form could be stored for several months at each hatchery, thus insuring an adequate supply at all times. Daily mixing of drugs with other diet ingredients could be eliminated. Drugs in dry meal diets could be more uniformly distributed, thereby increasing control of amounts fed.

In 1953, a broad spectrum antibiotic, aureomycin, was mixed with dry meals and pelleted. An animal feed supplement containing 10 grams of chlortetracycline (Aureomycin, trade mark of the American Cyanamid Company for the antibiotic chlortetracycline) per pound was used at a 1 percent level, furnishing 200 grams aureomycin activity per ton of finished feed. This level was based on that found

to be compatible to poultry feeds. This diet was fed for a six-month period to rainbow and brook trout fingerlings. Being a pilot experiment, it was conducted solely to determine the gross effect of aureomycin as a trout food additive. All trout reacted favorably, and it was not possible from the design of this experiment to attribute any advantages or disadvantages to the presence of the drug in the diet. To further test the hypothesis it was necessary, secondly, to wait for the disease to appear. It was felt that if trout contracted furunculosis in the usual manner rather than through mechanical injection of the disease-producing organism, the condition would be more representative of that occurring during normal hatchery-trout production. Since it was suspected that Aeromonas salmonicida is constantly present in hatchery water supplies where repeated outbreaks of furunculosis occur and since it is possible to rear trout in such waters, either the fish are more physiologically resistant at one time than another or the pathogen is not able to exert its full destructive force at all times due to variations in environmental conditions and particularly to variations in intercurrent subclinical infections. Therefore, it might be expected that injecting fish with pathogens to produce an artificial epidemic would produce results less valid than those from fish contracting furunculosis from endemic contamination.

Since it was already demonstrated that normal rainbow and brook trout were not appreciably affected by continuous feeding of aureomycin at a certain level, a diet containing this drug was kept in stock ready for instant use in the event furunculosis appeared in any group of trout.

An opportunity to test the disease-prevention properties of aureomycin was presented in September, 1955. Furunculosis was detected in a group of two-year-old brook trout. These trout had been reared in hatchery concrete tanks since hatching and had been sustained on an all-dry-meal diet. Immediately upon detection of the disease, all trout were counted. One-half, 106 in number, were placed in an adjacent tank, and one-half were allowed to remain in the original tank. A pelleted diet containing 200 grams of aureomycin per ton of feed was used for the medication. The group of 106 in the original tank were fed the medicated diet in amounts equal to 1.7 percent of body weight per day, and the other group continued to receive the pelleted diet fed before the disease was noted at 1.7 percent of body weight per day. Since a pelleted medicated diet was on hand, the experiment was in effect two hours after detecting the disease. Both groups were weighed periodically but emphasis was placed on mortality rather than growth increases.

During the course of the experiment, September, 1955, to March, 1956, both lots of fish were artificially spawned and the fertilized eggs incubated in water at 42° F. At the time of spawning the medicated group had been fed aureomycin continuously for two months. The total number of trout which died from each group is shown in Table 22.

As shown in Table 22, there was a wide difference between the number of fish surviving in the two groups. From these results it appears that aureomycin contributed to the well-being of the fish afflicted with furunculosis. Since the only known variable present in the diet was the level of aureomycin fed, it is necessary to conclude that there was a very good chance that the medicated diet either abetted recovery of those fish already afflicted and/or served as a preventive measure in those fish not yet under the influence of bacteria multiplication. Since, during this period, fish in both groups were spawned, a portion of mortality must be contributed to results of rough handling necessary during this operation. However, both groups were handled in a similar manner; therefore, it is unlikely that such a wide difference in mortality occurred due to spawning operations alone. Data pertaining to the progeny of both groups are included only to show that (1) egg production and fertilization is possible in fish suffering from furunculosis, and

Table 22. Function of aureomycin in brook trout diets.

Date	Medicated Diet		Nonmedicated Diet	
	Mortality	Total No.	Mortality	Total No.
	<u>Total Number at Start</u>			
		106		106
	<u>Before Spawning</u>			
September 30, 1955	5	101	5	101
October 31, 1955	8	93	5	96
November 18, 1955	5	88	34	62
	<u>After Spawning</u>			
November 30, 1955	1	87	14	48
December 31, 1955	9	78	18	30
January 31, 1956	11	67	4	26
February 29, 1956	9	60	4	22
Total number up to Febru- ary 29, 1956	48	60	84	22
Total percent mortality . .	43		79.2	
Percent of eggs hatched . . .		57.5		23.1

(2) continuous medication by feeding aureomycin for two months before spawning and under the conditions described above does not adversely affect fertilization and hatchability. Progeny are currently being reared on a medicated and nonmedicated diet to correspond with the diet received by the parent fish. It is planned to continue to feed the progeny as well as the parent fish this medicated diet in an attempt to determine the effect of aureomycin on reproduction when fed for longer periods. There is also a good chance that all groups will contract furunculosis again during the next year which, if it takes place, will provide an excellent opportunity to test the validity of the experiment just described.

It must be pointed out that the results described here are based on only one experiment. Further investigation is necessary to definitely prove the value of antibiotics as a means for preventing diseases in hatchery-reared trout.

DISCUSSION

Due to the basic nature of the studies reported herein, it was necessary to use certain experimental procedures which do not conform with methods usually used. An important difference was the manner of formulating the first diet for nutritional studies. Instead of formulating a diet solely for its nutritional quality, it was necessary to place greater emphasis on pelleting properties and performance of the pellet in a water medium.

The earliest experiments reported in this paper were conducted with trout whose diet, before pellet feeding started, consisted of relatively soft meat diets. It was found that the transition from feeding soft meat diets to feeding hard pellet diets required an adjustment period of several weeks. This adjustment period for rainbow trout may extend over four weeks.

From the experiments conducted, it could not be demonstrated conclusively that rainbow trout prefer one pellet size more than another. Trout are able to ingest large particles of soft pliable food but are reluctant to ingest large particles of coarse food which does not crush easily when moved through the pharyngeal area. The physical properties of trout foods need to be investigated further.

There is much room for improvement. Personal observations made during these experiments indicated that a dry diet in the form of ribbons or short tubes would be more suitable than solid pellets. Experimentation directed toward developing different forms of dry diets needs to be carried out not only from the standpoint of trout acceptance but from rate of digestion as well.

The tabular data portray better than words the results of the experiments conducted. To attempt an evaluation of individual dietary constituents on the basis of the data presented here would be unwise. All of them are acceptable as trout diet components and all warrant further study when considering their relationship to each other and their relative nutritional value as food for trout.

From Table 7 it is seen that Diet 1, when fed concurrently to two groups of rainbow trout, produced nearly identical results in all conditions shown. Conversion factors for all diets are small, including the raw beef liver control. Since a restricted form of feeding was employed throughout these experiments, higher conversion factors would be expected when trout are fed on a production scale. Usually greater volumes of food are fed for production purposes. Nevertheless, it should be pointed out that when raw meats are carefully fed, a conversion factors as low as 3.0 can be attained. This, however, is reflected in a slower growth rate per unit of time.

When fed on a production basis to brook trout, pelleted food conversion factors were one-half those of raw meat conversion factors (Table 17) and still produced a great percent gain in weight.

Diets 1 and 2 and Diets 3 and 4 can be compared in respect to diet variables. Diets 5 and 7 must be eliminated due to incomplete and unreliable data. Circumstances beyond the control of the investigator necessitated changing procedures two-thirds way through the experiment. The data are presented only to show that trout can be reared for twenty-eight weeks on these diets.

The dietary variables in Diets 1 and 2 are the types of yeast used. Diet 2, containing brewers yeast (Table 4), outperformed Diet 1 containing torula yeast in all respects, but recorded losses. In both groups receiving Diet 2 the recorded losses were approximately double those of groups receiving Diet 1. But, the total percent mortality for the groups receiving the two diets is reversed.

Groups fed Diets 3 and 4 were very similar to each other in all respects, with Diet 3 producing somewhat better results. All diets containing brewers yeast at a 5 percent level showed greater gains at lower costs, although with higher mortality, than those containing 5 percent torula yeast. On the basis of these experiments and considering all factors, both yeasts can be recommended at a 5 percent level for pelleted trout diets.

When evaluating the diets on the basis of legal-sized trout produced during a given time, Diet 6 must be rated higher than the rest. When the legal-sized trout produced in percent by weight is considered for each diet, all diets were about equal. But, when the legal-sized trout produced by number is considered, Diets 3 and 4 produced a lower number (Table 10). Diets 6, 8, 1, and 2, in that order, produced the highest percentage of legal-sized fish. Recorded losses in groups receiving Diet 1 were the lowest, and those receiving Diet 4 the highest. From the data presented it is concluded that pellet-meat combinations as fed during these experiments will consistently grow approximately as many legal-sized trout as a raw beef liver diet during comparable periods at a food cost one-third of that of raw liver diets and with less labor.

It is always a problem when large numbers of animals are used for experimentation to obtain size uniformity between individuals. From Table 9 it is obvious that the unaccountable losses for Diets 1, 4, and 6 far outnumber the recorded losses. At first glance and recalling the methods used, one might conclude that all the high losses are due to cannibalism. However, more careful analysis will reveal that this cannot be the only reason. All trout fed the same diet were treated in an identical manner, and the only difference between dual groups was dietary. There was a wide difference

(Table 9) in the unaccountable losses in groups fed the same diet (Diet 4) as well as different diets. Gross observations during the experimental period revealed that considerable "nipping" was going on in all but the raw beef liver control group. The "nipping" was confined to the dorsal and caudal fin areas. In some instances it became so severe that the whole fin was removed or fin-rays were left as independent rods. To the casual observer the condition resembled a serious case of fin rot. This "nipping" tendency appears to be more characteristic of rainbow trout than of brook trout. Some factor was operating in the control group which reduced this tendency. Whether dietary or otherwise is not known. It should be recalled that these rainbow trout were reared in concrete tanks of rather limited area.

The condition factor of rainbow trout allows the nutritionist some insight as to the relationship between weight and length. Among other things, numerical differences in this factor can be due to dietary factors. In Table 12 it was shown that Diet 8 (control) produced trout shorter but approximately as heavy as those from the large-sized groups fed pellet diets. From the equation shown, it can be demonstrated that when there is a weight increase without length increase the condition factor will increase.. This then means that, since the condition factors of trout fed pellets (Table 13) were

less than that of the control, the trout fed pellets were increasing in length more than meat-fed trout, but with less increase in breadth and depth of body dimensions.

Usually, gain in length or height is associated with growth of protein tissue, whereas depth and breadth gains are associated with fat deposition as well as protein tissue formation. Chemical analysis to establish fat-to-protein ratio was not carried out; therefore, it cannot be definitely concluded that pelleted diets will produce more protein tissues than a raw beef liver diet. It appears, however, that with more experimentation a dry pelleted diet could be formulated to produce, up to a reasonable point, the desired level of fat and protein ratio in trout.

Condition factors for small, medium, and large trout of the same age are shown in Table 14. Here again factors in the small and large groups are higher for Diet 8 than the others, but equal to the average in medium group.

Determining the well-being of trout in Michigan by red blood cell counts has been a common practice. Before 1953, when dry vegetable and animal meals were fed as a large portion of the total diet a dietary anemia developed in trout. The results of current experiments show that dry vegetable and animal meals, in the combinations used during this experiment, will not develop this anemic

condition in trout. Erythrocyte numbers below 900,000 are considered as an indication of the start of an anemic condition. As shown in Tables 17 and 18, rainbow and brook trout fed a pelleted dry diet produced numbers of red blood cells comparable to those of trout fed raw beef liver and greater numbers than those fed pork liver and melts. At no point throughout this experiment and from production trout in other hatcheries was it possible to demonstrate anemia to be due to pelleted dry foods even when fed one year without raw meat supplementation.

To demonstrate the practical application of the pelleted diets tested, the performance of Diet 1 when compared to a meat control is shown in Tables 15 and 16. These data were gained from the usual production records kept by state fish hatchery personnel in Michigan. As shown, feeding raw meat once every four or five days is not always necessary. All three diet combinations will produce brook trout at a higher rate of gain and at a lower cost per pound of fish reared than a raw meat diet. The difference in mortality between the groups was negligible.

From these experiments it can be concluded that brook trout will grow at a normal rate when fed a pelleted diet periodically supplemented with meat for a period of twenty-eight weeks and that when necessary meat supplementation can be withheld for as

long as three weeks without adversely affecting the rate of growth and without increasing mortality,

A direct and indirect saving due to feeding pelleted dry meals to hatchery-reared trout can be definitely demonstrated. The use of pellets instead of raw meats has effected a reduction of food costs per pound of fish raised, from 64 cents to 18 cents per pound of trout produced on a state-wide basis. It can be stated that as a direct result of the research described in this paper, the Michigan Department of Conservation was able to effect a direct saving in fish food expenditures of \$355,505 during the three-year period. Stated in another way, 60 percent more trout were produced on 40 percent less money spent for fish food.

SUMMARY AND CONCLUSIONS

In feeding trials conducted with rainbow and brook trout several pelleted diets were formulated and tested, the results of which have been presented.

The study was directed toward the ultimate goal of developing practical pelleted dry diets which could be fed to trout to produce strong, healthy, legal-sized fish in the shortest time possible and eliminate the need for raw meat mixtures as much as possible.

Large numbers of trout were used as experimental animals both on an experimental scale and on a production scale.

From the data obtained, the following conclusions were drawn:

1. The binding properties of feed constituents used for Diets 1 through 7 were suitable for pellet production. These combinations remain intact in pellet form for as long as eight minutes when placed in a water medium.

2. As determined by loss and gain of body weight, rainbow trout require one to two weeks to adjust from a relatively soft meat diet to a hard pelleted diet. Rainbow trout lose weight during the adjustment period but begin to regain it after the first two weeks of pellet feeding.

3. Rainbow trout averaging 4.5 inches in length will eat pellets $3/16$, $1/8$, and $3/32$ inch in diameter and $1/4$ inch long. The evidence from this study is inadequate to demonstrate definitely that any size pellet is preferred by rainbow trout 4.5 inches long.

4. Dry pellet diets can be fed at levels one-half to five-eighths lower than those recommended for raw meat mixtures without reducing trout growth rates.

5. The difference between Diet 1 (containing torula yeast) and Diet 2 (containing brewers yeast) and between Diet 2 and Diet 8 (control) was statistically significant. Under the conditions described and in the combinations used, brewers yeast was superior to torula yeast when determined by percent gain in weight of rainbow trout. Also statistical analysis showed Diet 2 to be superior to Diet 8 (control) and Diets 1, 3, 4, and 6 to be equal to Diet 8.

6. Feeding the dry diets described in this paper to rainbow and brook trout did not cause a reduction in numbers of red blood cells per millimeter of blood as was originally thought. Counts were as high and higher than those from rainbow and brook trout fed an all-beef liver diet. At no time during these experiments and in hatcheries where the diets were fed on a production scale was nutritional anemia evident. Feeding Diet 1 and Diet 2 for

twelve months to rainbow and brook trout without raw meat supplementation did not cause a reduction in numbers of red blood cells.

7. Feeding a raw meat supplement can be dispensed with, on a production scale, for as long as three weeks without adversely affecting trout growth rate.

8. All of the diet ingredients listed in Table 4 are suitable, in the combinations shown and with a weekly meat supplementation, to rear rainbow and brook trout on a production scale.

9. The dry pelleted rations used during these experiments are superior to raw meat diets in enough respects to warrant their adoption for full-scale hatchery feeding. A direct result of the adoption of dry pelleted diets 1 and 2 by all the state fish hatcheries in Michigan as standard diets increased trout production (in pounds) by 60 percent and reduced fish food expenditure by 40 percent.

10. As demonstrated by one experiment, antibiotics may play a major role in bacterial disease prevention in trout when fed as a constituent of dry pelleted rations.

11. Suggestions for future studies are indicated.

BIBLIOGRAPHY

Agersborg, H. P. K.

1934. An inexpensive balanced diet for trout and salmon. Trans. Am. Fish. Soc. 64, 155-162.

Brockway, Donald R.

1953. Fish food pellets show promise. Prog. Fish Cult. 15 (2), pp. 92-93.

Churchill, W. S.

1944. The food of trout. Wisconsin Conserv. Bull. 9, No. 5, 3-6.

Davis, H. S.

1935. Cheaper trout foods. Prog. Fish Cult. No. 9, 7-10.

Davis, H. S., and Lord, R. F.

1929. The use of substitutes for fresh meat in the diet of trout. Trans. Am. Fish Soc. 59, 160-167.

Duel, Charles R., Haskell, David C., and Tunison, A. V.

1942. The New York State Fish Hatchery feeding Chart. Fisheries Research Bull. No. 3, N.Y. Conser. Dept., Albany, N.Y.

Donaldson, L. R.

1935. The use of salmon by-products as food for young king salmon. Trans. Am. Fish. Soc. 65, 165-171.

Einarsen, A. S., and Royal, L.

1929. What shall we feed? Trans. Am. Fish. Soc. 59, 268-271.

Foster, F. J., Pelnar, J., and Hagen, W.

1939. Preliminary results of an experiment on the inclusion of apple flour in foods fed to young salmonoids. Prog. Fish Cult. No. 47, 49-51.

Gutsell, J. S.

1939. Fingerling trout feeding experiments, Leetown, 1938.
Prog. Fish Cult. No. 45, 32-41.

Haempel, O.

1927. The significance of vitamins in the food of domestic animals, especially fish. Allg. Fischerei-Zeitg. 2, 20-23; Biol. Ab. 4, No. 2, 4135 (1930).

Hagen, W.

1940. Suiting the diet to the hatchery. Prog. Fish Cult. No. 51, 11-15.

Heard, R. D. H.

1930. Investigations on the nutrition of speckled trout. Trans. Am. Fish. Soc. 60, 140-145.

Hewitt, E. W.

1937. Some recent work on fatty livers in trout. Prog. Fish Cult. No. 27, 11-15.

Ide, F. P.

1942. Availability of aquatic insects as food of the speckled trout, Salvelinus fontinalis. Trans. North Amer. Wildlife Conf. 7, 442-450.

Idyll, C.

1942. Food of rainbow, cutthroat and brown trout in the Cowichan River system, British Columbia. J. Fish. Res. Bd. Can. 5, No. 5, 448-458.

McCay, C. M.

1934. Growth. Prog. Fish. Cult. No. 1, 2-7.

McCay, C. M.

- 1937a. Observations on the nutrition of trout. The progressive Fish Cultust, U.S. Bureau of Fisheries, Memo. 1-131, No. 30, pp. 1-24.

McCay, C. M.

- 1937b. The biochemistry of fish. Annual Review of Biochemistry, Vol. VI, pp. 445-468.

McCay, C. M.

1939. The vitamins. (A symposium). pp. 599-600, The American Medical Assoc., Chicago.

McCay, C. M., and Dilley, W. E.

1927. Factor H in the nutrition of trout. Trans. Am. Fish. Soc. 57, 250-260.

McCay, C. M., and Phillips, A. M.

1940. Feeds for the fish hatcheries. Prog. Fish Cult. No. 52, 18-21.

McCay, C. M., and Tunison, A. V.

1935. Report of experimental work at the Cortland (N.Y.) experimental hatchery for the year 1934. Prog. Fish Cult. No. 7, 8-9.

McCay, C. M., Tunison, A. V., Crowell, M., and Paul, H.

1936. The calcium and phosphorous content of the body of the brook trout in relation to age, growth, and food. J. Biol. Chem. 114, No. 1, 259-263.

McCay, C. M., Tunison, A. V., Crowell, M., Tressler, D. K., McDonald, S. P., Titcomb, J. W., and Cobb, E. W.

1931. The nutritional requirements of front and chemical composition of the entire trout body. Trans. Am. Fish. Soc. 61, 58-82.

McCraw, Bruce M.

1952. Furunculosis of fish. Special Scientific report: Fisheries No. 84.

McLaren, B. A., Herman, E. F., and Elvehjem, C. A.

1947. Nutrition of trout, studies with practical diets. Proc. Soc. Exptl. Biol. Med. 65, 97-101.

McLaren, Barbara A., Keller, Elithabeth, O'Donnell, D. John, and Elvehjem, C. A.

1947. The nutrition of rainbow trout. I. Studies of vitamin requirements. Arch. Biochem. 15, No. 2 (169-178).

Phillips, A. M., Brockway, D. R., Rodgers, E. O., Robertson, R. L., Goodell, Herbert, Thompson, J. A., and Willoughby, Harvey.

1947. The nutrition of trout. Fish. Res. Bull., No. 10, Cortland Hatchery Rept., No. 16, 35 pp.

Phillips, A. M., Tunison, A. V., Fenn, A. H., Mitchell, C. R., and McCay, C. M.

1940. The nutrition of trout. Cortland Hatchery Rept. No. 9, 30 pp., N.Y. State Conservation Dept., Albany, N.Y.

Raney, E. C., and Lachner, E. A.

1941. Autumn food of recently planted young brown trout in small streams of central New York. Trans. Am. Fish. Soc. 71, 106-111.

Sekine, H., and Kakizaki, Y.

1931. Biochemical studies on salmonidae, VII. J. Agr. Chem. Soc. Japan 7, 36-40; C.A. 25, No. 13, 3401 (1931).

Snedecor, George W.

Statistical Methods. 4th edition. Iowa State College Press, Ames, Iowa.

Stiles, E. W., and Russ, H. C.

1938. Meat diet supplements, and their effect on growth and health of fingerling trout. Prog. Fish. Cult. No. 37, 10-16.

Thompson, W. T.

1929. A rival to liver. Trans. Am. Fish Soc. 59, 168-173.

Titcomb, John W., Cobb, Eben W., Crowell, Mary F., and McCay, C. M.

1928. The nutritional requirements and growth rates of brook trout. Trans. Am. Fish Soc. Vol. 58, pp. 205-231.

Titcomb, J. W., Cobb, E. W., Crowell, M. and McCay, C. M.

1929. The relative value of plant and animal by-products as feeds for brook trout and the basic nutritional requirements of brook trout in terms of proteins, carbohydrates, vitamins, inorganic elements and roughage. Trans. Am. Fish. Soc. 59, 126-145.

Tunison, A. V., Brockway, D. R., Maxwell J. M., Dorr, A. L., and McCay, C. M.

1942. The nutrition of trout. Fish. Res. Bull. No. 4, N.Y. State Conserv. Dept., Bur. Fish Culture, Albany, N.Y., Cortland Hatchery Rept. No. 11, 45 pp.

Tunison, A. V., Brockway, D. R., Schoffer, H. B., Maxwell, C. M., McKay, C. M., Palm, C. E., and Webster, D. A.

1943. The nutrition of trout. Cortland Hatchery Report No. 12. New York Conservation Department, Albany, New York.

Tunison, A. V., and McCay, C. M.

1933. The nutritional requirements of brook trout. Trans. Am. Fish. Am. Fish Soc. 63, 167-177.

Tunison, A. V., and McCay, C. M.

1935a. The nutritional requirements of trout. Prog. Fish Cult. No. 11, 17.

Tunison, A. V., and McCay, C. M.

1935b. The nutritional requirements of trout. Trans. Am. Fish. Soc. 65, 359-375.

Tunison, A. V., and Phillips.

1939. The nutrition of Trout. Cortland Hatchery Report No. 8 for the year 1939, New York State Conservation Department, Albany, N.Y.

Tunison, A. V., Phillips, A. M., Shaffer, H. B., Maxwell, J. M., Brockway, D. R., and McCay, C. M.

1944. The nutrition of trout. Fish Res. Bull. No. 6, N.Y. State Conserv. Dept., Albany, N.Y., Cortland Hatchery Rept. No. 13, 20 pp.

Wales, J. H., and Moore, Myron.

1938. Progress Report of Trout Feeding Experiments, 1937. California Fish and Game, 24, 126.

Welles, G. D.

1944. Trout and their favorite food. Game Breeder and Sportsman 49, No. 7, 78-79, 84-85.

Wilkinson, J. T.

1938. Notes on the use of supplements for fresh meat in the propagation of brook, rainbow, and brown trout in Michigan. Trans. Am. Fish. Soc. 68, 96-117.

Willoughby, Harvey.

1953. Use of pellets as trout food.
Prog. Fish Cult. 15 (3): 127-128.

Wolf, Harold.

1953. Colored Fish-Food Pellets.
Prog. Fish Cult. 15 (4): 182.

Wolf, L. E.

1945. Dietary gill disease of trout. Fish. Res. Bull. No. 7, N.Y. State Conserv. Dept., Bureau Fish Culture, Albany, N.Y., 32 pp.

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