

CHANGES IN MINERAL, CRUDE FAT, AND MOISTURE LEVELS
OF CHANNEL CATFISH (ICTALURUS PUNCTATUS)
AS EFFECTED BY SUPPLEMENTAL DIETS

by 1050 710

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INTRODUCTION

Channel catfish, Ictalurus punctatus (Rafinesque), has become an important sport and food species. From information gathered from many nutritional studies, successful supplemental feeds have been developed for pond culture of channel catfish. Various feed requirements have been established in an attempt to achieve maximum growth at least cost.

Vitamins C and D are important in absorption and utilization of calcium and phosphorus in vertebrates. Effects of dietary addition of these vitamins on the mineral retention of fingerling (age group I) channel catfish have not been studied. The quantity of vitamin C and D₃ was therefore varied in three experimental diets in the first segment of this study.

Calcium and phosphorus are known to be important in vertebrate maintenance and growth. Effects of mineral content of age group II channel catfish diets have not been studied. The quantity of these two minerals were varied in three experimental diets in the second segment of this study.

In addition to the effect on mineral retention, the author was interested in what effects these six diets would have on the fish's growth. Body weight gain was used as the index of fish growth. Crude fat and moisture content of age group I and II fish were determined throughout both segments of this study to determine their contribution to weight gains and to determine diet and seasonal variations in crude fat and moisture levels.

The material in this thesis is presented in the following order: a general review of literature followed by three, separate, publishable units on (1) dietary vitamin C and D, (2) dietary calcium and phosphorus, and (3) moisture and crude fat levels in the fish.

REVIEW OF LITERATURE

Vitamins C and D₃

Vitamin C was named by J. C. Drummond in 1920 and the crystalline form of the compound was named ascorbic acid by Szent-Gyorgyi and Hayworth in 1933. Ascorbic acid is a reducing agent for hydrogen transport. It is necessary for formation of collagen, bone, and normal cartilage; bone repair; and wound healing. In deficient fish, intabulated L-ascorbic acid is rapidly mobilized and fixed in areas of rapid collagen synthesis and is concentrated in thick collagen of skin, cartilagenous bone, pituitary and adrenal glands of the anterior kidney (Halver, 1972).

Using chemical methods, Fontaine and Hatey (1954) found an abundance of ascorbic acid in the interrenal region of both selachians (cartiagenous fish) and teleosts (fishes having bony skeletons and rayed fins). But the nutritional vitamin C requirements of fish have been highly debated. Studies by Kitamura et al. (1967); Poston (1967); Halver, Ashley, and Smith (1969); and Lovell (1973) have indicated unfavorable growth in rainbow trout (Salmo gardneri), brook trout (Salvelinus fontinalis), coho salmon (Oncorhynchus kisutch), and channel catfish (Ictalurus punctatus) on diets free of ascorbic acid. Ikeda and Sato (1964) found that when ¹⁴C glucose was ingested by large carp (Cyprinus carpio) ¹⁴C ascorbic acid could be found in the blood and effluent waters of aquariums used to hold the carp but insufficient vitamin C was synthesized to satisfy requirements of young carp in 20°C water. The requirements for vitamin C are thought to be related to stress, growth rate, size of fish and other nutrients in the diet; 200 mg/kg of diet is considered a compromise level for trout and salmon in 10° - 15°C freshwater systems (Halver 1972). The minimum amount of ascorbic acid required has been reported at 10 mg/100g dry diet (Snieszko 1972).

Dupree (1966), however, found no differences in weight gain or mortality of fingerling channel catfish fed vitamin C-deficient and control diets. Post-period examination exposed no abnormal conditions of gill filaments, stomachs, spleens, intestines, or kidneys. Primbs and Sinnhuber (1971), who concluded that ascorbic acid is not essential in the diet of rainbow trout under normal conditions, suggested that lordosis and scoliosis were not caused by an ascorbic acid deficiency but rather by hypervitaminosis A.

Vitamin D₃ is an animal sterol derivative formed by UV-irradiation of 7-dehydrocholesterol. It is involved in the homeostasis of calcium and inorganic phosphorus; alkaline phosphatase activity; intestinal absorption of calcium; and influences action of parathyroid hormone on bone (Dam and Sondergaard 1964; West et al. 1966) and is secondarily responsible for kidney function with respect to calcium and phosphorus (Wasserman 1963). The principle stores of vitamin D are in the liver (Wasserman 1963). The mechanism for vitamin D effect on calcium may be: the vitamin (1) is concerned in the active transport of calcium or (2) is involved in increasing permeability of cells of the intestinal mucosa to the mineral (Maynard and Loosi 1969). It has been demonstrated in chickens that ⁴⁵Ca appears in the blood at a faster rate when the diet contained vitamin D₃ (Keane et al. 1956). Vitamin D has been shown to have a growth promoting effect on albino rats fed a low-calcium diet adequately supplied with phosphorus and other dietary essentials. Addition of calcium to this low-calcium, adequate phosphorus diet could not increase growth to levels induced by vitamin D alone. When dietary Ca/P ratio was 4.5/1.0 vitamin D depressed growth (Steinbock and Herting 1955). Fournier and Dupuis (1964) found the calcium in serum and other tissues of rats was 20% higher than those fed a vitamin D supplemented diet than those fed an unsupplemented diet.

Studies on vitamin D requirements in fish have been limited in number. Jewell et al. (1933) indicated that channel catfish and goldfish (Carassius

auratus) needed that vitamin D provided by crude cod liver oil in their diets, but the diets used were probably deficient in other nutrients which the cod liver oil provided. Vitamin D₃ has been shown to be a better source of vitamin D than was D₂ (Ashley 1972). Vitamin D has been shown to not be a dietary requirement for brook trout (Phillips et al. 1955), in fact hypovitaminosis D has not been reported in fish but hypervitaminosis D is known (Halver 1972).

Calcium and phosphorus

For most vertebrates, 1% of the body calcium occurs outside the bones (Maynard and Loosi 1969). It is distributed throughout the organs and tissues where it exists, at least in part, in the colloidal tissues.

The large amounts of phosphorus found in tissues other than bone are present in organic compounds such as phosphoprotein, nucleoprotein, phospholipids and hexose phosphate.

Adequate calcium and phosphorus nutrition is dependent upon a sufficient supply of both minerals, a suitable ratio between them, and the presence of vitamin D (Maynard and Loosi 1969). With plenty of vitamin D in the ration, the ratio becomes less important and more efficient utilization is made of the amount of calcium and phosphorus present in vertebrate diets. Absence of vitamin D causes reduction in assimilation even when the other factors are optimum.

In fish, the level of phosphorus in different parts of the body can be correlated with the amount of bone structure present. Phillips et al. (1953) listed the parts of the fish body in decreasing order of amounts of phosphorus: head, skin, gills, remainder of body, viscera, and gastro-intestinal tract. In the same study, the head, jaws and opercula were found to contain the most calcium; the gastro-intestinal tract the least; and the size of the fish did not influence the amount of calcium present.

McCay et al. (1936) demonstrated that calcium and phosphorus levels varied according to age, growth and supply of food in hatchery raised trout. In studies

on nonfeeding brook trout, it was found that calcium is absorbed from the water, through the gills, in amounts dependent upon the chemical composition of the water (Phillips et al. 1955). Increased concentration of minerals other than calcium in the water increased the ability to absorb and metabolize the calcium. Other studies on brook trout (Phillips et al. 1956 and 1957) showed that in brook trout calcium is absorbed from the water in at least the same magnitude of calcium absorbed from food and continues to be used even though calcium from food is available. Brook trout fed low calcium diets absorb more calcium than trout fed high calcium diets and changes in the composition of the water had no effect on the assimilation of calcium from the feed. The major portion of calcium deposited in the fish body is as bone salt and the blood is the medium of internal transport (Phillips et al. 1960a). Only 1/400 as much phosphorus is absorbed from the water as calcium by brook trout (Phillips et al. 1958). The source of phosphorus for structural growth in brook trout is dietary (Phillips et al. 1958 and 1960b). Cold water retards the assimilation of phosphorus in food and calcium present in the food proportionally depresses the utilization of phosphorus in the food of brook trout. Phosphorus is most efficiently utilized at a calcium/phosphorus ratio of 1/1 (Phillips et al. 1958). In later studies, Phillips (1962) found more phosphorus was utilized from an all meat diet than a meat-meal diet and blood-phosphorus was higher in temporarily starved or the all meat diet fed trout tested in cold water than in hot water. There was no difference in the blood phosphorus of trout fed the meat-meal diet at the two temperatures.

Rainbow trout have been reported to absorb and conserve dietary calcium more efficiently and dissolved calcium less efficiently than brook trout and brown trout (Podoliak and Holden 1966). The skin and skeleton of the rainbow function as a buffer system that can absorb and release calcium ions in response to narrow limits of supply and demand. The study also showed that dietary phosphorus interfered with absorption and retention of dietary calcium.

Several studies have reported levels of various minerals in fish other than trout. Menhaden fish meal was found to be 3.9 to 7.6% calcium and 2.6 - 4.7% phosphorus (Knutzman and Ambrose 1967); commercial fish meal was 5.4% calcium, 3.4% phosphorus, and 0.4% potassium (Morrison 1959); 100 grams of salmon muscle contained 32 mg magnesium, 120 mg sodium, and 340 mg potassium (Spector 1956); buffalo fish (Ictiobus sp.) fillets averaged 55 mg sodium and 295 mg potassium per 100 gram sample and carp fillets averaged 48 mg sodium and 288 mg potassium per 100 gram sample (Thurston et al. 1959).

Studies by Dove (1972) employed supplemental diets with the following Ca/P percentages: (1) 0.315/0.175; (2) 0.78/0.38; and (3) 1.5/0.9. Pappas (1972) used supplemental diets containing (1) 1.5/1.0; (2) 1.9/1.0; and (3) 2.8/1.8 Ca/P percentages. Both studies used age group I channel catfish and reported higher mineral retention, better feed conversions, and faster growth in fish fed high calcium and phosphorus levels. Dove (1972) reported continual reduction of bone calcium and phosphorus during the summer growing season. Pappas (1972) reported phosphorus levels in eviscerated fish and bones were more variable between diets than calcium levels and that calcium and phosphorus levels were higher at the end of the feeding period than at the start. Pappas also found better results when dicalcium phosphate was used than when just plant and animal sources of calcium and phosphorus were used.

Crude fat and moisture

Brown (1957) reported 80% as the average water content of fish flesh along with extreme values of 53 and 89% have been recorded for certain fish at various season and locations. Moisture and fat content vary inversely and fat content may vary from place to place in the same fish (Lagler 1962). In brook trout, fat is deposited at the expense of water without differences due to water temperature or size of the fish (Phillips et al. 1960c). Neither the increase nor the decrease in water content of the brook trout is at a constant rate but rather a trend

toward increase and decrease with apparent dips and peaks at somewhat regular intervals.

Fat content of nine species of fish, atlantic herring (Clupea h. harengus), white suckers (Catostomus commersonii), carp, burbot (Lota lota), smelt (Osmerus mordax), lake trout (Salvelinus namaycush), whitefish (Coregonus clupeaformis), yellow perch (Perca flavescens), walleye pike (Stizostedion vitreum) varied widely with whitefish, trout and carp having a high fat content. Moisture in the nine species ranged from 69.2 to 82.6, with lower levels recorded in fat fish and higher levels in lean fish (Ingalls et al. 1950).

In adult yellow perch, water remained fairly constant while fat varied seasonally between 1.5 and 5.0% (Pearse 1925). In largemouth bass (Micropterus salmoides) and pumpkinseed (Lepomis gibbosus) moisture was higher in starved fish and fat was higher in fed fish. Moisture content of longear sunfish (Lepomis megalotis) varied only 3% while moisture content of green sunfish (Lepomis cyanellus) varied 8% (Gerking 1952). In fall and spring studies on bluegill (Lepomis macrochirus) by Gerking (1955), the moisture was at 74.10% in both seasons and fat was 2.33% in the spring and 3.03% in the fall. Analyses of carp bodies (Heper and Chervenski 1965) showed 68.8% moisture and 12.23% fat.

In analyses of brook trout, Pearse (1925) found water content to increase from 61 to 83% during the first six months of development and after the first year remained fairly constant at 75%. Fat varied between 1.68 and 7.80% with seasonal fluctuations. In a comparative study of hatchery and wild brook trout and hatchery brown trout (Salmo trutta), Phillips (1959) found 71.50% moisture and 3.40% fat in wild brook trout; and 71.2% moisture and 6.5% fat in hatchery brook trout; and 68.6% moisture and 6.3% fat in brown trout.

In a comparative study of 10 salmonid fishes under hatchery and wild conditions, Wood et al. (1957) found higher lipid values for hatchery fish (15.10 - 28.70%) than wild fish (11.10 - 18.60) of the same age and species. In a study

on carbohydrate requirements of chinook salmon (Oncorhynchus tshawytska), Buhler and Halver (1961) reported moisture ranged from 77 to 81% and fat on a dry basis ranged from 18.7 to 36.6%.

Fat content of siscowet (Salvelinus n. siscowet) and lake trout from Lake Superior ranged from 32.5 to 88.8% and 6.60 to 62.3% respectively (Eshmeyer and Phillips 1965).

The concentration of fat, water-solubles, protein, and water vary seasonally (Damberg 1964) in cod (Gadus morhua). During spawning, there is a decrease in fat (-20%) and protein (-5%) paralleled by an increase in water-solubles (+10%) and water (+5%). The return of these major components to their average annual values occurs at various rates.

Black bullhead (Ictalurus m. melas) fillets have been found to contain 79 to 83.1% water and 0.8 to 4.4% fat (Thurston et al. (1959). Spring and fall analyses of age group I catfish (30-100 grams in weight) revealed seasonal differences; 76.99% moisture and 1.76% fat in the spring compared with 73.53% moisture and 5.93% fat in the fall (Nail 1962). Suppes et al. (1967) found seasonal variations in fat and protein in age group I channel catfish. The study also showed that fat gained during a 132-day feeding period was lost during a 244-day starvation period and provided 70% of the energy for metabolism during the starvation period. As fat decreased, moisture increased during the starvation period.

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Effects of dietary addition of vitamin C and/or D₃ on
growth and mineral levels of fingerling channel catfish¹

by

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Effects of dietary addition of vitamin C and/or D₃ on
growth and mineral levels of fingerling channel catfish

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Abstract

Biweekly from May to September 1972 and monthly from September 1972 to February 1973 calcium, phosphorus, sodium, chlorine, manganese and potassium were determined, using neutron activation, on eviscerated bodies and fat-free skeletons of fingerling channel catfish. Fish were fed one of three diets with added vitamin C, vitamin D₃, or both from May to September 1972. Body weight gains, survival rates, and feed conversion were determined for the May to September period.

During the feeding period, there were significant sampling-date differences ($P < .05$) for calcium in eviscerated fish and highly significant sampling-date-diet interactions ($P < .01$) for calcium in bone and phosphorus in both eviscerated fish and bone. During the nonfeeding period there were significant sampling-date differences ($P < .05$) for calcium and phosphorus. (Though sodium, chlorine, manganese, and potassium were determined during both periods, differences were not analyzed.) During the feeding period, fish weight increased. Survival rate and feed conversions ranged from 94.8 to 97.6% and 1.06 to 1.13, respectively, during the feeding period.

Effects of dietary addition of vitamin C and/or D₃ on
growth and mineral levels of fingerling channel catfish

C. A. Launer

In fish vitamin C (ascorbic acid) functions as a reducing agent for hydrogen transport. It is necessary for collagen, normal cartilage, and bone formation; bone repair; and wound healing (Halver 1972). Vitamin D is involved in the homeostasis of calcium and inorganic phosphorus, alkaline phosphatase activity, intestinal absorption of calcium, and action of the parathyroid hormone on bone (Dam and Sondergaard 1964; West et al. 1966). Ashley (1972) indicated vitamin D₃ is a better source of vitamin D than is D₂.

This study was initiated in the spring of 1972 at Tuttle Creek Fisheries Laboratory near Manhattan, Kansas, to determine how dietary addition of vitamin C and D₃ would affect mineral levels and growth of fingerling channel catfish, Ictalurus punctatus (Rafinesque). Objectives were to determine levels of calcium, phosphorus, sodium, chlorine, manganese, and potassium in eviscerated bodies and fat-free skeletons at intervals during the feeding (May to September 1972) and nonfeeding (September 1972 to February 1973) periods.

MATERIALS AND METHODS

Each of 12 polyethylene-lined ponds (each 578 m²) was stocked with 3,637 grams of fingerling channel catfish (average of 580 fish) in April 1972. In the study we used three pelleted, sinking diets (Z-58, Z-59, and Z-60, Table 1) with vitamin C, vitamin D₃, or both added. Fish in each of three regimens (four ponds each) were assigned one diet and fed

Table 1. Protein, fat, fiber, energy, calcium, phosphorus, vitamin C and vitamin D₃ determinations on diets Z-58, Z-59, and Z-60.

| Item | Diet | | |
|---|-------|--------------------|--------|
| | Z-58 | Z-59 | Z-60 |
| Protein % ^a | 30 | 30 | 30 |
| Protein % ^b | 28.7 | 29.1 | 30.2 |
| Fat % | 4.8 | 4.8 | 4.8 |
| Fiber % | 4.3 | 3.8 | 3.9 |
| Metabolizable energy (Kcal/kg) ^a | 2222 | 2222 | 2222 |
| Calcium % ^a | 2.9 | 2.9 | 2.9 |
| Calcium % ^c | 2.72 | 2.60 | 2.74 |
| Calcium % ^d | 1.47 | 1.42 | 1.51 |
| Phosphorus % ^a | 1.9 | 1.9 | 1.9 |
| Phosphorus % ^e | 2.00 | 1.98 | 2.03 |
| Phosphorus % ^d | 1.11 | 1.09 | 1.19 |
| Vitamin C (mg/kg) ^a | 0.00 | 99 | 99 |
| Vitamin C (mg/kg) ^f | 0.00 | 131.12 | 131.56 |
| Vitamin D ₃ (I.U./kg) ^a | 330 | 0.0 | 330 |
| Vitamin D ₃ (I.U./kg) ^g | 363.0 | 169.4 ^h | 297.0 |

^aCalculated from values in ingredient analyses tables for poultry (NRC 1969).

^bDetermined by AOAC methods (1960).

^cDetermined by flame ionization atomic absorption using strontium-chloride to prevent phosphorus interference by K.S.U. An. Sci. Nutr. Lab.

^dDetermined by neutron activation (Eckhoff *et al.* 1972).

^eDetermined by colorimetric spectrophotometry by K.S.U. An. Sci. Nutr. Lab.

^fDetermined by chromatographic-UV spectrophotometric assay (Quadri, 1973).

^gDetermined by biological (rat) analyses by Warf Inst., Inc., Madison, Wisc.

^hResulting from naturally occurring low levels in feed ingredients rather than from vitamin D supplementation.

six days a week at 3% mean total body weight from May 1 to September 14, 1972. Fifty fish from three ponds of each regimen were seined and weighed biweekly (seven times) from May 27 to August 29 to determine the amount of feed needed to maintain the feeding rate (3%-of-body-weight). On September 14, when feeding was terminated, all fish were removed, counted, and weighed. After weight gains, survival rate, and feed conversions were determined for the feeding period, 160 fingerlings fed the diet containing both vitamin C and D₃ were transferred to a small observation pond to facilitate sampling during the winter.

Thirty fingerlings in the fourth pond of each regimen were removed for mineral analyses on April 16, May 18, and biweekly thereafter during the feeding period. Body minerals were analyzed in 20 of those fish; bone minerals in 10. Monthly during the nonfeeding period (September 1972 to February 1973), 20 fish were removed; body minerals were analyzed in 10 fish; bone minerals in 10.

For body mineral analyses, fish were eviscerated, finely ground through a meat grinder, and mixed for uniformity of sample; then a portion of the mixture was placed in a tarred vial and frozen for storage. Each sample was taken from a composite of two fish during the feeding period (when the fish were small) and from one fish during the nonfeeding period.

Bone samples were obtained by placing eviscerated fish in hot water until the flesh was loosened from the bones. All bones, including the ossified fin rays, were then removed and brushed under cool running water to remove the flesh. Bones were air-dried; placed in petroleum ether and allowed to stand at room temperature for 24 hours to remove fat; and then air-dried for 24 hours and weighed. Each skeleton was finely ground using

a mortar and pestle, then mixed for uniformity of sample; a portion of the sample was placed in a tarred vial and frozen for storage.

Mineral levels were determined in all fish samples by neutron activation* (Eckhoff *et al.* 1972), and biweekly pond-water samples were analyzed for calcium and phosphorus by atomic absorption and colorimetric spectrophotometry, respectively. A two-way, unequal subclass analysis of variance was used to evaluate calcium and phosphorus content during the feeding period; a one-way analysis, during the nonfeeding period (Snedecor and Cockran 1967). Two-way analysis of variance was used to evaluate pond-water samples (Snedecor and Cockran 1967).

RESULTS

Table 2 shows the mean body weight gain per pond, feed conversion, and survival rate for each regimen during the feeding period. No growth abnormalities such as lordosis or scoliosis were noted in fish in any regimen.

The mean calcium levels in eviscerated fish for feeding and nonfeeding periods (Figure 1) ranged from 0.28% for Z-59-fed fish on July 27 to 0.86% (initial level) on April 16. During the feeding period, sampling-date differences were highly significant ($P < .01$) but diet differences and sampling date-diet interaction were not ($P > .70$). Mean calcium levels in eviscerated fish originally fed the Z-60 diet and then not supplementally fed after September 14 showed highly significant sampling-date differences ($P < .01$).

The mean phosphorus levels in eviscerated fish for feeding and nonfeeding periods (Figure 1) ranged from 0.15% for Z-59-fed fish on August 22 to 0.73% phosphorus for the Z-59-fed fish on June 18. There was a highly significant sampling date-diet interaction ($P < .01$) during the feeding period. During the nonfeeding period, phosphorus levels for eviscerated fish showed significant sampling-date differences ($P < .01$).

*Method for case NAA-1

Table 2. Body-weight-gain, feed-conversion, and survival rate means of fingerling channel catfish (in 3 ponds) fed diets Z-58, Z-59, and Z-60 from May 1 to September 14, 1972.

| <u>Diet</u> | <u>Vitamin</u> | <u>Weight gain/pond</u> ^a | <u>Feed conversion</u> ^a | <u>Survival rate</u> ^a |
|-------------|--------------------|--------------------------------------|-------------------------------------|-----------------------------------|
| Z-58 | D ₃ | 49,261 g | 1.07 | 97.6% |
| Z-59 | C | 46,684 g | 1.13 | 94.8% |
| Z-60 | D ₃ + C | 49,627 g | 1.06 | 94.6% |

^aMean of 3 ponds.

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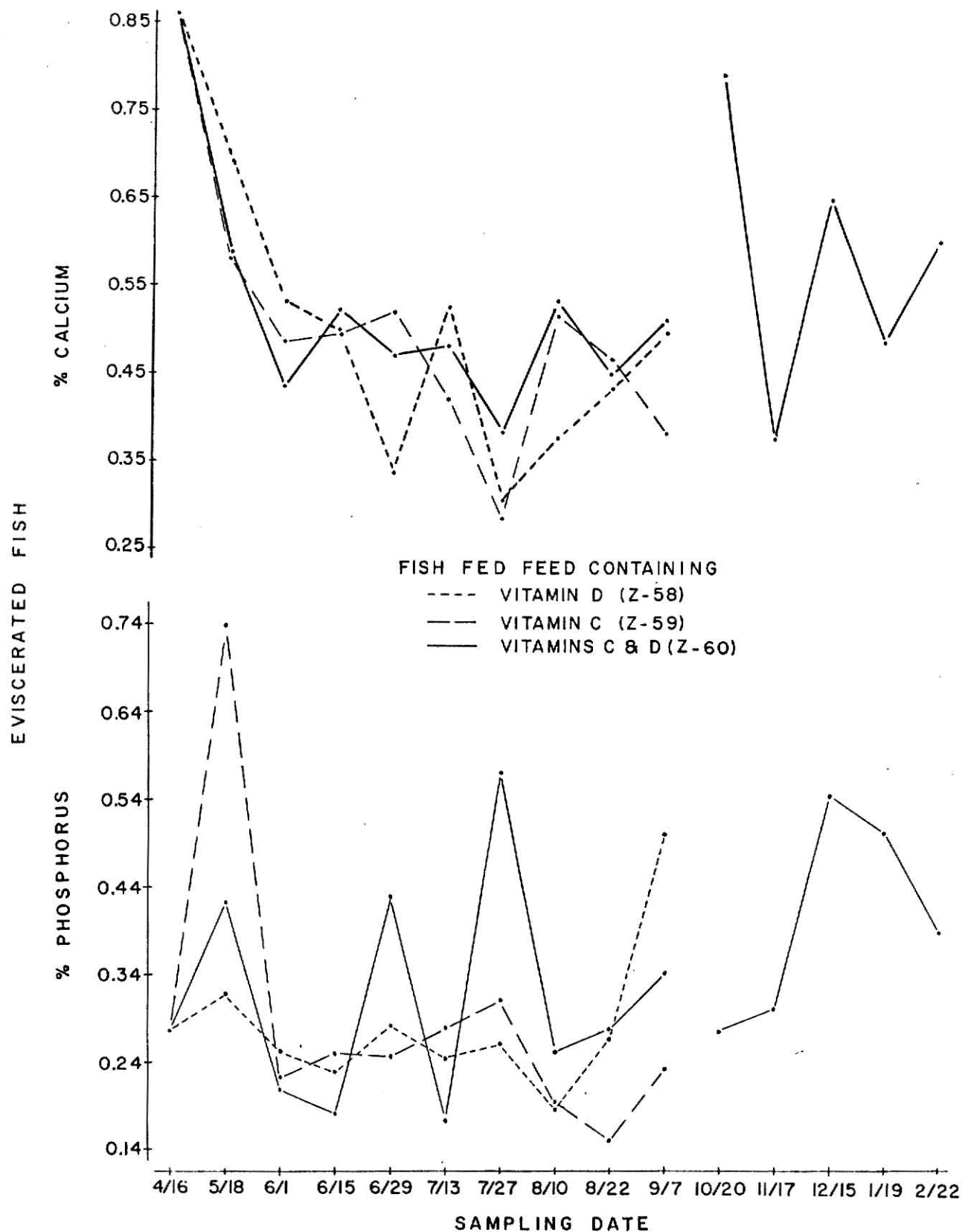


Figure 1. Mean % Ca and P in fingerlings fed three diets. Determinations were made April 16 and then biweekly during the feeding period (May 1 to Sept. 14, 1972) and monthly during a nonfeeding period (Sept. 14, 1972 to Feb. 22, 1973).

The mean calcium levels in fat-free skeletons for feeding and non-feeding period (Figure 2) ranged from 8.49% for Z-59-fed fish on June 1 to 15.25% for Z-60-fed fish on August 10. There was a highly significant sampling date-diet interaction during the feeding period ($P < .01$). During the nonfeeding period, calcium in bones decreased slowly but highly significantly ($P < .01$).

Phosphorus in fat-free skeletons for feeding and nonfeeding period (Figure 2) ranged from 1.77% for Z-60-fed fish on September 7 to 8.82% for Z-59-fed fish on August 10. During the feeding period, sampling date-diet interaction was highly significant ($P < .01$). During the nonfeeding period, phosphorus in the bones increased highly significantly ($P < .01$).

Levels were determined but not statistically analyzed for sodium, chlorine, manganese, and potassium (Table 3).

Neither calcium nor phosphorus in pond-water samples differed significantly ($P > .10$) by date or regimen.

DISCUSSION

The differences in the diets between calculated and AOAC determinations of calcium and phosphorus (Table 1) indicated that levels were slightly lower than expected. Atomic absorption and spectrophotometric analyses differed from neutron activation analyses analytically.

Studies by Kitamura et al. (1967); Poston (1967); Halver, Ashley, and Smith (1969); and Lovell (1973) indicated unfavorable growth in rainbow trout, brook trout, coho salmon, and channel catfish on diets free of ascorbic acid. Dupree (1966), however, found no differences in weight gain or mortality of fingerling channel catfish fed vitamin C-deficient and control diets. Primbs and Sinnhuber (1971), who concluded that ascorbic

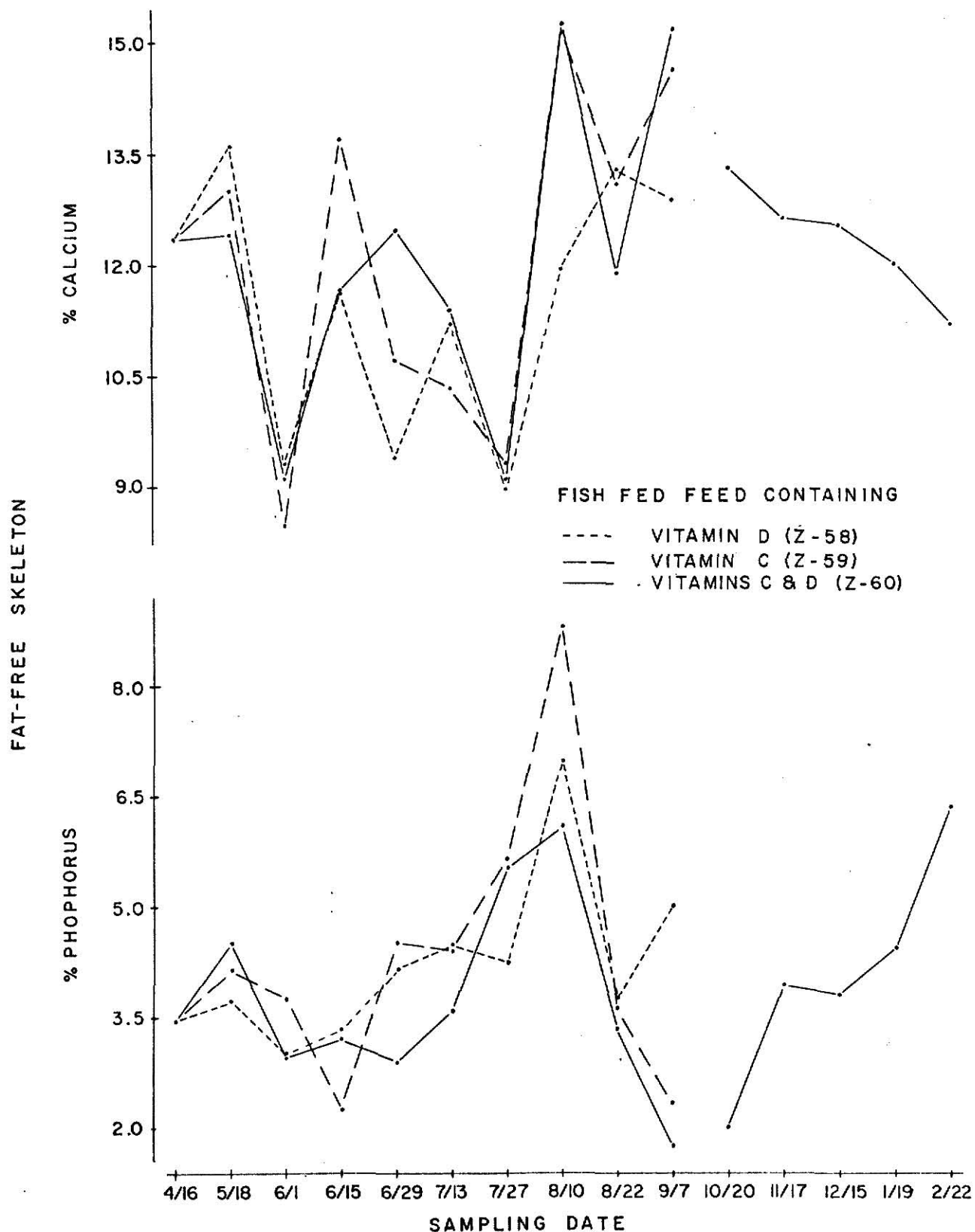


Figure 2. Mean % Ca and P in skeletons of fingerlings. Determinations were made April 16 and then biweekly during the feeding period (May 1 to Sept. 14, 1972) and monthly during a nonfeeding period (Sept. 14, 1972 to Feb. 22, 1973).

Table 3. Sodium, chlorine, manganese, and potassium in parts per million in eviscerated bodies and fat-free skeletons of fingerling channel catfish during a feeding period (May 1 to September 14, 1972) and a subsequent nonfeeding period (September 14, 1972, to February 22, 1973).

| <u>Mineral</u> | <u>Feeding period</u> | | <u>Nonfeeding period</u> | |
|---------------------------------|-----------------------|---------------------------|--------------------------|---------------------------|
| | <u>Mean</u> | <u>Standard Deviation</u> | <u>Mean</u> | <u>Standard Deviation</u> |
| Sodium in eviscerated fish | 673.71 | 152.72(278) ^a | 483.28 | 98.83(50) ^a |
| Sodium in fat-free skeletons | 1516.80 | 341.15(279) | 1799.12 | 218.78(43) |
| Chlorine in eviscerated fish | 756.92 | 268.51(278) | 893.02 | 188.11(50) |
| Chlorine in fat-free skeletons | 638.05 | 417.73(273) | 253.54 | 20.73(43) |
| Manganese in eviscerated fish | 3.52 | 1.49(276) | 2.74 | 1.43(50) |
| Manganese in fat-free skeletons | 24.60 | 13.34(279) | 20.19 | 5.02(43) |
| Potassium in eviscerated fish | 7300.36 | 2782.93(275) | 4293.83 | 2782.42(49) |
| Potassium in fat-free skeletons | 7909.02 | 3770.46(272) | 2384.17 | 1476.29(33) |

^aNumbers in parentheses indicate number of samples analyzed.

acid is not essential in the diet of rainbow trout under normal conditions, suggested that lordosis and scoliosis were not caused by an ascorbic acid deficiency but rather by hypervitaminosis A.

Jewell et al. (1933) indicated that catfish and goldfish needed the vitamin D provided by crude cod liver oil in their diets but the diets might have been deficient in other vitamins provided by cod liver oil. Halver (1972) reported that hypervitaminosis D is known but that hypovitaminosis D is not known in fish.

In this study, similar weight gains, feed conversions, and survival rates for fingerling channel catfish fed diets containing vitamin C and/or vitamin D₃ indicated no superiority in growth was achieved by adding those vitamins to the diet. The sampling date-diet interactions indicated no direct relationship between added vitamin C and/or D₃ and the calcium and phosphorus in eviscerated bodies and fat-free skeletons of fingerling channel catfish.

Of the minerals in Table 3, manganese was the element in lowest concentration in eviscerated bodies and fat-free skeleton while potassium was the element in highest concentration. Chlorine was the only element found at a higher concentration in eviscerated fish than in fat-free skeleton. Sodium in fat-free skeletons and chlorine in eviscerated fish were at higher concentrations during the nonfeeding period than during the feeding period.

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Effects of dietary calcium and phosphorus levels on growth
and retention of certain minerals in age group II channel catfish¹

by

C. A. Launer²

¹Contribution No. 1232, Division of Biology and Contribution No. 863, Department of Grain Science and Industry. Investigations were cooperative with the Federal Aid to Fisheries, Project F-12-R; Kansas Forestry, Fish and Game Commission; Kansas Agricultural Experiment Station; and Department of Nuclear Engineering Kansas State University.

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Effects of dietary calcium and phosphorus levels on growth
and retention of certain minerals in age group II channel catfish

C. A. Launer

Abstract

From May to September 1972, groups of age group II channel catfish were fed one of three diets of differing calcium-phosphorus content. Biweekly during the same period, calcium, phosphorus, sodium, chlorine, manganese, and potassium were determined, by neutron activation analysis, on eviscerated bodies and fat-free skeleton samples of fish. Body weight gains, survival rates, and feed conversions were also determined.

Calcium-phosphorus levels in the diet had no direct effect on body and bone calcium or phosphorus levels. Sodium, chlorine, manganese, and potassium were determined during the period without regard to diet regimen differences. The fish fed the intermediate calcium-phosphorus diet had the best weight gains and feed conversions similar to the fish fed the high calcium-phosphorus diet.

Effects of dietary calcium and phosphorus levels on growth
and retention of certain minerals in age group II channel catfish

C. A. Launer

This study was initiated in the spring of 1973 at the Tuttle Creek Fisheries Laboratory near Manhattan, Kansas, to determine how the calcium-phosphorus content of the diet would affect the mineral content and growth of age group II channel catfish, Ictalurus punctatus (Rafinesque). Objectives were to determine the calcium, phosphorus, sodium, chlorine, manganese, and potassium levels in eviscerated bodies and in fat-free skeleton at predetermined intervals during the feeding period (May 21 to September 14) as well as determining fish weight gains, feed conversions, and survival rates.

Materials and methods

In May 1973, six polyethylene-lined ponds were each stocked with 29,550 grams of age group II channel catfish (average of 275 fish per pond; 107 g/fish). Ponds were managed in pairs and the fish were fed one of three pelleted, sinking diets (Z-50, Z-51, Z-67, Table 1) of different calcium-phosphorus content. The fish were fed once daily, six days a week from May 21 to September 14 at 2% mean total fish body weight. On September 14, all fish were removed, counted, and weighed.

Twenty fish were weighed and prepared for analysis at the time of stocking (May 18, 1973); thereafter, 10 fish were removed from each pond biweekly during the feeding period. Of the 20 fish (10 from each pond of a pair of ponds) given the same diet and removed on the same sampling date, 10 were analyzed for body-mineral and 10 for bone-mineral content.

For body-mineral analysis, each fish was eviscerated, individually fine-ground through a meat grinder and mixed for uniformity of sample; then a portion of the mixture was placed in a tarred vial, sealed, and frozen for storage.

Table 1. Protein, fat, fiber, energy, calcium and phosphorus determinations on diets Z-50, Z-51, and Z-67.

| Item | Diet | | |
|---|------|------|------|
| | Z-50 | Z-51 | Z-67 |
| Protein % ^a | 25.0 | 25.0 | 25.0 |
| Protein % ^b | 24.6 | 26.6 | 25.4 |
| Fat % | 3.9 | 5.6 | 3.6 |
| Fiber % | 6.6 | 6.1 | 5.7 |
| Metabolizable energy (Kcal/kg) ^a | 1870 | 1870 | 1870 |
| Calcium % ^a | 1.9 | 2.8 | 5.0 |
| Calcium % ^c | 1.64 | 2.84 | 3.60 |
| Calcium % ^d | 0.93 | 1.43 | 1.49 |
| Phosphorus % ^a | 1.0 | 1.8 | 2.5 |
| Phosphorus % ^e | 1.16 | 2.15 | 2.60 |

^aCalculated from values in ingredient analyses tables for poultry (NRC 1969).

^bDetermined by AOAC methods (1960).

^cDetermined by flame ionization atomic absorption using strontium-chloride to prevent phosphorus interference by K.S.U. An. Sci. Nutr. Lab.

^dDetermined by neutron activation analysis (Eckhoff et al. 1972, case NAA-1).

^eDetermined by colorometric spectrophotometry by K.S.U. An. Sci. Nutr. Lab.

Bone samples were obtained by placing eviscerated fish in hot water until the flesh was loosened from the bones. All bones, including the ossified fin rays, were then removed and brushed under cool running water to remove the remaining flesh. Bones were air-dried, placed in petroleum ether and allowed to stand at room temperature for 24 hours to remove fat, and then air-dried for 24 hours and weighed. Each skeleton was finely ground using a mortar and pestle, then mixed for uniformity of sample; a portion of the sample was placed in a tarred vial, sealed, and frozen for storage.

Mineral levels were determined in all fish samples by neutron activation analysis (Eckhoff et al. 1972, case NAA-1), and pond-water samples obtained biweekly were analyzed for calcium and phosphorus by atomic absorption and colorometric spectrophotometry, respectively. A two-way unequal subclass analysis of variance (Snedecor and Cockran 1967) was used to evaluate calcium and phosphorus content in eviscerated bodies and fat-free skeletons. Two-way analysis of variance (Snedecor and Cockran 1967) was used to evaluate pond-water samples, but sampling date-regimen interaction could not be determined because the water samples from the two ponds in each regimen were pooled for analysis at each sampling date. T-test for significant of differences between two independent sample means (Williams 1969) was used to compare the percentage of bone in fish from the three regimens.

Results

Body weight gains, feed conversions, and survival rates for each regimen (Table 2) were determined after harvesting on September 14.

The mean calcium levels in eviscerated fish (Figure 1) ranged from 0.24% for Z-51 fed fish on July 2 or 0.89% for Z-67 fed fish on July 17. During the study, sampling-date differences were significant ($P < .02$) but diet differences ($P > .48$) and sampling date-diet interaction ($P > .67$) were not.

Table 2. Weight gain, feed conversion and survival rate of channel catfish fed diets Z-50, Z-51, and Z-67 from May 18 to September 14.

| <u>Diet</u> | <u>Weight gain/fish^a</u> | <u>Feed conversion</u> | <u>Survival rate^a</u> |
|-------------|-------------------------------------|------------------------|----------------------------------|
| Z-50 | 170.5 g | 1.77 | 98.9 |
| Z-51 | 194.0 g | 1.51 | 99.8 |
| Z-67 | 180.0 g | 1.56 | 100.0 |

^aBased on fish recovered at harvest.

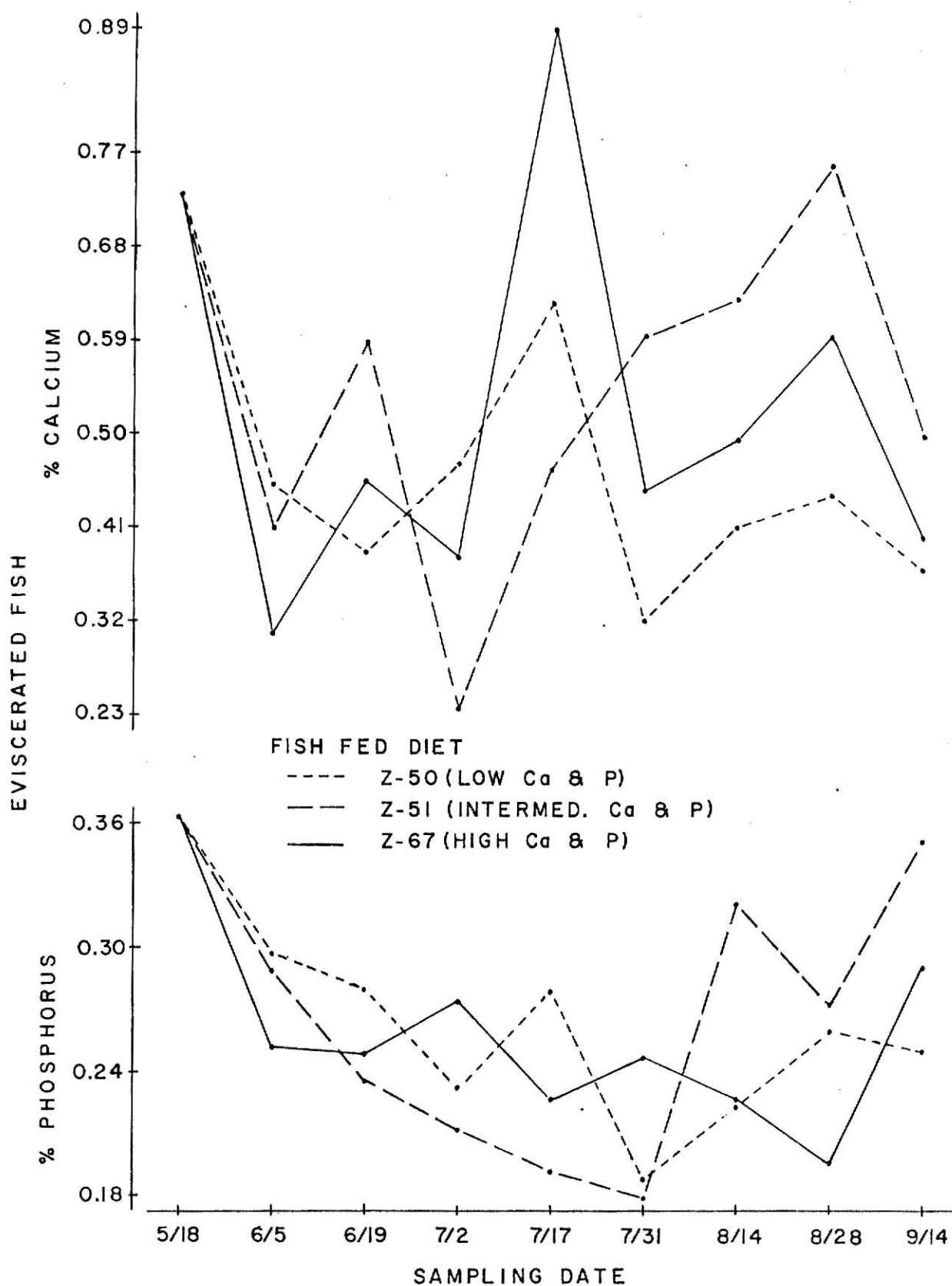


Figure 1. Mean % Ca and P in age group II fish fed three diets. Determinations were made May 18 and biweekly thereafter until Sept. 14. Fish were fed six days a week from May 21 to Sept. 14.

The mean phosphorus levels in eviscerated fish (Figure 1) ranged from 0.18% for Z-51 fed fish on July 31 to 0.36% (initial level) on May 18. During the study, sampling-date differences were highly significant ($P < .001$) but diet differences ($P > .86$) and sampling date-diet interactions ($P > .39$) were not.

The mean calcium levels in fat-free skeletons (Figure 2) ranged from 12.23% for Z-67 fed fish on June 5 to 22.35% for Z-67 fed fish on July 31. During the study, sampling date-diet interactions were highly significant ($P < .001$). The significant interaction prevented discussing sampling-date and diet differences without considering both variables at the same time.

The mean phosphorus levels in fat-free skeletons (Figure 2) ranged from 3.42% for Z-51 fed fish on June 19 to 6.57% for Z-51 fed fish on September 14. During the study, sampling date-diet interactions were highly significant ($P < .001$).

Calcium and phosphorus in pond-water samples (Table 3) revealed that for calcium, sampling-date differences were highly significant ($P < .001$) but regimen differences were not ($P > .53$); and that for phosphorus, both sampling-date and regimen differences were highly significant ($P < .002$ and $P < .004$, respectively). Phosphorus in pond water from the Z-67 regimen was significantly higher ($P < .05$) than in the other two regimens. There was no significant differences ($P > .05$) between the phosphorus levels in pond-water samples from the Z-50 and Z-51 regimens.

The means and standard deviation for sodium, chlorine, manganese, and potassium levels in fish from all 3 regimens are listed in Table 4. Skeleton weights were used to determine the amount of bone per fish (Table 5) and T-tests revealed no significant differences ($P > .05$) in the percentage of bone in fish from the different regimens.

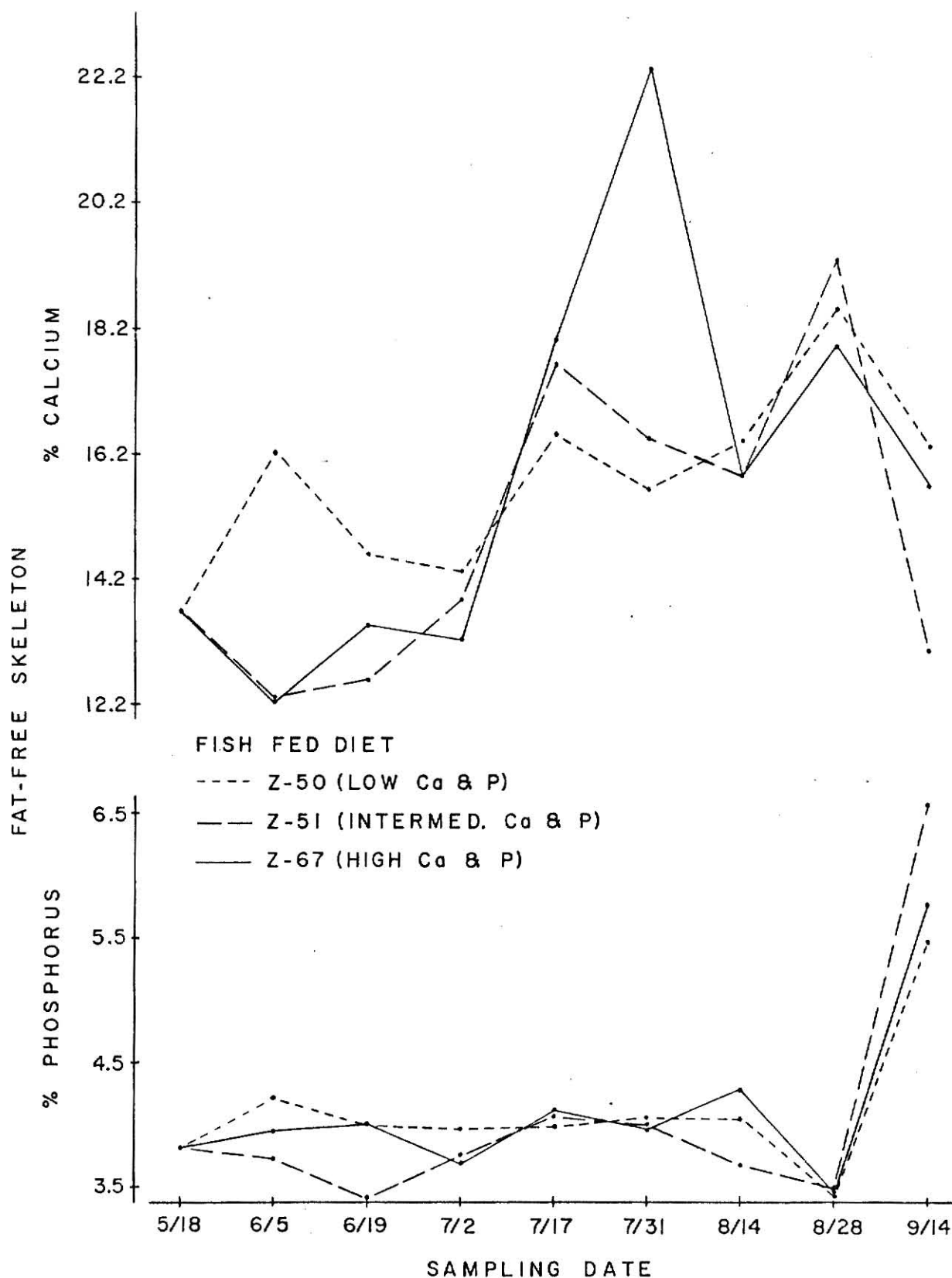


Figure 2. Mean % Ca and P in skeletons of age group II fish fed three diets. Determinations were made May 18 and biweekly thereafter until Sept. 14. Fish were fed six days a week from May 21 to Sept. 14.

Table 3. Calcium^a and phosphorus^b in parts per million in pond-water samples taken from ponds of regimens Z-50, Z-51, and Z-67.

| <u>Sampling date</u> | <u>Regimen</u> | | | | | |
|----------------------|----------------|----------|-------------|----------|-------------|----------|
| | <u>Z-50</u> | | <u>Z-51</u> | | <u>Z-67</u> | |
| | <u>Ca</u> | <u>P</u> | <u>Ca</u> | <u>P</u> | <u>Ca</u> | <u>P</u> |
| 6/5 | 10.2 | 0.94 | 10.6 | 1.28 | 12.0 | 1.03 |
| 6/19 | 9.0 | 2.48 | 6.6 | 1.40 | 10.3 | 3.61 |
| 7/2 | 7.2 | 3.27 | 7.4 | 2.83 | 11.7 | 3.27 |
| 7/17 | 7.3 | 4.38 | 7.6 | 4.39 | 10.4 | 3.30 |
| 7/31 | 10.2 | 3.13 | 8.8 | 2.74 | 9.0 | 3.96 |
| 8/14 | 3.8 | 4.49 | 6.2 | 4.46 | 6.7 | 2.56 |
| 8/28 | 7.9 | 3.25 | 10.0 | 2.87 | 12.0 | 2.44 |
| 9/14 | 11.8 | 4.02 | 9.3 | 3.20 | 12.7 | 2.99 |

^a Determined by flame ionization atomic absorption using strontium-chlorine to prevent phosphorus interference by K.S.U. An. Sci. Nutr. Lab.

^b Determined by colorometric spectrophotometry by K.S.U. An. Sci. Nutr. Lab.

Table 4. Sodium, chlorine, manganese, and potassium in parts per million (ppm) in eviscerated bodies and fat-free skeletons of age group II channel catfish sampled biweekly from May 18 to September 14, 1973.

| <u>Mineral</u> | <u>Mean</u> | <u>Standard deviation</u> |
|---------------------------------|-------------|---------------------------|
| Sodium in eviscerated fish | 411.60 | 206.83(250) ^a |
| Sodium in fat-free skeletons | 1616.54 | 428.72(249) |
| Chlorine in eviscerated fish | 988.31 | 607.50(250) |
| Chlorine in fat-free skeletons | 740.25 | 3510.55(247) |
| Manganese in eviscerated fish | 4.23 | 2.59(249) |
| Manganese in fat-free skeletons | 51.80 | 17.64(248) |
| Potassium in eviscerated fish | 3357.10 | 2475.81(250) |
| Potassium in fat-free skeletons | 2749.04 | 1894.33(202) |

^a Numbers in parentheses indicate number of samples analyzed and analyses were made on fish from all three regimens.

Table 5. Body weight and bone percentage of fish sampled biweekly from May 18 to September 14, 1973.

| Sampling Date | Fish fed | | Fish fed | | Fish fed | |
|------------------|--------------------------|---------------------|--------------------------|---------------------|--------------------------|---------------------|
| | diet Z-50 | | diet Z-51 | | diet Z-67 | |
| | Body weight ^a | % Bone ^b | Body weight ^a | % Bone ^b | Body weight ^a | % Bone ^b |
| 5/18 | 93.9 g | 4.91 | 93.9 g | 4.91 | 93.9 g | 4.91 |
| 6/5 | 124.8 | 4.14 | 142.1 | 3.78 | 142.3 | 4.22 |
| 6/19 | 153.9 | 4.16 | 138.3 | 4.48 | 136.5 | 4.60 |
| 7/2 | 153.3 | 4.43 | 149.2 | 4.80 | 183.1 | 4.75 |
| 7/14 | 141.4 | 5.23 | 164.5 | 4.75 | 145.1 | 4.98 |
| 7/31 | 179.1 | 4.38 | 169.3 | 5.49 | 205.1 | 4.71 |
| 8/14 | 183.1 | 4.97 | 169.8 | 5.00 | 202.7 | 4.95 |
| 8/24 | 211.9 | 4.92 | 300.4 | 4.24 | 215.9 | 4.22 |
| 9/14 | 310.6 | 4.24 | 243.4 | 4.17 | 240.6 | 4.42 |
| Mean | | 4.60 | | 4.62 | | 4.64 |

^aMean weight of 10 fish

^bBones were defatted and air dried

Discussion

The difference in the Z-67 diet between calculated and AOAC determinations for calcium (Table 1) indicated that there was an error made in adding the diet ingredients. Atomic absorption analysis differed from neutron activation analysis analytically.

Studies by Dove (1972) employed three supplemental diets with the following Ca/P percentages: (1) 0.315/0.175; (2) 0.78/0.38; and (3) 1.5/0.9. Pappas (1972) used three supplemental diets containing (1) 1.5/1.0; (2) 1.9/1.0; and (3) 2.8/1.8 Ca/P percentages. Both studies used age group I channel catfish fed at 3% body weight and reported higher mineral retention, better feed conversions, and faster growth in fish fed the high calcium-phosphorus levels. Dove reported continual reduction of bone calcium and phosphorus during the summer growing season. Pappas reported phosphorus levels in eviscerated fish and bones were more variable between diet regimens than calcium and that calcium and phosphorus levels were higher at the end of the feeding period than at the start.

In this study, calcium and phosphorus contents in eviscerated bodies of age group II channel catfish showed no differences due to the diet fed. The calcium and phosphorus sampling date-diet interactions in fat-free skeletons indicated no direct correlation between calcium-phosphorus levels in the diet and those in the skeletons. In eviscerated bodies and fat-free skeletons, calcium varied more than did phosphorus.

As fish get larger, their relative rate of growth slows down and their mineral requirements may decrease with size and rate of growth. This could explain the differences in results between Dove and Pappas' studies on age group I fish and this study on age group II channel catfish.

No correlation was apparent between levels of calcium and phosphorus in pond water and levels of those minerals in the bodies and skeletons of the fish or the weight gains and feed conversions of the fish. Bone weights averaged 4.6% of the total body weight of the fish in all three regimens.

Of the minerals in Table 4, manganese was the element in lowest concentration in both eviscerated bodies and fat-free skeletons while potassium was the element in highest concentration. Both chlorine and potassium were found in higher concentrations in eviscerated bodies than in fat-free skeletons.

Data on feed conversions indicated fish fed the low calcium-phosphorus diet (Z-50) did not convert feed to body weight as efficiently as did fish fed the intermediate (Z-51) and high (Z-67) calcium-phosphorus diets. Fish fed the Z-51 and Z-67 diets had nearly equal feed conversions but fish fed the Z-51 diet had higher weight gains per fish. Survival of fish in all 3 regimens was excellent.

In summary, this study indicated that different dietary levels of calcium and phosphorus were not directly related to calcium and phosphorus retention in age group II channel catfish, whereas studies on age group I channel catfish (Dove 1972; Pappas 1972) did show a relationship. In this study, fish fed the intermediate calcium-phosphorus diet had the greatest weight gains and their feed conversions were similar to those of fish fed the high calcium-phosphorus diet. Fish fed the low calcium-phosphorus diet had the poorest feed conversions and weight gains.

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Dietary and seasonal variations of
crude fat and moisture levels in age group

I and II channel catfish¹

by

C. A. Launer²

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C. A. Launer

Abstract

Crude fat and moisture were determined on livers and eviscerated bodies of age group I channel catfish during feeding and nonfeeding periods and on livers and eviscerated bodies of age group II channel catfish during a feeding period. Age group I fish were fed one of three 30% protein diets from May to September 1972 and not fed from September 1972 to February 1973. From May to September 1973, age group II fish were fed one of three 25% protein diets.

Percentages of moisture and crude fat in livers were not related in either age group I or II channel catfish. Livers were not a major area of fat storage. Crude fat increased in bodies of catfish of both age groups during feeding periods but decreased in bodies of age group I fish during the nonfeeding period. During the feeding period, moisture levels in the bodies of age group I channel catfish showed no relation to the crude fat percentages, but were inversely related to crude fat percentages in the bodies of age group I fish during the nonfeeding period and the bodies of age group II channel catfish during the feeding period. Body tissues (excluding viscera and liver) were primary storage areas for fat.

Dietary and seasonal variations of crude fat and
moisture levels in age group I and II channel catfish

C. A. Launer

In this study on dietary and seasonal variations of moisture and crude fat in supplementally fed channel catfish, Ictalurus punctatus (Rafinesque), we determined the amount of moisture and crude fat in eviscerated bodies and livers of age group I and II catfish during feeding periods and of age group I catfish during a nonfeeding period.

Materials and methods

In April 1972, three polyethylene-lined ponds (578 m² each) were stocked with 3,637 grams of age group I channel catfish (average of 580 fish; 6.27 g/fish). Fish in each pond were fed one of three 30% protein, sinking pellet diets (Z-58, Z-59, and Z-60, Table 1). The fish were fed once daily, six days a week, from May 1 to September 14, 1972, at 3% mean total fish body weight. Thirty fish were removed from each pond on April 16, May 18, and biweekly thereafter until September 7, 1972. Twenty of the 30 fish removed from each pond on each sampling date were eviscerated (to eliminate digestive-tract-content variability) and finely ground through a meat grinder in groups of two fish. Each ground composite of two eviscerated fish was mixed for uniformity, placed in a plastic bag, and frozen until analyzed. Livers of all 30 fish were removed, weighed, and frozen as a composite.

On September 14, 1972, 160 of the fish that had been fed the Z-60 diet were transferred to a small observation pond to facilitate sampling during the non-feeding period (September 14, 1972 to February 22, 1973). Twenty of these fish were removed monthly. Livers from all 20 were removed, weighed and frozen as a composite; the bodies of 10 were eviscerated and individually fine-ground, bagged, and frozen.

Table 1. Proximate and vitamin analyses of diets Z-58, Z-59 and Z-60.

| Item | Diet | | |
|---|-------|--------|--------|
| | Z-58 | Z-59 | Z-60 |
| Protein % ^a | 30.0 | 30.0 | 30.0 |
| Protein % ^b | 28.7 | 29.1 | 30.2 |
| Fat % ^a | 4.8 | 4.8 | 4.9 |
| Fiber % ^a | 4.3 | 3.8 | 3.9 |
| Ash % ^a | 11.8 | 12.1 | 12.8 |
| Moisture % ^a | 10.7 | 10.7 | 10.1 |
| Vitamin C (mg/kg) ^c | 0.0 | 131.12 | 131.56 |
| Vitamin D ₃ (I.U./kg) ^d | 363.0 | 169.4 | 297.0 |
| Metabolizable energy (Kcals/kg) ^a | 2222 | 2222 | 2222 |

^aCalculated from values in ingredient analyses table for poultry (NRC 1969).

^bDetermined by AOAC methods (1960).

^cDetermined by chromatographic-UV spectrophotometric assay (Quadri 1973).

^dDetermined by biological (rat) analyses by Warf Inst., Inc., Madison, Wisc.

In May 1973, six polyethylene-lined ponds were stocked with 29,550 grams of age group II channel catfish (average of 275 fish; 107 g/fish). Ponds were managed in duplicate and the fish were fed one of three 25% protein, sinking-pellet diets (Z-50, Z-51, and Z-67, Table 2). The fish were fed once daily six days a week, from May 21 to September 14, 1973, at 2% mean total fish body weight. Twenty fish were prepared for analysis at the time of stocking (May 18, 1973) and, thereafter, 10 fish were removed from each of the six ponds biweekly during the feeding period. Of the 20 fish fed the same diet and removed on each sampling date, 10 fish were eviscerated and individually fine-ground, placed in a plastic bag, and frozen for storage. The livers of all 20 fish were removed, weighed and frozen as a composite.

During the feeding and nonfeeding periods, percentages of moisture and crude fat in the eviscerated bodies of age group I and II fish were determined using the following procedure: Five 4-gram samples -- one sample from each of five bags of ground fish -- were pooled to make a 20-gram composite sample (each 20-gram sample was composed of fish that had been fed the same diet, were of the same age group, and had been removed from the ponds on the same date). Each 20-gram composite sample was emulsified in 100 ml of deionized water, freeze dried, and then weighed to compute the moisture level. Two grams of each freeze-dried sample was then analyzed for crude fat using the AOCS method (Aa 4-38) using petroleum ether as the extracting solvent.

During the feeding and nonfeeding periods, percentages of moisture and crude fat in the livers of age group I and II fish were determined using the following procedure: a 20-gram portion was removed from each liver composite sample, emulsified in 100 ml of deionized water, freeze dried and then analyzed as above for moisture and crude fat in eviscerated bodies.

Table 2. Proximate and mineral analyses of diets Z-50, Z-51 and Z-67.

| Item | Diet | | |
|---|------|------|------|
| | Z-50 | Z-51 | Z-67 |
| Protein % ^a | 25.0 | 25.0 | 25.0 |
| Protein % ^b | 24.6 | 26.6 | 25.4 |
| Fat % | 3.9 | 5.6 | 3.6 |
| Fiber % | 6.6 | 6.1 | 5.7 |
| Moisture % | 12.5 | 10.0 | 10.2 |
| Ash % | 8.8 | 12.9 | 15.3 |
| Calcium % ^c | 1. | 2.84 | 3.60 |
| Phosphorus % ^d | 1.16 | 2.15 | 2.60 |
| Metabolizable energy (Kcal/kg) ^a | 1870 | 1870 | 1870 |

^aCalculated from values in ingredient analyses tables for poultry (NRC 1969).

^bDetermined by AOAC methods (1969).

^cDetermined by flame ionization atomic absorption using strontium chloride to prevent phosphorus interference by K.S.U. An. Sci. Nutr. Lab.

^dDetermined by colorometric spectrophotometry by K.S.U. An. Sci. Nutr. Lab.

Analyses of crude fat in eviscerated fish bodies were run on four determinations per diet regimen per sampling date. Analyses of crude fat in livers and of moisture in eviscerate fish bodies were run on two determinations per diet regimen per sampling date. Because of the small amount of livers available, analyses of moisture in livers were run on one determination per diet regimen per sampling date. Percentages of crude fat in both livers and eviscerated fish bodies were recorded on a dry weight basis and total crude fat content of livers and fish on a wet weight basis.

Two-way analysis of variance (Snedecor and Cockran 1967) was used to evaluate percentages of moisture and crude fat in the eviscerated bodies and in the livers of age group I and II fish sampled during their respective feeding periods. In the analyses, sampling date-diet interactions were determined for the moisture and crude fat in the eviscerated bodies but not in the livers, because only one composite liver sample was analyzed per regimen per sampling date. Where the sampling date-diet interaction could not be determined, the statistical tests were unavoidably weighted toward the nonsignificant conclusion. One-way analysis of variance (Snedecor and Cockran 1967) was used to evaluate percentages of moisture and crude fat in livers of age group I fish sampled during the nonfeeding period. Percentages of moisture in the livers during the nonfeeding period could not be statistically analyzed because there was only one sample per sampling date and only one group of fish studied.

Results

There were no significant sampling date or diet differences ($P > 0.5$) in the percentages of moisture and crude fat in livers of age group I channel catfish (Figure 1) during the feeding period. The sampling date-diet interaction could not be determined. There was a significant difference ($P < .05$) in the percentages of crude fat in the livers at the different sampling dates during the nonfeeding period. Percentages of moisture in the livers during the nonfeeding period were determined but not statistically analyzed.

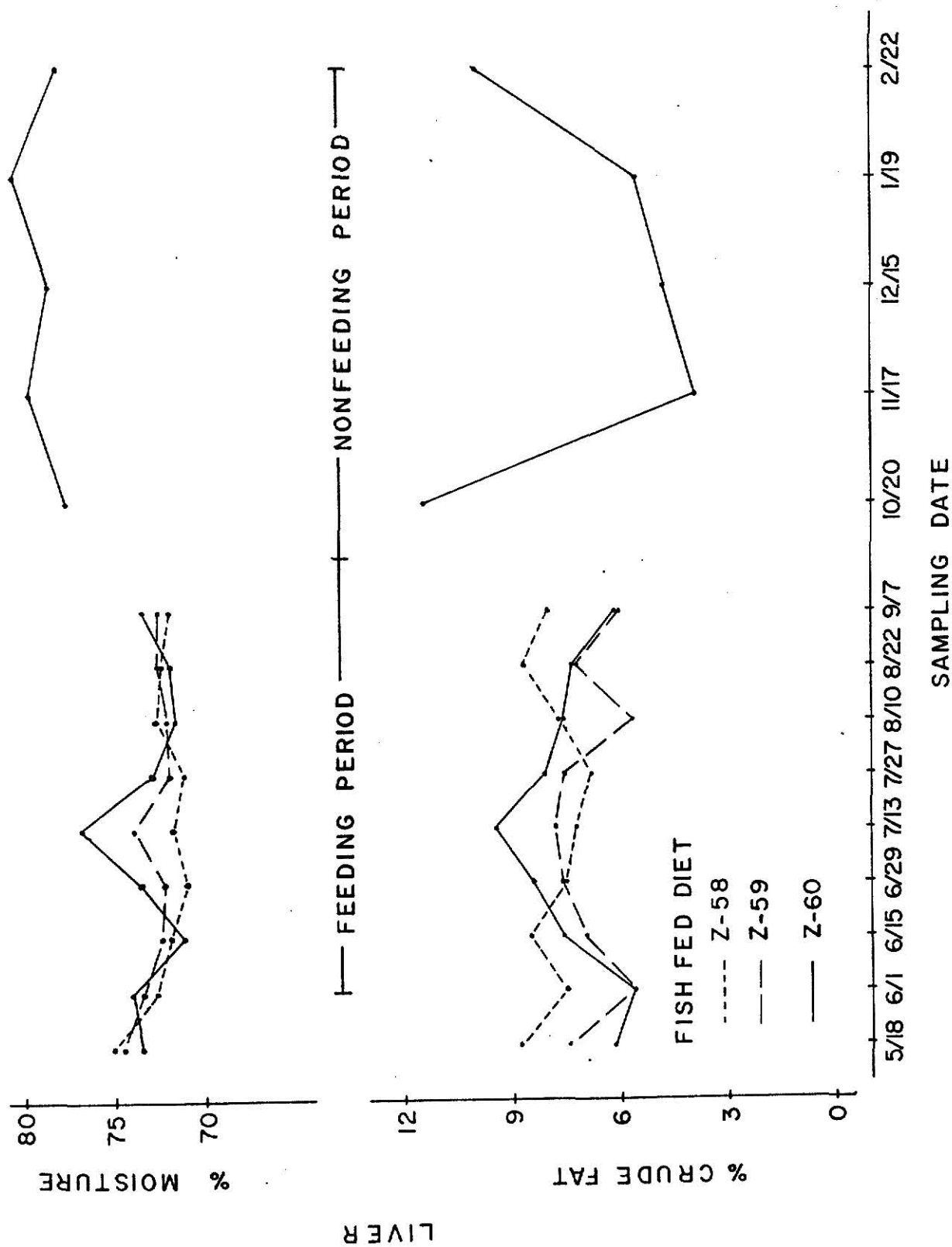


Figure 1. Moisture and crude fat percentages in livers of age group I fish fed three diets from June 1 to Sept. 14, 1972, and not fed from Sept. 14, 1972 to Feb. 22, 1973.

There were significant sampling-diet differences ($P < .05$) in the percentages of moisture and crude fat in livers of supplementally fed age group II fish (Figure 2) but no significant diet differences ($P > .10$). The sampling date-diet interaction could not be determined.

There were significant sampling-date and diet differences ($P < .05$) but no significant sampling date-diet interaction ($P > .10$) in the percentages of crude fat in the eviscerated bodies of age group I channel catfish (Figure 3) during the feeding period. The percentages of crude fat were statistically higher in fish fed the Z-58 diet than in those fed the Z-60 diet. There were no statistical differences ($P > .05$) between the percentages of crude fat in the fish fed the Z-59 diet and those fed either of the other diets. During the non-feeding period, there were significant sampling date differences ($P < .05$) in the percentages of crude fat. From May 18 to September 14, 1973, percentages of crude fat in the eviscerated bodies of age group II fish (Figure 4) varied significantly ($P < .05$) but neither diet differences nor sampling date-diet interactions were significant ($P > .35$).

During the feeding period, sampling date-diet interaction was highly significant ($P < .008$) for the percentage of moisture in the eviscerated bodies of age group I channel catfish (Figure 3). That interaction prevents our discussing date or diet without considering both variables at the same time. During the nonfeeding period, percentages of moisture in the eviscerated bodies of fish were significantly different ($P < .05$) at the different sampling dates.

Percentages of moisture in the eviscerated bodies of supplementally fed age group II channel catfish (Figure 4) showed highly significant sampling-date differences ($P < .001$), but neither diet differences ($P > .88$) nor sampling date-diet interactions ($P > .44$) were significant during the feeding period.

Tables 3, 4, and 5 and 6 show, respectively, for channel catfish of both age groups, body-weight gains and feed conversions during the respective feeding

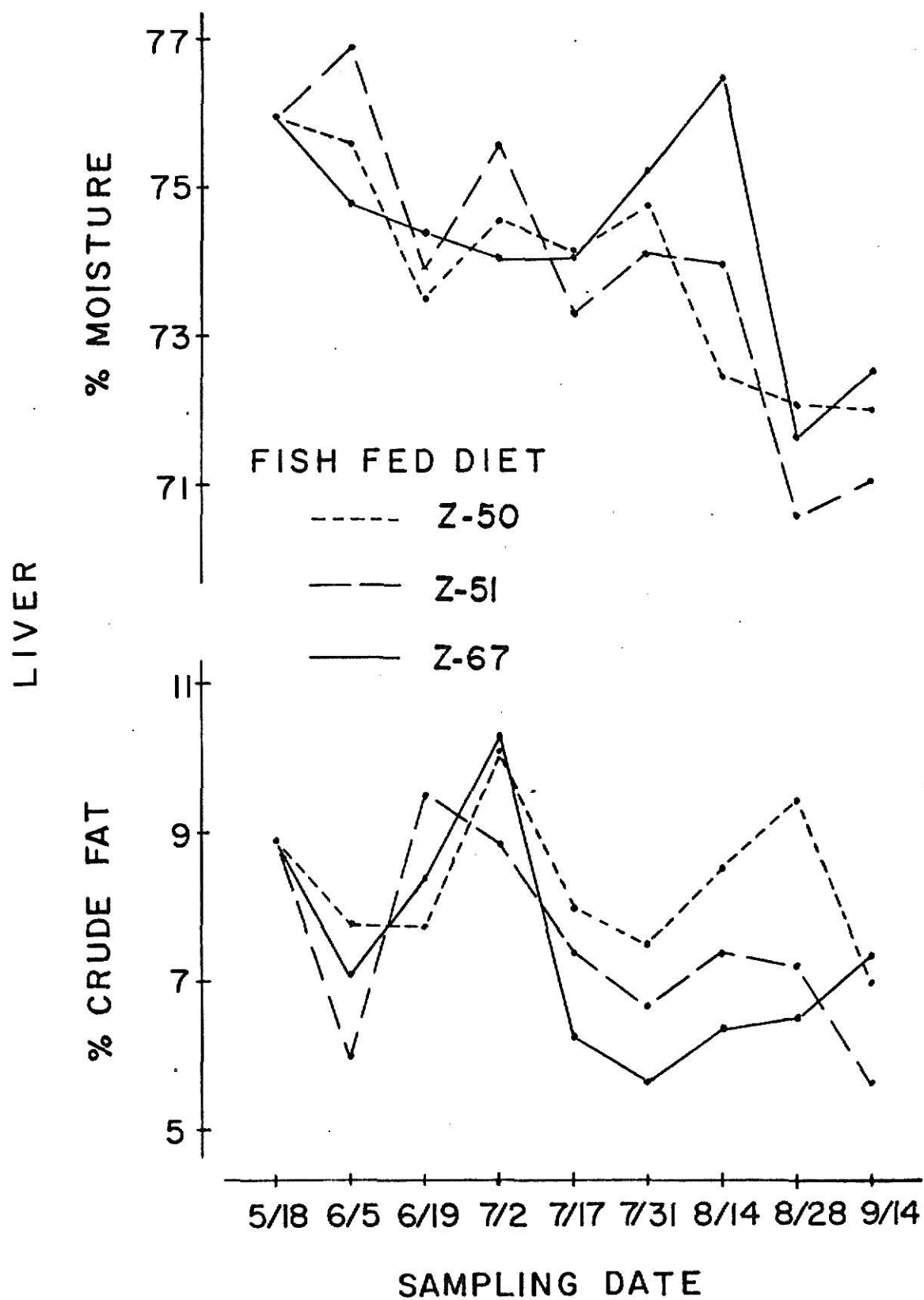


Figure 2. Moisture and crude fat percentages in livers of age group II fish fed three diets from May 21 to Sept. 14, 1973.

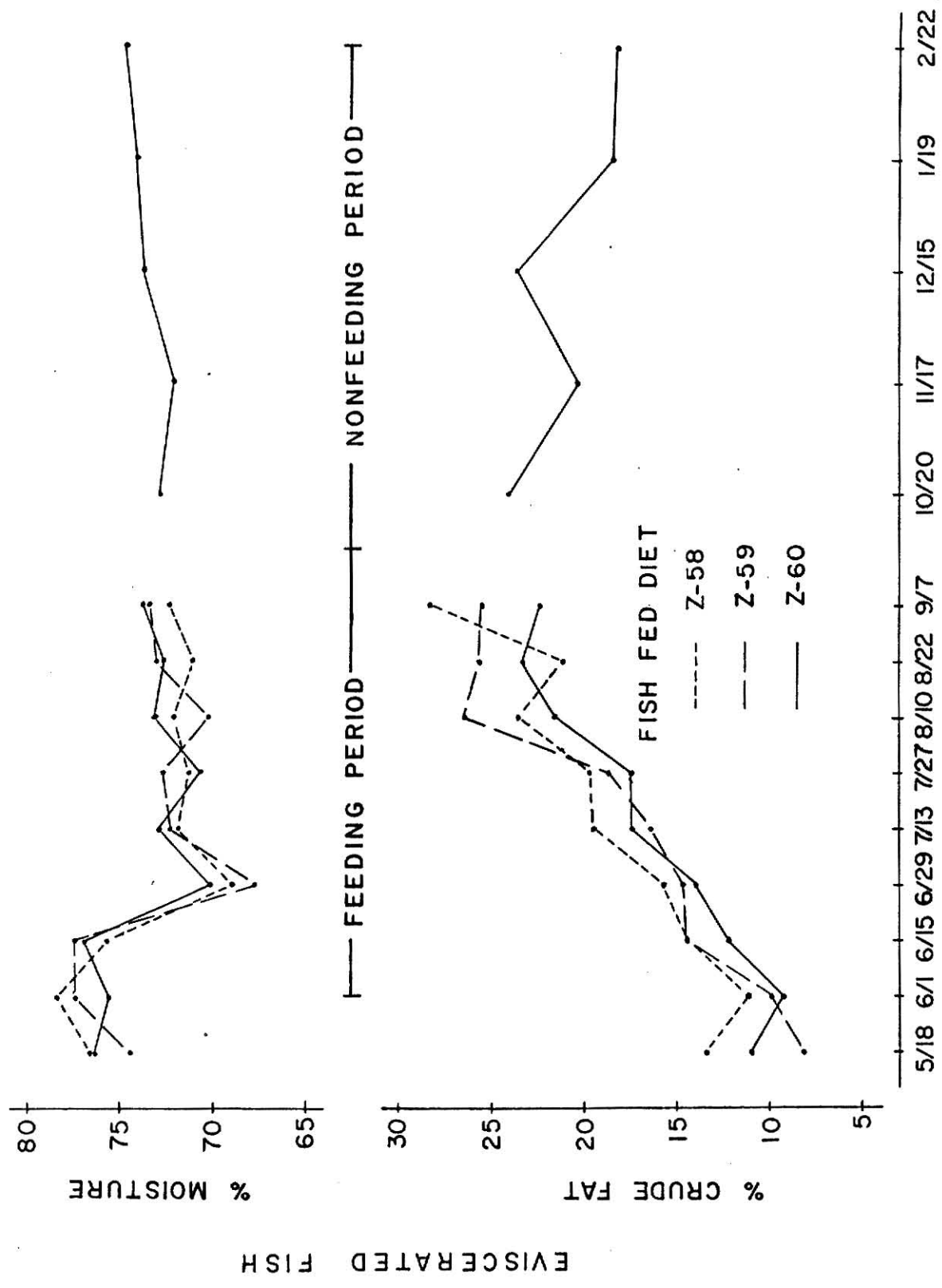


Figure 3. Moisture and crude fat percentages in age group I fish fed three diets from June 1 to Sept. 14, 1972, and not fed from Sept. 14, 1972 to Feb. 22, 1973.

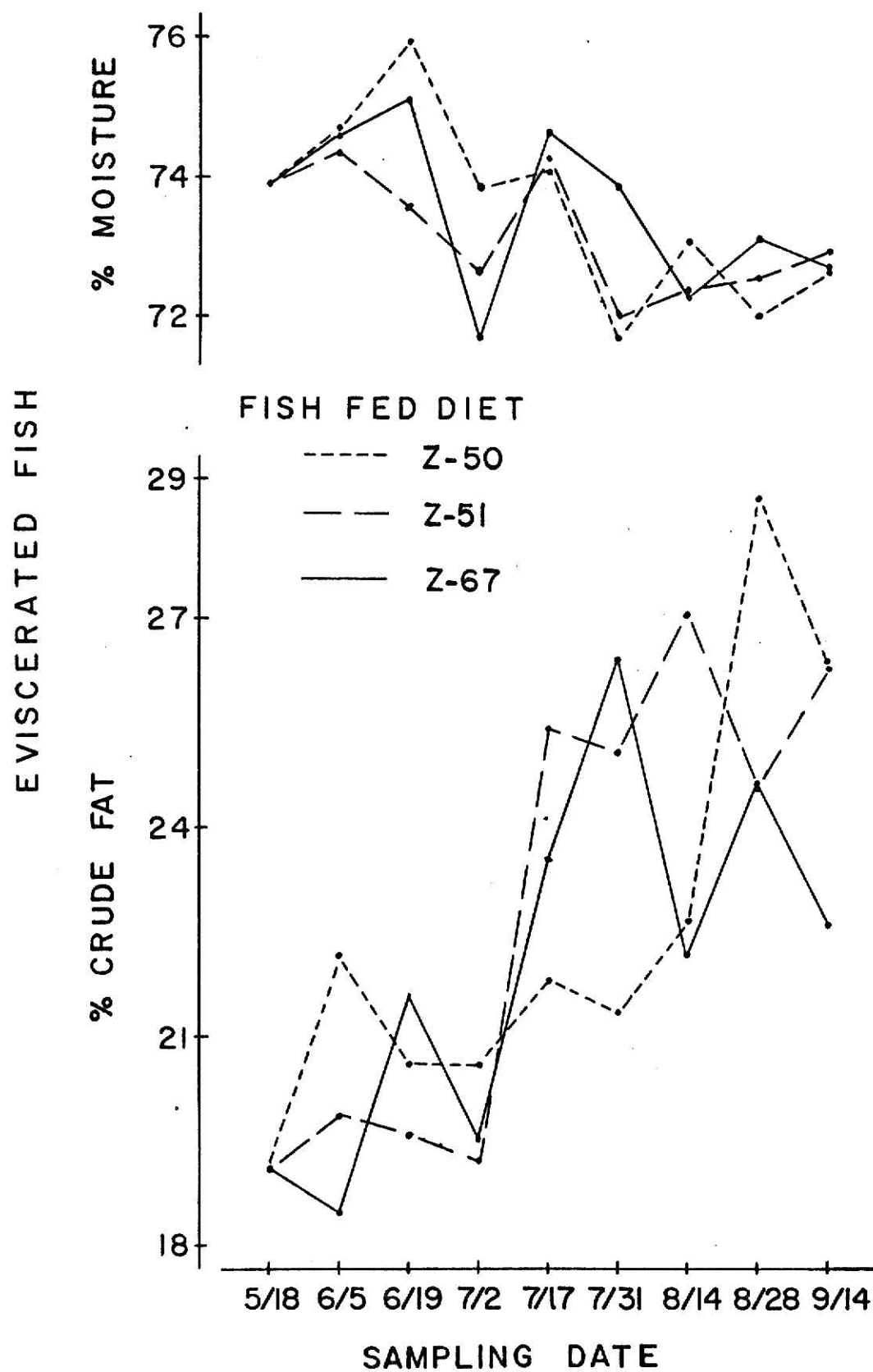


Figure 4. Moisture and crude fat percentages in age group II fish fed three diets from May 21 to Sept. 14, 1973.

Table 3. Body weight gains and feed conversions for age group I and II channel catfish determined from stocking and harvesting weights.

| <u>Age group</u> | <u>Fish fed diet</u> | <u>Weight gain/pond</u> | <u>Feed conversion</u> |
|------------------|----------------------|-------------------------|------------------------|
| I | Z-58 | 49,261 g | 1.07 |
| I | Z-59 | 46,684 g | 1.13 |
| I | Z-60 | 49,627 g | 1.06 |
| II | Z-50 | 46,120 g | 1.77 |
| II | Z-51 | 53,156 g | 1.51 |
| II | Z-67 | 50,040 g | 1.61 |

Table 4. Average weight of livers in age group I and II channel catfish and average weight of crude fat per liver.

| <u>Sampling date</u> | <u>Age class</u> | <u>Weight/liver</u> | <u>Crude fat/liver^a</u> |
|----------------------|------------------|--------------------------|------------------------------------|
| 5/18/72 | I | 0.20 g (30) ^b | 0.004 g |
| 6/1/72 | I | 0.29 (30) | 0.005 |
| 6/15/72 | I | 0.42 (30) | 0.010 |
| 6/29/72 | I | 0.49 (30) | 0.011 |
| 7/13/72 | I | 0.69 (30) | 0.014 |
| 7/27/72 | I | 0.86 (30) | 0.018 |
| 8/10/72 | I | 1.25 (30) | 0.024 |
| 8/22/72 | I | 1.33 (30) | 0.028 |
| 9/7/72 | I | 1.87 (30) | 0.038 |
| 10/20/72 | I | 1.46 (20) | 0.037 |
| 11/17/72 | I | 2.08 (20) | 0.016 |
| 12/15/72 | I | 2.28 (20) | 0.022 |
| 1/19/73 | II | 1.41 (20) | 0.015 |
| 2/22/73 | II | 1.08 (20) | 0.023 |
| 5/18/73 | II | 1.23 (20) | 0.029 |
| 6/5/73 | II | 2.50 (20) | 0.042 |
| 6/19/73 | II | 2.26 (20) | 0.050 |
| 7/2/73 | II | 2.53 (20) | 0.063 |
| 7/17/73 | II | 3.16 (20) | 0.060 |
| 7/31/73 | II | 3.26 (20) | 0.055 |
| 8/14/73 | II | 3.01 (20) | 0.057 |
| 8/28/73 | II | 3.33 (20) | 0.073 |
| 9/14/73 | II | 3.12 (20) | 0.059 |

^aOn a wet weight basis.

^bNumbers in parentheses indicate number of livers weighed.

Table 5. Average weight of age group I channel catfish and weight of crude fat per fish.

| <u>Sampling date</u> | <u>Regimen</u> | <u>Weight/fish</u> | <u>Crude fat/fish^a</u> |
|----------------------|----------------|--------------------|-----------------------------------|
| 5/18/72 | Z-58 | 9.6 g | 0.30 g |
| | Z-59 | 8.1 | 0.17 |
| | Z-60 | 8.6 | 0.22 |
| 6/1/72 | Z-58 | 15.0 | 0.38 |
| | Z-59 | 15.3 | 0.32 |
| | Z-60 | 14.3 | 0.32 |
| 6/15/72 | Z-58 | 20.3 | 0.71 |
| | Z-59 | 19.6 | 0.64 |
| | Z-60 | 18.0 | 0.51 |
| 6/29/72 | Z-58 | 25.3 | 1.24 |
| | Z-59 | 26.3 | 1.25 |
| | Z-60 | 25.6 | 1.07 |
| 7/13/72 | Z-58 | 33.3 | 1.83 |
| | Z-59 | 31.6 | 1.43 |
| | Z-60 | 36.0 | 1.71 |
| 7/27/72 | Z-58 | 42.6 | 2.42 |
| | Z-59 | 51.0 | 2.61 |
| | Z-60 | 48.6 | 2.49 |
| 8/10/72 | Z-58 | 55.5 | 3.65 |
| | Z-59 | 61.8 | 4.87 |
| | Z-60 | 62.3 | 3.60 |
| 8/22/72 | Z-58 | 71.3 | 4.37 |
| | Z-59 | 71.3 | 4.94 |
| | Z-60 | 83.6 | 5.57 |

Table 5. continued.

| | | | |
|----------|------|-------|------|
| 9/7/72 | Z-58 | 88.9 | 6.96 |
| | Z-59 | 97.7 | 6.64 |
| | Z-60 | 100.0 | 6.14 |
| 10/20/72 | Z-60 | 128.5 | 8.40 |
| 11/17/72 | Z-60 | 109.6 | 5.92 |
| 12/15/72 | Z-60 | 113.6 | 7.04 |
| 1/19/73 | Z-60 | 75.9 | 3.62 |
| 2/22/73 | Z-60 | 81.0 | 3.73 |

^aOn a wet weight basis.

Table 6. Range and mean of body weights of age group II channel catfish and average weight of crude fat per fish.

| <u>Sampling date</u> | <u>Weight of fish</u> | | <u>Crude fat/fish^a</u> |
|----------------------|-----------------------|-------------|-----------------------------------|
| | <u>Range</u> | <u>Mean</u> | |
| 5/18 | 52-160 | 93.5 g | 4.66 g |
| 6/5 | 73-276 | 146.5 | 7.52 |
| 6/19 | 68-241 | 138.1 | 6.41 |
| 7/2 | 73-244 | 144.7 | 7.94 |
| 7/17 | 85-294 | 159.1 | 9.56 |
| 7/31 | 90-332 | 192.7 | 12.27 |
| 8/14 | 86-380 | 186.9 | 12.27 |
| 8/28 | 55-428 | 226.5 | 16.14 |
| 9/14 | 102-564 | 280.8 | 19.16 |

^aOn a wet weight basis.

periods for the diet regimens used, total weight of crude fat in liver for the entire study, and total weight of crude fat in eviscerated bodies for the entire study.

Discussion

Because percentages of moisture and crude fat in livers of age group I channel catfish (Figure 1) varied little (a maximum difference of 1.16 g of water in the 20-gram samples and 0.008 g crude fat in the 2-gram samples analyzed) during the feeding period, livers apparently maintained an equilibrium of approximately 73% moisture and 7.5% crude fat. Percentages of crude fat in the livers analyzed during the nonfeeding period dropped during the first month and then during the next three months gradually increased to 1.5% (or 0.03 g) lower than that recorded at the first sampling date of the nonfeeding period. Physiological studies of the nonfed channel catfish would be required to explain those changes. The liver moisture percentages for the nonfeeding period were unexplainably higher than those recorded during the feeding period.

During the feeding period, the percentage of moisture in the livers of supplementally fed age group II channel catfish tended to decrease, while percentages of crude fat seemed to fluctuate about a 7.5% level (Figure 2). Suppes et al. (1967) reported that in their study the lowest percentages of moisture in livers of age group II channel catfish occurred near the middle of the feeding period; in our study the lowest percentage was recorded at the end of the feeding period. Both studies, however, recorded that age group II fish had lower percentages of moisture in their livers at the end than at the beginning of the feeding period. Suppes et al. (1967) found higher percentage of crude fat in the livers of age group II fish than we found. That might have been because of differences in the feeding rate; Suppes' fish were fed at 4% (Suppes 1967) and ours at 2%.

During the feeding periods for age group I and II fish, the percentages of

crude fat in livers (on a dry weight basis) stayed constant (Figures 1 and 2), while liver weight and total crude fat in livers (on a wet weight basis) increased (Table 4). That indicated liver was not a major fat storage area.

Diet-related differences in percentages of crude fat (Figure 3) and total crude fat (Table 5) in the eviscerated bodies of age group I channel catfish were not reflected in either weight gains or feed conversion during the feeding period (Table 3). Percentages of crude fat in the fish bodies increased during the feeding period but tended to decrease during the nonfeeding period, indicating fish used stored fat for energy and body maintenance in the absence of supplemental diets. As in supplementally fed age group I fish, percentages of crude fat increased in the eviscerated bodies of age group II channel catfish sampled during the feeding period, indicated body tissues (excluding viscera and liver) were major storage areas for fat. Also in age class II fish, there was no correlation between the percentage of fat (Figure 4) or total fat (Table 6) in the bodies and weight gain or feed conversion during the feeding period (Table 3). That indicated fat content was not a good indicator of weight gains or feed conversions of supplementally fed channel catfish of either age group.

Reasons for changes in the moisture levels in the bodies of age group I fish (Figure 3) during the feeding period are not clear. Pearse (1925) found that moisture in 0-6-month-old brook trout was as high as 83% but stabilized at 75% after the trout were a year old. Moisture increased in the eviscerated bodies of age group I fish (Figure 3) during the nonfeeding period and decreased in those of age group II catfish (Figure 4) during the feeding period. Though moisture was not related to the percentage of crude fat in the bodies of supplementally fed age group I catfish, it was related to the percentage of crude fat in the bodies of unfed age group I catfish and of supplementally fed age group II fish. As crude fat percentages decreased in the bodies of

unfed age group I fish, moisture levels increased; and as crude fat percentages increased in the bodies of supplementally fed age group II fish, moisture levels decreased. Similar inverse moisture-fat relationships have been reported for trout (Phillips et al. 1960) and for age class II channel catfish (Suppes 1967).

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APPENDIX

Calcium determination in feeds

Weigh 2 gm. into acid-washed crucibles. Ash at 600°C , increasing the temperature slowly. Proceed with standard atomic absorption analyses procedures using strontium chloride to prevent phosphorus interference. Read duplicate samples on a Jarrell-Ash flame emission-atomic absorption spectrophotometer at 4226.5 Å.

Phosphorus determination in feeds

Reagents: MS Solution: Dissolve 5 gm. sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) in about 500 ml. water. Add 14 ml. conc. H_2SO_4 , and bring to 1 liter with deionized water. (Stable indefinitely if stored in polyethylene bottles.)

Elon Solution: Dissolve 1 gm. elon (p-methylaminophenol sulfate) in 100 ml. 3% sodium bisulfite (NaHSO_3). Prepared fresh weekly. Store under refrigeration.

Standards: From potassium dihydrogen phosphate (KH_2PO_4) or other pure anhydrous phosphorus compound, prepare standards containing 20, 40, 60, 100, and 200 mcg phosphorus per ml.

Procedure: Weight 2 gm. into acid-washed crucibles. Ash at 600°C , increasing the temperature slowly. Dissolve in 5 ml. 6N HCl, bring to a boil, and bring to 100 ml. Add 0.3 ml. to 4 ml. MS solution. Mix, and add 0.5 ml. elon solution. Mix again, and let stand one hour. Read on colorimetric spectrophotometer at 700 mμ.

Calculations: From the standards, and the Beer's Law relationship, $A = abC$, where A =absorbance; a =absorptivity; b =path length (usually 1 cm., and generally omitted from the equation); C =concentration in units corresponding to a , compute $1/a = C/A$. Thus, for unknowns, $C = A(1/a)$. $1/a$ is a constant, and can be used in a calculator as a constant multiplier.

Calcium determination in pond water

To clean test tubes add 1 ml. of pond water and 4 ml. of 1% strontium. Mix well. Do in duplicate. Use standards ranging from 1 - 50 ppm calcium, plus a blank. Samples are then read on a Jarrell-Ash flame emission-atomic absorption spectrophotometer at 4226.5 Å.

Phosphorus determination in pond water

Reagents: MS Solution: Dissolve 5 gm. sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) in about 500 ml. water. Add 14 ml. conc. H_2SO_4 , bring to 1 liter with deionized water.

Elon Solution: Dissolve 1 gm. elon (p-methylaminophenol sulfate) in 100 ml. 3% sodium bisulfite (NaHSO_3). Prepare fresh weekly and store under refrigeration.

Standards: 40 micrograms P/ml.: .1757 mg. KH_2PO_4 to a tared liter storage bottle. Add approximately 500 ml. distilled/deionized water, plus 2.5 ml. 1:1 H_2SO_4 and a few drops of CHCl_3 (as preservative). Take to a total volume of 1000 ml.

1 microgram P/ml: 25 ml. of the 40 mcg. standard, plus 3 or 4 drops of CHCl_3 to 1000 ml.

Intermediate standards: Prepare standards of 5, 10, 15, 20, 25, and 30 mcg. from the 40 mcg. standard. Prepare one blank, too.

Equipment: Two 25 ml. volumetric flasks; two 25 ml. test tubes; and one clean pipette per sample. All equipment should be rinsed with MS solution and then with deionized water before using - including pipettes.

Procedure: Using 25 ml. flasks, add 15 ml. pondwater, 5 ml. MS solution 1 ml. Elon solution and add deionized water to fill to volume. Stopper and mix well. Use both volumetrics for one sample at the same time. (Get better results when both are made simultaneously). Transfer contents of volumetrics to test tube - fill it well and dump the rest out. Rinse volumetrics two or three times with deionized water and drain as much water out as possible.

Let test tubes sit at least 30 minutes, but not more than three hours. Turn DU on - wait an additional 30 minutes before running samples. Read on colorimetric spectrophotometer at 700 mu.

Table I. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of calcium in eviscerated bodies of age group I channel catfish fed from May 1 to September 14.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|--------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 9 | 60,456,160 | 0.0000 |
| Diet | 2 | 1,490,343 | 0.7102 |
| Sampline date-diet interaction | 18 | 3,340,554 | 0.7369 |
| Residual | 266 | 4,348,692 | |
| Total | 295 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % calcium*</u> |
|----------------------|------------------------|
| 4/16 | 0.8605 |
| 5/18 | 0.6205 |
| 6/1 | 0.4854 ^a |
| 6/15 | 0.5058 ^a |
| 6/29 | 0.4399 ^a |
| 7/13 | 0.4777 ^a |
| 7/27 | 0.3265 |
| 8/10 | 0.4767 ^a |
| 8/22 | 0.4508 ^a |
| 9/7 | 0.4645 ^a |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table II. One-way analysis of variance table (A) and summary of statistically significant levels (B) of calcium in eviscerated bodies of age group I channel catfish fed the Z-60 diet from May 1 to September 14, 1972.

A. One-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 14 | 18,878,592 | .0054 |
| Residual | 133 | 7,915,059 | |
| Total | 147 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % calcium*</u> |
|----------------------|-------------------------|
| 4/16 | 0.8604 ^a |
| 5/18 | 0.5880 ^{a,b,c} |
| 6/1 | 0.4367 ^c |
| 6/15 | 0.5227 ^{b,c} |
| 6/29 | 0.4728 ^{b,c} |
| 7/13 | 0.4826 ^{b,c} |
| 7/27 | 0.3845 ^c |
| 8/10 | 0.5345 ^{b,c} |
| 8/22 | 0.4512 ^c |
| 9/7 | 0.5123 ^{b,c} |
| 10/20 | 0.7914 ^{a,b} |
| 11/17 | 0.3776 ^c |
| 12/15 | 0.6500 ^{a,b,c} |
| 1/19 | 0.4870 ^{b,c} |
| 2/22 | 0.6037 ^{a,b,c} |

*Means sharing the same superscript letter are not significantly different at the 99% level.

Table III. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of phosphorus in eviscerated bodies of age group I channel catfish fed from May 1 to September 14, 1972.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|--------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 9 | 0.1764 | 0.0000 |
| Diet | 2 | 0.0165 | 0.5891 |
| Sampling date-diet interaction | 18 | 0.0995 | 0.0000 |
| Residual | 203 | 0.0310 | |
| Total | 232 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % phosphorus*</u> | | |
|----------------------|---------------------------|---------------------------|---------------------------|
| | <u>Fish fed diet Z-58</u> | <u>Fish fed diet Z-59</u> | <u>Fish fed diet Z-60</u> |
| 4/16 | 0.2774 ^{d,e,f,g} | 0.2774 ^{d,e,f,g} | 0.2774 ^{d,e,f,g} |
| 5/18 | 0.3185 ^{d,e,f,g} | 0.7387 ^a | 0.4239 ^{b,c,d,e} |
| 6/1 | 0.2637 ^{d,e,f,g} | 0.2209 ^{f,g} | 0.2079 ^{f,g} |
| 6/15 | 0.2419 ^{e,f,g} | 0.2516 ^{e,f,g} | 0.1812 ^{f,g} |
| 6/29 | 0.2837 ^{d,e,f,g} | 0.2487 ^{e,f,g} | 0.4269 ^{b,c,d} |
| 7/13 | 0.2462 ^{e,f,g} | 0.2788 ^{d,e,f,g} | 0.1749 ^{f,g} |
| 7/27 | 0.2599 ^{d,e,f,g} | 0.3059 ^{d,e,f,g} | 0.5712 ^{a,b} |
| 8/10 | 0.1866 ^{f,g} | 0.1949 ^{f,g} | 0.2522 ^{e,f,g} |
| 8/22 | 0.2659 ^{d,e,f,g} | 0.1499 ^g | 0.2771 ^{d,e,f,g} |
| 9/7 | 0.5099 ^{b,c} | 0.2333 ^{e,f,g} | 0.3412 ^{c,d,e,f} |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table IV. One-way analysis of variance table (A) and summary of statistically significant levels (B) of phosphorus in eviscerated bodies of age group I channel catfish fed the Z-60 diet from May 1 to September 14, 1972.

A. One-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|----------------|---------------------------|---------------------|--------------------|
| Sampling date. | 14 | 0.1417 | 0.00001 |
| Residual | 113 | 0.0354 | |
| Total | 127 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % phosphorus*</u> |
|----------------------|-----------------------------|
| 4/16 | 0.277 ^{b, c, d} |
| 5/18 | 0.424 ^{a, b, c} |
| 6/1 | 0.208 ^{c, d} |
| 6/15 | 0.181 ^d |
| 6/29 | 0.427 ^{a, b, c} |
| 7/13 | 0.175 ^d |
| 7/27 | 0.571 ^a |
| 8/10 | 0.252 ^{c, d} |
| 8/22 | 0.277 ^{b, c, d} |
| 9/7 | 0.341 ^{a, b, c, d} |
| 10/20 | 0.276 ^{c, d} |
| 11/17 | 0.301 ^{b, c, d} |
| 12/15 | 0.545 ^a |
| 1/19 | 0.502 ^{a, b} |
| 2/22 | 0.389 ^{a, b, c, d} |

*Means sharing the same superscript letter are not significantly different at the 99% level.

Table V. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of calcium in fat-free skeletons of age group I channel catfish fed from May 1 to September 14, 1972.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|--------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 9 | 10,121,854,976 | 0.0000 |
| Diet | 2 | 1,325,093,120 | 0.0046 |
| Sampling date-diet interaction | 18 | 987,577,343 | 0.0000 |
| Residual | 268 | 240,878,480 | |
| Total | 297 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % calcium*</u> | | |
|----------------------|---------------------------|---------------------------|---------------------------|
| | <u>Fish fed diet Z-58</u> | <u>Fish fed diet Z-59</u> | <u>Fish fed diet Z-60</u> |
| 4/16 | 12.36 ^{c,d,e,f} | 12.36 ^{c,d,e,f} | 12.36 ^{c,d,e,f} |
| 5/18 | 13.61 ^{b,c} | 13.02 ^{c,d,e} | 12.43 ^{c,d,e,f} |
| 6/1 | 9.33 ^{j,k} | 8.49 ^k | 9.13 ^{j,k} |
| 6/15 | 11.64 ^{e,f,g,h} | 13.71 ^{b,c} | 11.68 ^{e,f,g,h} |
| 6/29 | 9.41 ^{i,j,k} | 10.75 ^{g,h,i} | 12.63 ^{c,d,e,f} |
| 7/13 | 11.24 ^{f,g,h} | 10.35 ^{h,i,j} | 11.41 ^{f,g,h} |
| 7/27 | 8.96 ^{j,k} | 9.35 ^{j,k} | 9.13 ^{j,k} |
| 8/10 | 11.97 ^{d,e,f,g} | 15.15 ^a | 15.25 ^a |
| 8/22 | 13.28 ^{b,c,d} | 13.13 ^{c,d} | 11.90 ^{d,e,f,g} |
| 9/7 | 12.89 ^{c,d,e} | 14.65 ^{a,b} | 15.20 ^a |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table VI. One-way analysis of variance table (A) and summary of statistically significant levels (B) of calcium in fat-free skeletons of age group I channel catfish fed the Z-60 diet from May 1 to September 14, 1972.

A. One-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 14 | 2,833,608,192 | 0.0000 |
| Residual | 127 | 258,827,424 | |
| Total | 141 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % calcium*</u> |
|----------------------|------------------------|
| 4/16 | 12.36 ^{c,d} |
| 5/18 | 12.43 ^{c,d} |
| 6/1 | 9.13 ^e |
| 6/15 | 11.68 ^{c,d} |
| 6/29 | 12.63 ^{b,c,d} |
| 7/13 | 11.41 ^d |
| 7/27 | 9.13 ^e |
| 8/10 | 15.25 ^a |
| 8/22 | 11.90 ^{c,d} |
| 9/7 | 15.20 ^{a,b} |
| 10/20 | 13.30 ^{b,c} |
| 11/17 | 12.62 ^{b,c} |
| 12/15 | 12.55 ^{c,d} |
| 1/19 | 12.05 ^{c,d} |
| 2/22 | 11.22 ^d |

*Means sharing the same superscript letter are not significantly different at the 99% level.

Table VII. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of the phosphorus in fat-free skeletons of age group I channel catfish fed from May 1 to September 14, 1972.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|--------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 9 | 46.46 | 0.0000 |
| Diet | 2 | 6.67 | 0.0031 |
| Sampling date-diet interaction | 18 | 6.92 | 0.0000 |
| Residual | 251 | 1.13 | |
| Total | 280 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % phosphorus*</u> | | |
|----------------------|-----------------------------|-----------------------------|---------------------------|
| | <u>Fish fed diet Z-58</u> | <u>Fish fed diet Z-59</u> | <u>Fish fed diet Z-60</u> |
| 4/16 | 3.47 ^{g,h,i,j} | 3.47 ^{g,h,i,j} | 3.47 ^{g,h,i,j} |
| 5/18 | 3.72 ^{f,g,h,i,j} | 4.15 ^{d,e,f,g,h,i} | 4.53 ^{d,e,f} |
| 6/1 | 3.01 ^{h,i,j,k} | 3.77 ^{e,f,g,h,i,j} | 2.99 ^{i,j,k} |
| 6/15 | 3.36 ^{h,i,j} | 2.28 ^{k,l} | 4.53 ^{d,e,f} |
| 6/29 | 4.17 ^{d,e,f,g,h} | 4.54 ^{d,e,f} | 2.91 ^{j,k} |
| 7/13 | 4.49 ^{d,e,f} | 4.41 ^{d,e,f,g} | 3.59 ^{f,g,h,i,j} |
| 7/27 | 4.27 ^{d,e,f,g,h} | 4.69 ^{c,d,e} | 5.55 ^{b,c} |
| 8/10 | 6.98 ^a | 8.83 | 6.11 ^{a,b} |
| 8/22 | 3.79 ^{e,f,g,h,i,j} | 3.63 ^{f,g,h,i,j} | 3.35 ^{h,i,j} |
| 9/7 | 5.03 ^{c,d} | 2.34 ^{k,l} | 1.77 ^l |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table VIII. One-way analysis of variance table (A) and summary of statistically significant levels (B) of phosphorus in fat-free skeletons of age group I channel catfish fed the Z-60 diet from May 1 to September 14, 1972.

A. One-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 14 | 15.95 | 0.0000 |
| Residual | 122 | 0.82 | |
| Total | 136 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % phosphorus*</u> |
|----------------------|----------------------------|
| 4/16 | 3.467 ^{d,e,f} |
| 5/18 | 4.534 ^{b,c} |
| 6/1 | 2.986 ^{e,f,g,h,i} |
| 6/15 | 3.212 ^{e,f,g} |
| 6/29 | 2.914 ^{f,g,h} |
| 7/13 | 3.592 ^{c,d,e,f} |
| 7/27 | 5.551 ^{a,b} |
| 8/10 | 6.110 ^a |
| 8/22 | 3.355 ^{d,e,f} |
| 9/7 | 1.774 ⁱ |
| 10/20 | 2.183 ^{g,h,i} |
| 11/17 | 3.957 ^{c,d,e} |
| 12/15 | 3.835 ^{c,d,e,f} |
| 1/9 | 4.470 ^{b,c,d} |
| 2/22 | 6.354 ^a |

*Means sharing the same superscript letter are not significantly different at the 99% level.

Table IX. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of calcium in eviscerated bodies of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean square</u> | <u>Probability</u> |
|--------------------------------|---------------------------|--------------------|--------------------|
| Sampling date | 8 | 0.3661 | 0.0109 |
| Diet | 2 | 0.1317 | 0.4805 |
| Sampling date-diet interaction | 16 | 0.1449 | 0.6752 |
| Residual | 238 | 0.1791 | |
| Total | 264 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % calcium*</u> |
|----------------------|---------------------------|
| 5/18 | 0.7299 ^d |
| 6/5 | 0.3900 ^{a,b} |
| 6/19 | 0.4779 ^{a,b,c} |
| 7/2 | 0.3636 ^a |
| 7/17 | 0.6622 ^{c,d} |
| 7/31 | 0.4613 ^{a,b,c} |
| 8/14 | 0.5141 ^{a,b,c,d} |
| 8/28 | 0.6000 ^{b,c,d} |
| 9/14 | 0.4245 ^{a,b} |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table X. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of phosphorus in eviscerated bodies of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean square</u> | <u>Probability</u> |
|--------------------------------|---------------------------|--------------------|--------------------|
| Sampling date | 8 | 0.0595 | 0.0001 |
| Diet | 2 | 0.0021 | 0.8623 |
| Sampling date-diet interaction | 16 | 0.0153 | 0.3987 |
| Residual | 233 | 0.1449 | |
| Total | 259 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % phosphorus*</u> |
|----------------------|---------------------------|
| 5/18 | 0.3628 |
| 6/5 | 0.2799 ^{b,c} |
| 6/19 | 0.2555 ^{a,b,c} |
| 7/2 | 0.2398 ^{a,b,c} |
| 7/17 | 0.2327 ^{a,b} |
| 7/31 | 0.2045 ^a |
| 8/14 | 0.2564 ^{a,b,c} |
| 8/28 | 0.2426 ^{a,b,c} |
| 9/14 | 0.2981 ^c |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XI. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of calcium in fat-free skeletons of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean square</u> | <u>Probability</u> |
|--------------------------------|---------------------------|--------------------|--------------------|
| Sampling date | 8 | 137.53 | 0.0000 |
| Diet | 2 | 28.36 | 0.0088 |
| Sampling date-diet interaction | 16 | 26.08 | 0.0000 |
| Residual | 242 | 5.88 | |
| Total | 268 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % calcium*</u> | | |
|----------------------|------------------------------|----------------------------|---------------------------|
| | <u>Fish fed diet Z-50</u> | <u>Fish fed diet Z-51</u> | <u>Fish fed diet Z-67</u> |
| 5/18 | 13.69 ^{a,b,c,d} | 13.69 ^{a,b,c,d} | 13.69 ^{a,b,c,d} |
| 6/5 | 16.26 ^{f,g,h} | 12.30 ^a | 12.23 ^a |
| 6/19 | 14.59 ^{b,c,d,e,f} | 12.59 ^{a,b} | 13.45 ^{a,b,c} |
| 7/2 | 14.33 ^{a,b,c,d,e,f} | 13.87 ^{a,b,c,d,e} | 13.22 ^{a,b,c} |
| 7/17 | 18.53 ^{i,j} | 17.64 ^{g,h,i,j} | 18.03 ^{h,i,j} |
| 7/31 | 15.66 ^{d,e,f,g} | 16.44 ^{f,g,h,i} | 22.35 |
| 8/14 | 16.42 ^{f,g,h,i} | 15.86 ^{e,f,g,h} | 15.88 ^{e,f,g,h} |
| 8/28 | 18.52 ^{i,j} | 19.30 ^j | 17.94 ^{h,i,j} |
| 9/14 | 16.31 ^{f,g,h} | 13.07 ^{a,b,c} | 15.71 ^{d,e,f,g} |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XII. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of phosphorus in eviscerated bodies of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean square</u> | <u>Probability</u> |
|--------------------------------|---------------------------|--------------------|--------------------|
| Sampling date | 8 | 15.6655 | 0.0000 |
| Diet | 2 | 0.1062 | 0.7142 |
| Sampling date-diet interaction | 16 | 0.7641 | 0.0021 |
| Residual | 240 | 0.3150 | |
| Total | 266 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % phosphorus*</u> | | |
|----------------------|---------------------------|---------------------------|---------------------------|
| | <u>Fish fed diet Z-50</u> | <u>Fish fed diet Z-51</u> | <u>Fish fed diet Z-67</u> |
| 5/18 | 3.8291 ^{a,b} | 3.8291 ^{a,b} | 3.8291 ^{a,b} |
| 6/5 | 4.2619 ^b | 3.7352 ^{a,b} | 3.9649 ^{a,b} |
| 6/19 | 4.0034 ^b | 3.4162 ^a | 4.0120 ^b |
| 7/2 | 3.9728 ^{a,b} | 3.7455 ^{a,b} | 3.6971 ^{a,b} |
| 7/17 | 3.9979 ^b | 4.0816 ^b | 4.1345 ^b |
| 7/31 | 4.0597 ^b | 4.0123 ^b | 3.9763 ^{a,b} |
| 8/14 | 4.0491 ^b | 3.6886 ^{a,b} | 4.3058 ^b |
| 8/28 | 3.4264 ^a | 3.4913 ^a | 3.4505 ^a |
| 9/14 | 5.4767 ^c | 6.5731 | 5.7837 ^c |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XIII. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of crude fat on a dry weight basis in eviscerated bodies of age group I channel catfish fed from May 1 to September 14, 1972.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|--------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 400.98 | P<.01 |
| Diet | 2 | 30.21 | P<.05 |
| Sampling date-diet interaction | 16 | 12.40 | P>.05 |
| Residual | 27 | 7.78 | |
| Total | 59 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % crude fat*</u> |
|----------------------|--------------------------|
| 5/18 | 10.94 ^a |
| 6/1 | 10.18 ^a |
| 6/15 | 13.95 ^b |
| 6/29 | 14.67 ^b |
| 7/13 | 17.82 ^c |
| 7/27 | 18.67 ^c |
| 8/10 | 23.82 ^d |
| 8/22 | 23.82 ^d |
| 9/7 | 25.82 ^d |
| <u>Diet</u> | |
| Z-58 | 18.64 ^e |
| Z-59 | 17.78 ^{e, f} |
| Z-60 | 16.81 ^f |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XIV. One-way analysis of variance table (A) and summary of statistically significant levels (B) of crude fat, on a dry weight basis, in eviscerated bodies of age group I channel catfish fed diet Z-60 from May 1 to September 14, 1972.

A. One-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 13 | 100.50 | 0.0000 |
| Residual | 14 | 9.82 | |
| Total | 27 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % crude fat*</u> |
|----------------------|--------------------------|
| 5/18 | 11.08 ^{d,e} |
| 6/1 | 9.30 ^e |
| 6/15 | 12.34 ^{d,e} |
| 6/29 | 14.04 ^d |
| 7/13 | 17.52 ^c |
| 7/27 | 17.55 ^c |
| 8/10 | 21.59 ^{a,b} |
| 8/22 | 24.43 ^a |
| 9/7 | 23.43 ^a |
| 10/20 | 24.13 ^a |
| 11/17 | 19.44 ^{b,c} |
| 12/15 | 23.66 ^a |
| 1/19 | 18.46 ^{b,c} |
| 2/22 | 18.30 ^c |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XV. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of moisture in the eviscerated bodies of age group I channel catfish fed from May 1 to September 14, 1972.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 45.8969 | 0.0000 |
| Diet | 2 | 1.3750 | 0.1094 |
| Sampling date-diet interactions | 16 | 1.6571 | 0.0072 |
| Residual | 27 | 0.5717 | |
| Total | 53 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % moisture*</u> | | |
|----------------------|---------------------------|---------------------------|---------------------------|
| | <u>Fish fed diet Z-58</u> | <u>Fish fed diet Z-59</u> | <u>Fish fed diet Z-60</u> |
| 5/18 | 76.61 ^{a,b} | 74.47 ^a | 76.43 ^{a,b} |
| 6/1 | 77.41 ^a | 77.42 ^a | 75.66 ^b |
| 6/15 | 75.78 ^b | 76.45 ^{a,b} | 77.00 ^{a,b} |
| 6/29 | 68.95 ^{j,k} | 67.83 ^k | 70.15 ^{i,j} |
| 7/13 | 71.85 ^{e,f,g} | 72.34 ^{c,d,e,f} | 72.93 ^{c,d,e} |
| 7/27 | 71.34 ^{f,g,h,i} | 71.74 ^{e,f,g,h} | 70.78 ^{g,h,i} |
| 8/10 | 72.15 ^{d,e,f,g} | 70.29 ^{h,i,j} | 73.20 ^{c,d,e} |
| 8/22 | 71.17 ^{f,g,h,i} | 73.07 ^{c,d,e} | 72.70 ^{c,d,e,f} |
| 9/7 | 72.39 ^{c,d,e,f} | 73.52 ^{c,d} | 73.78 ^c |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XVI. One-way analysis of variance table (A) and summary of statistically significant levels (B) of moisture in the eviscerated bodies of age group I channel catfish fed the Z-60 diet from May 1 to September 14, 1972.

A. One-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 13 | 7.6184 | 0.00009 |
| Residual | 14 | 0.8150 | |
| Total | 27 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % moisture*</u> |
|----------------------|--------------------------|
| 5/18 | 76.43 ^{a,b} |
| 6/1 | 75.66 ^{a,b,c} |
| 6/15 | 77.00 ^a |
| 6/29 | 70.15 ^h |
| 7/13 | 72.93 ^{e,f} |
| 7/27 | 70.78 ^{g,h} |
| 8/10 | 73.20 ^{d,e,f} |
| 8/22 | 72.70 ^{e,f,g} |
| 9/7 | 73.78 ^{c,d,e,f} |
| 10/20 | 72.93 ^{e,f} |
| 11/17 | 72.20 ^{f,g} |
| 12/15 | 73.82 ^{c,d,e,f} |
| 1/19 | 74.17 ^{c,d,e} |
| 2/22 | 74.87 ^{b,c,d} |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XVII. Two-way analysis of variance table for levels of crude fat, on a dry weight basis, in livers of age group I channel catfish fed from May 1 to September 14, 1972.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|-------------------------------|-------------------------|--------------------|
| Sampling date | 8 | 1.0813 | 0.3026 |
| Diet | 2 | 2.2469 | 0.0948 |
| Residual | 16 | 0.8201 | |
| Total | 26 | | |

Table XVIII. One-way analysis of variance table (A) and summary of statistically significant levels (B) of crude fat, on a dry weight basis, in the livers of age group I channel catfish fed the Z-60 diet from May 1 to September 14, 1972.

A. One-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 13 | 9.1639 | 0.0498 |
| Residual | 14 | 3.6513 | |
| Total | 27 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % crude fat*</u> |
|----------------------|---------------------------|
| 5/18 | 6.75 ^{b,c,d,e} |
| 6/1 | 5.59 ^{c,d,e} |
| 6/15 | 7.58 ^{a,b,c,d,e} |
| 6/29 | 8.45 ^{a,b,c,d} |
| 7/13 | 9.47 ^{a,b,c} |
| 7/27 | 8.12 ^{a,b,c,d} |
| 8/10 | 7.61 ^{a,b,c,d,e} |
| 8/22 | 7.33 ^{b,c,d,e} |
| 9/7 | 6.15 ^{b,c,d,e} |
| 10/20 | 11.54 ^a |
| 11/17 | 3.87 ^e |
| 12/15 | 4.63 ^{d,e} |
| 1/19 | 5.53 ^{c,d,e} |
| 2/22 | 9.96 ^{a,b} |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XIX. Two-way analysis of variance table of moisture levels in the livers of age group I channel catfish fed from May 1 to September 14, 1972.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|-------------------------------|-------------------------|--------------------|
| Sampling date | 8 | 2.5554 | 0.0943 |
| Diet | 2 | 2.5680 | 0.1501 |
| Residual | 16 | 1.2001 | |
| Total | 26 | | |

Table XX. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of crude fat, on a dry weight basis, in eviscerated bodies of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|--------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 40.04 | 0.00005 |
| Diet | 2 | 5.49 | 0.39024 |
| Sampling date-diet interaction | 16 | 6.51 | 0.35943 |
| Residual | 27 | 5.63 | |
| Total | 53 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % crude fat*</u> |
|----------------------|--------------------------|
| 5/18 | 19.11 ^a |
| 6/5 | 20.16 ^a |
| 6/19 | 20.60 ^a |
| 7/2 | 19.75 ^a |
| 7/17 | 23.60 ^b |
| 7/31 | 24.27 ^b |
| 8/14 | 23.94 ^b |
| 8/28 | 25.99 ^b |
| 9/14 | 25.90 ^b |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XXI. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of moisture in eviscerated bodies of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|--------------------------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 6.1379 | 0.00032 |
| Diet | 2 | 0.1327 | 0.88683 |
| Sampling date-diet interaction | 16 | 1.1562 | 0.44099 |
| Residual | 27 | 1.1002 | |
| Total | 53 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % moisture*</u> |
|----------------------|-------------------------|
| 5/18 | 73.93 ^{a,b} |
| 6/5 | 74.57 ^a |
| 6/19 | 74.85 ^a |
| 7/2 | 72.70 ^{b,c} |
| 7/17 | 74.52 ^a |
| 7/31 | 72.50 ^c |
| 8/14 | 72.55 ^c |
| 8/28 | 72.54 ^c |
| 9/14 | 72.74 ^{b,c} |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XXII. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of crude fat, on a dry weight basis, in livers of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 3.9834 | 0.01827 |
| Diet | 2 | 2.1632 | 0.19172 |
| Residual | 16 | 1.1792 | |
| Total | 26 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % crude fat*</u> |
|----------------------|--------------------------|
| 5/18 | 8.91 ^{a,b,c} |
| 6/5 | 6.98 ^{c,d} |
| 6/19 | 9.20 ^{a,b} |
| 7/2 | 9.73 ^a |
| 7/17 | 7.21 ^{b,c,d} |
| 7/31 | 6.61 ^d |
| 8/14 | 7.42 ^{b,c,d} |
| 8/28 | 7.71 ^{a,b,c,d} |
| 9/14 | 6.75 ^d |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XXIII. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of moisture in livers of age group II channel catfish fed from May 21 to September 14, 1973.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 7.0804 | 0.00012 |
| Diet | 2 | 0.7155 | 0.42584 |
| Residual | 16 | 0.7942 | |
| Total | 26 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Mean % moisture*</u> |
|----------------------|-------------------------|
| 5/18 | 75.94 ^a |
| 6/5 | 75.75 ^a |
| 6/19 | 73.92 ^b |
| 7/2 | 74.38 ^{a,b} |
| 7/17 | 73.83 ^b |
| 7/31 | 74.68 ^{a,b} |
| 8/14 | 74.27 ^{a,b} |
| 8/28 | 71.43 ^c |
| 9/14 | 71.83 ^c |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XXIV. Two-way analysis of variance table for calcium (A) and phosphorus (B) in water samples taken from ponds of regimens Z-58, Z-59, and Z-60.

A. Two-way analysis of variance table for calcium.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 40.9959 | 0.1777 |
| Regimen | 2 | 0.5515 | 0.9776 |
| Residual | 16 | 24.3059 | |
| Total | 26 | | |

B. Two-way analysis of variance table for phosphorus.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 8 | 8.4696 | 0.3294 |
| Regimen | 2 | 16.8245 | 0.1134 |
| Residual | 16 | 6.7249 | |
| Total | 26 | | |

Table XXV. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of calcium in water samples taken from ponds of regimens Z-50, Z-51, and Z-67.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 7 | 2.5495 | 0.0044 |
| Regimen | 2 | 0.3255 | 0.5235 |
| Residual | 14 | 0.4936 | |
| Total | 23 | | |

B. Summary of statistically significant data.

| <u>Sampling date</u> | <u>Calcium in parts per million*</u> |
|----------------------|--------------------------------------|
| 6/5 | 1.0833 |
| 6/19 | 2.4967 ^a |
| 7/2 | 3.1233 ^{a,b} |
| 7/17 | 4.0233 ^b |
| 7/31 | 3.2767 ^{a,b} |
| 8/14 | 3.8367 ^b |
| 8/28 | 2.8533 ^{a,b} |
| 9/14 | 3.4033 ^{a,b} |

*Means sharing the same superscript letter are not significantly different at the 95% level.

Table XXVI. Two-way analysis of variance table (A) and summary of statistically significant levels (B) of phosphorus in water samples taken from ponds of regimens Z-50, Z-51, and Z-67.

A. Two-way analysis of variance table.

| <u>Source</u> | <u>Degrees of freedom</u> | <u>Mean squares</u> | <u>Probability</u> |
|---------------|---------------------------|---------------------|--------------------|
| Sampling date | 7 | 9.4790 | 0.0020 |
| Regimen | 2 | 13.3013 | 0.0036 |
| Residual | 14 | 1.5408 | |
| Total | 23 | | |

B. Summary of statistically significant levels.

| <u>Sampling date</u> | <u>Phosphorus in parts per million*</u> |
|----------------------|---|
| 6/5 | 10.9333 ^{a,b} |
| 6/19 | 8.6333 ^c |
| 7/2 | 8.7667 ^{b,c} |
| 7/17 | 8.4333 ^c |
| 7/31 | 9.3333 ^{a,b,c} |
| 8/14 | 5.5667 |
| 8/28 | 9.9667 ^{a,b,c} |
| 9/14 | 11.2667 ^a |

*Means sharing the same superscript letter are not significantly different at the 95% level.

CHANGES IN MINERAL, CRUDE FAT, AND MOISTURE LEVELS
OF CHANNEL CATFISH (ICTALURUS PUNCTATUS)
AS EFFECTED BY SUPPLEMENTAL DIETS

by

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Age group I and II channel catfish, Ictalurus punctatus (Rafinesque), were used in a two part study. In April 1973, age group I fish were stocked in 12 polyethylene-lined ponds. Fish in regimens of four ponds each were fed one of three pelleted feeds containing vitamin C and/or vitamin D₃ from May 1 to September 14, 1972, and not fed from September 14, 1972 to February 22, 1973.

Age group II channel catfish were stocked in six ponds in May 1973. Fish in regimens of two ponds each were fed one of three pelleted feeds of differing calcium-phosphorus content from June 7 to September 14, 1973.

Proximate and mineral analyses were made biweekly during the feeding periods of both age group I and II fish and monthly during the nonfeeding period for the age group I fish. Eviscerated fish were fine-ground with a meat grinder and analyzed for moisture, crude fat, calcium, phosphorus, sodium, chlorine, manganese and potassium. Bones were analyzed for calcium, phosphorus, sodium, chlorine, manganese and potassium. Livers of both age group I and II fish were analyzed for moisture and crude fat.

There was no direct relationship between the dietary addition of vitamins C and/or D₃ and mineral retention, growth, or feed conversions during the feeding period for age group I channel catfish. During the nonfeeding period there were significant sampling-date differences ($P < .05$) for calcium and phosphorus.

There was no direct relationship between the dietary calcium-phosphorus levels and mineral retention in age group II channel catfish. The intermediate calcium-phosphorus-diet fed fish, however, had the best weight gains and feed conversions. Skeletons averaged 4.6% of the body weight of fish on each diet.

Moisture and crude fat levels in livers were not related in either age group I or II channel catfish. Livers were not a major area of fat storage. Crude fat levels in bodies of age group I and II fish increased during the feeding periods and crude fat levels decreased in bodies of age group I cat-

fish during the nonfeeding period. During the feeding period, moisture levels in the bodies of age group I channel catfish showed no relation to the crude fat levels but were inversely related to crude fat levels in both the bodies of age group I fish during the nonfeeding period and the bodies of age group II channel catfish during the feeding period. Body tissues (excluding viscera and liver) were storage areas for fat.