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THE OPTIMAL PROTEIN:METABOLIZABLE ENERGY RATIO
AND TOTAL PROTEIN REQUIREMENT FOR
YELLOW PERCH IN A SINGLE-PASS, FLOW-THROUGH SYSTEM

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By

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ABSTRACT

THE OPTIMAL PROTEIN:METABOLIZABLE ENERGY RATIO
AND TOTAL PROTEIN REQUIREMENT FOR
YELLOW PERCH IN A SINGLE-PASS, FLOW-THROUGH SYSTEM

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Cost-effective intensive culture of yellow perch requires a better understanding of their nutritional requirements. Studies were designed to estimate: 1) the quantitative essential amino acid requirements, 2) the acceptability of semipurified diets supplemented with crystalline amino acids, 3) the optimal protein:metabolizable energy ratio (P:ME), and 4) the protein requirement at the optimal P:ME for yellow perch fingerlings.

The amino acid profiles of yellow perch skinless fillets, whole bodies, and eggs were similar to like values reported for other fishes. The amino acid content of the semipurified diets was based on the amino acid profile of yellow perch fillets. Yellow perch grew well for at least 10 weeks on these diets but required an initial five-day feed-transition period for the semipurified diets to be accepted. The optimal P:ME was determined to be 91 mg protein/Kcal estimated metabolizable energy of dry diet. At a P:ME of 91, dietary protein could be lowered to 26%.

This thesis is dedicated to my loving parents

Robert and Jill Ramseyer

and to my grandparents

Daniel and Willa Ramseyer and Joseph and Violet Conway

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KEY TO SCIENTIFIC NOMENCLATURE

List of common and scientific names of fishes cited in the thesis.

Ayu		*Plecoglossus altivelis*
Bass,	striped	*Morone saxatilis*
Bream		*Abramis brama*
Burbot		*Lota lota*
Carp,	bighead	*Aristichrhys nobilis*
	common	*Cyprinus carpio*
	crucian	*Carassius carassius*
Catfish,	channel	*Ictalurus punctatus*
	walking	*Clarias batrachus*
Cod		*Gadus morhua*
Dolphin fish		*Coryphaena hippurus*
Drum,	red	*Sciaenops ocellatus*
Eel,	European silver	*Anguilla anguilla*
Milkfish		*Chanos chanos*
Opaleye		*Girella nigricans*
Perch,	European	*Perca fluviatilis*
	yellow	*Perca flavescens*
Pike,	northern	*Esox lucius*
Pike-perch		*Stizostedion lucioperca*
Puffer fish		*Fugu rubripes*
Roach		*Rutilus rutilus*

Salmon,	Atlantic	*Salmo salar*
	cherry	*Oncorhyncus masou*
	chum	*O. Keta*
	coho	*O. kisutch*
Sea bream,	red	*Chrysophrys major*
Tilapia,	Nile	*Oreochromis niloticus*
Trout,	brook	*Salvelinus fontinalis*
	rainbow	*Oncorhynchus mykiss*
Toadfish		*Opsanus tau*
Turbot		*Scophthalamus maximus*
Walleye		*Stizostedion vitreum*
Yellow tail		*Seriola quinqueradiata*

INTRODUCTION

Yellow perch, Perca flavescens, is an economically important fish in the Midwest. It is prized not only by anglers, but by the commercial fishing industry as well. The desirability of the species, together with a fluctuating natural supply (Craig 1987; Hile and Jobes 1941) and increasing restrictions on the commercial harvest have led to high market values ($ 12.60 per pound retail) and an interest in the potential of yellow perch aquaculture.

Yellow perch have been raised in the laboratory with some success (Benoit 1968; Huh et al. 1976; Reinitz and Austin 1980; Starr 1989). Calbert and Huh (1976) reported that yellow perch cultured in their laboratory produced a 5% greater fillet yield than wild yellow perch and were of excellent flavor quality.

Some facilities for the intensive commercial culture of yellow perch already exist. However, since little is known about the specific nutritional requirements of yellow perch, they are being raised on salmonid feeds (D. Smith, Freshwater Farms of Ohio; J. Malison, University of Wisconsin-Madison; C. Starr, Bay Port Aquaculture Systems, Michigan, pers. comm.). There is a general consensus among

the commercial perch culturists that salmonid feeds are not entirely suitable for the yellow perch, and that diets lower in protein content should be examined. Work by Starr (1989) has shown that yellow perch raised on trout feeds accumulate significantly higher levels of body cavity fat than wild yellow perch. Increased body fat indicates inappropriate levels of dietary protein and/or energy.

It is believed that fish, like mammals, eat to satisfy energy needs (Lee and Putnam 1973; Poston 1975; Mitchell 1962). Bromley (1980) found that turbot fed in excess consumed the same number of calories per gram body weight per day regardless of dietary composition. Therefore it is important that the ratio of dietary protein (P) to energy (E; expressed as gross, digestible, or metabolizable energy) be at its optimum. If the dietary P:E ratio is lower than optimal (i.e. excess energy), fish will stop eating before enough protein is consumed to meet their growth potential. Further, the extra calories consumed beyond metabolic requirements may be stored as fat (Bromley 1980; Page and Andrews 1973).

Fish can efficiently deaminate amino acids and use the carbon skeleton for de novo fatty acid synthesis (Sargent et al. 1989). Henderson and Sargent (1981) suggested that amino acids are preferred over glucose as the carbon source for fatty acid biosynthesis in rainbow trout (see Key to Scientific Nomentclature for scientific names of fishes

cited in this thesis). Therefore the result of feeding fish diets with a greater than optimal P:E ratio (i.e. excess protein or individual amino acids) may also result in fatty fish. This situation is not only a costly waste of protein, it can result in a build-up of ammonia in the culture system as well (Beamish and Thomas 1984). Since the shelf life of harvested fish is inversely related to fat content, excessively fatty fish represent a potential waste of both feeds and product.

It is important to determine the optimal dietary P:E ratio for yellow perch so that the efficiency of perch aquaculture operations can be optimized. Therefore the objectives of these experiments were:

1) To estimate the quantitative essential amino acid requirements of yellow perch in order to formulate the protein portion of the experimental diets,
2) To determine the acceptability of semipurified diets for the growth of yellow perch,
3) To determine the optimal protein-to-energy ratio using experimental diets based on the results of objectives 1 and 2, and
4) To determine the optimal protein and energy levels at the optimal protein-to-energy ratio.

LITERATURE REVIEW

A. PROTEIN, CARBOHYDRATE, AND LIPID IN FISH DIETS

1. Protein

The protein requirement of fish is best described as a requirement for individual amino acids in specific relative amounts. Amino acids are used to make new and replacement proteins and as an energy source. All fish require the same ten essential amino acids (EAA): arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, differing only in the amounts of each EAA required (NRC 1981).

Essential amino acids function in amounts relative to each other, so a deficiency in one EAA will cause a relative surplus of the others. Excess amino acids are readily converted to glucose and/or fat by the fish or simply deaminated and excreted (Cowey and Sargent 1979; Sargent et al. 1989). Therefore an understanding of the proper balance of EAAs for yellow perch is essential before trying to determine the optimal protein-to-energy ratio (P:ME).

Unfortunately, feeding trials to determine actual quantitative EAA requirements of fishes are laborious, costly, and beyond the scope of the present study. For

these reasons other investigators have developed an indirect method for estimating these requirements. The EAA composition of the whole body, muscle, or egg has been used to estimate EAA requirements (Ketola 1982; Arai 1981; Fuller et al. 1979).

The usefulness of this method was demonstrated by Arai (1981), who fed coho salmon casein diets supplemented with amino acids to reflect the essential amino acid ratio (A/E), calculated as: A/E = [(individual EAA content/total EAA content+Cys+Tyr)*1000], of coho fry whole bodies. These fish showed better growth and feed efficiency than fish given fish meal diets. Ogata et al. (1983) also found that a casein diet supplemented with amino acids to simulate the A/E ratio of cherry salmon whole body protein resulted in better protein and feed efficiency of cherry salmon than casein only, casein + amino acids simulating cherry salmon eyed egg A/E ratio, or fish meal diets.

Supporting this method of estimation, Wilson and Poe (1985) found a high degree of correlation (r=0.96) between the A/E ratios of known dietary EAA requirements and the whole body A/E composition of channel catfish. They also found a weaker correlation (r=0.68) between the A/E ratios of dietary requirements and egg EAA composition. Gatlin (1987) found the A/E ratios of Cyprinid baitfish whole bodies to correlate well (r=0.87) with established carp EAA requirements when expressed as A/E ratios. Fuller et al.

(1979) found that the elimination of endogenous nitrogen in growing pigs was minimized when the amino acid profile of the feed was similar to that of pig whole body. Finally, Boorman (1980) noted a good correlation between the AA requirements and muscle AA composition of growing pigs and chicks.

Whole body, muscle, and egg AA profiles have all been used as estimators of dietary AA requirements. Since the three estimators have not been compared in any one fish growth study, there is some debate over which method should be used. The above findings of Poe and Wilson (1985) and Ogino et al. (1983) indicate that the egg AA pattern is less indicative of actual requirements than whole body. Cowey and Tacon (1982) promoted muscle as the intuitive choice, since the greatest proportion of body weight gain in growing animals is from muscle accretion.

There is a potential problem with using muscle or whole body EAA profiles as estimators, however, since the differing protein turnover rates of various tissues are not considered. For example, although the gill makes up only a small portion of total body protein in the rainbow trout, it has a protein synthesis rate of about 12 times that of muscle (Fauconneau 1985). The foregut synthesizes up to 33 times as much protein as does the muscle. Therefore any differences in the A/E ratios of gill, foregut and muscle,

would be magnified by the protein synthesis demands of the various tissues. The result could be reduced growth.

Ostrowski and Divakaran (1989) examined this question with the dolphin fish and found the differing metabolic rates of tissues to be irrelevant since gill, intestine, and muscle A/E ratios were very similar. The amino acid composition of yellow perch tissues other than muscle and whole body (determined in this study) is unavailable. Given the success of correlating amino acid requirements to whole body and muscle amino acid values in both aquatic and terrestrial animals, it will be assumed that, as for the dolphin fish, protein turnover considerations are of minor importance in the estimation of essential amino acids for yellow perch.

2. Carbohydrates

Six-carbon sugars are the basic energy source of all animals and can be derived from carbohydrates, lipid, and protein (Smith 1989). However not all fish can derive energy efficiently from carbohydrates. The extent of carbohydrate utilization depends on the ability of the fish to digest dietary carbohydrates and absorb and utilize glucose.

In the wild, the diet of a carnivorous fish such as the rainbow trout typically consists of 50% protein and 50% fat on a dry matter basis (Smith 1989). Such animals which lack

carbohydrate in their natural diet would not be expected to have as great a capacity for its digestion or use. Yet the various carbohydrases that are required to hydrolyze starch can be found even in piscivorous fishes (Kuz'mina 1984; Ushiyama et al. 1965).

Fish (1960) found amylase activity in the diffuse pancreas of the European perch, the biological equivalent of yellow perch (Thorpe 1977). Kuz'mina (1984) has shown that the α-amylase activity in the lumen and intestinal mucosa of European perch is intermediate between that of the piscivorous burbot, pike-perch and pike and the benthic-feeding bream and roach. This pattern was also found for saccharase activity, but not maltase activity (Kuz'mina 1984). In addition to the above fishes, maltase activity has been found in the rainbow trout (Kitamikado and Tachino 1960), chum salmon (Ushiyama et al. 1965), ayu and red sea bream (Kawai and Ikeda 1971), and common carp(Vonk and Western 1984). These studies make it clear that even carnivorous fish do have some capacity to digest carbohydrate, and that perch have higher levels of carbohydrase activity than strictly carnivorous fishes (Kuz'mina 1984).

Because many fishes are limited in carbohydrase activity (Kuz'mina 1984; Fish 1960), the digestibility of carbohydrate is dependant upon carbohydrate complexity and the level of inclusion in the diet. Kaushik et al. (1989a)

have shown that Siberian sturgeon can utilize gelatinized or extruded starch well, but not raw starch. Likewise, rainbow trout do not use untreated corn as efficiently as corn which has been popped, exploded, flaked, or extruded (Luquet and Bergot 1976; Pfeffer et al. 1991). Singh and Nose (1967) assessed the digestibility of glucose, sucrose, lactose, dextrin, and potato α-starch in rainbow trout diets. At 20% inclusion in the diet, they found that the mono- and disaccharides had digestibilities exceeding 94%, whereas dextrin and starch had digestibilities of 77.2% and 69.2%, respectively.

Studies with rainbow trout have shown an inverse relationship between dietary carbohydrate concentration and digestibility. Inaba et al. (1963) found that the digestibility of cooked α-starch fell from 90% to 48.2% when dietary starch content was raised from 11.5% to 40.2%, respectively. Singh and Nose (1967) found similar results for trout fed dextrin at 20 and 60% of dietary dry matter; digestibility fell from 77.2 to 45.5%, respectively. Singh and Nose (1967) attributed the differences in digestibility to insufficient amounts of α-amylase in the rainbow trout intestine.

Once starch is digested, it must be absorbed. But absorption does not appear to be a barrier to carbohydrate utilization. Furuichi and Yone (1980) found that over 90% of dietary dextrin was digested and absorbed by carp, an

omnivore, red sea bream, a semi-carnivore, and yellow tail, a strict carnivore. This occurred at dextrin levels of 10% to 40% of the diet.

The cellular uptake of glucose from blood is facilitated by insulin in mammals (Hunt and Groff 1990). Fish and mammalian insulin are very similar in structure (Christiansen and Klungsøyr 1987). In contrast to mammals, however, fish are naturally diabetic-like (Palmer and Ryman 1972). Furuichi and Yone (1981) demonstrated that the degree of this diabetes-like condition is greater for carnivorous than for omnivorous fish. They have shown that carnivorous species such as yellow tail are slower at reducing a glucose load than the omnivorous carp due to insufficient (rather than ineffectual) amounts of insulin.

Low insulin levels in carnivorous fish probably reflect the high protein-low carbohydrate content of their natural diets. Not surprisingly, it has been found that amino acids have a much greater stimulatory affect than glucose on insulin secretion in toadfish (Tashime and Cahill 1968), cod and rainbow trout (Thorpe and Ince 1977), and European silver eel (Ince and Thorpe 1977; Ince 1979 and 1980). In fact, Hilton and Atkinson (1982) found increased dietary glucose levels had no effect on insulin secretion in the rainbow trout. Additionally, fish do not adapt the amount of insulin secreted in response to long-term increases of dietary carbohydrates (Yone 1979; Kaushik et al. 1989b).

The most important rate-limiting step in the utilization of glucose by fish however seems to be phosphorylation (Newsholme and Start 1973). Before glucose can enter the glycolytic pathway or be converted to glycogen or fat, it first must be converted to glucose-6-phosphate. This is done by the enzymes hexokinase in the muscle and glucokinase in the liver. In many animals, increases in blood glucose triggers an increase in glucokinase. But Cowey et al. (1977) did not observe any increase in hexokinase or glucokinase activity in rainbow trout in response to increasing dietary dextrin from 0-50%. Walton and Cowey (1982) also note that hexokinase is present in the least amount of all the glycolytic enzymes in rainbow trout. Interestingly, glucokinase is deficient in humans with diabetes mellitus (Hunt and Groff 1990).

It seems likely that the poor use of carbohydrates in carnivorous fish is the result of both digestive and metabolic inadequacies. That is, some combination of limited amounts of enzymes for converting carbohydrate to glucose, and the limited ability to use glucose once it is absorbed. Therefore the level of dietary carbohydrate must be carefully chosen, especially for carnivorous fish.

Yellow perch are facultative carnivores, eating zooplankton, benthos, and small fish in the wild (Hayes 1988). The extent to which yellow perch can utilize carbohydrate is unknown, but Kuz'mina (1984) has shown that

the European perch may have a greater ability to utilize carbohydrates than the more strictly carnivorous fishes.

3. Dietary lipids

Dietary lipid is important as a dense, highly digestible energy source, a source of essential fatty acids (EFA), and as a carrier of fat-soluble vitamins. Dietary lipids also have the important role of sparing dietary protein in fish which do not utilize carbohydrates efficiently (i.e. carnivorous fish) (Watanabe 1982; NRC 1981).

The EFA requirements differ from species to species and are influenced by several factors such as salinity, temperature, and total dietary lipid content. For example, freshwater fish do not have the desaturases necessary to form linoleate (18:2n-6) or linolenate (18:3n-3) (Sargent et al. 1989). These fatty acids must be obtained through the diet and are therefore essential nutrients (Table 1). However marine fish such as red sea bream, yellow tail, opaleye, and turbot seem limited in their ability to elongate and desaturate 18:3n-3, 18:2n-6 and 18:1n-9 (Owen et al. 1975; Fujii and Yone 1976a,b; Yone 1978). Therefore their EFA requirements are more efficiently satisfied with longer chain fatty acids (Gatesoupe et al. 1977).

Temperature may also affect the EFA requirements of fishes; the requirements of warmwater fish seem to be lower

Table 1. Essential fatty acid requirements of some freshwater fishes.

Species	omega-3	omega-6	Reference
Rainbow trout	1.0	---	NRC 1981
Coho salmon	1 to 2.5	<1.0	Yu et al. 1979
Carp	1.0	1.0	Watanabe et al. 1975a.b; Takeuchi et al. 1977a
Channel catfish	<1.0		Stickney et al. 1983
Eel	0.5	0.5	

than for coldwater fish. This may relate to problems of membrane fluidity in colder environments. There is also evidence that the total dietary lipid level may increase EFA requirements (Watanabe 1982; Takeuchi and Watanabe 1977b).

Apparently EFA requirements have yet to be determined for coolwater fish such as yellow perch. However if EFAs are included in the diet in amounts satisfactory for salmonids, the fishes with the highest known requirements and lower optimal growth temperatures, they should be adequate for yellow perch.

4. Protein-sparing

Both lipids and carbohydrates have been shown to "spare" dietary protein from use for energy requirements, leaving it available for growth and other functions (Watanabe 1977). This protein-sparing action has been demonstrated in many species including rainbow trout (Takeuchi et al. 1978a; Kaushik and Oliva-Teles 1985), coho salmon (Yu and Sinnhuber 1981), channel catfish (Gatlin and Stickney 1982), and carp (Watanabe et al. 1987). And since lipids and carbohydrates are usually a cheaper source of dietary energy than protein, commercial diet manufacturers like to use maximal amounts of each. However, there is a point beyond which additional dietary carbohydrates or lipids are no longer beneficial.

Elevated levels of carbohydrate can have a negative effect on the growth and health of fish. Growth was depressed for carp, red sea bream, and yellow tail when dietary dextrin levels surpassed 40, 30, and 20%, respectively (Furuichi and Yone 1980). Hilton and Atkinson (1982) found that rainbow trout cannot utilize over 14% dietary cerelose (α-glucose). Palmer and Ryman (1972) found high levels of carbohydrates in carp diets were correlated with increased liver size, mostly due to glycogen accumulation. Similar results have been found for Siberian sturgeon and rainbow trout (Walton 1986; Medela et al. 1991). Hilton and Dixon (1982) found that increased glycogen can impair liver function in the rainbow trout.

Determining optimal amount of lipid in the diet is important since excess lipid may result in fatty fish or reduced growth (low protein accretion) (Williams and Robinson 1988; Satia 1974; Reinitz et al. 1978; Takeuchi et al. 1978a; Dupree 1969; Andrews et al. 1978). Diets too low in fats either may not have a protein-sparing effect or may result in depressed growth associated with EFA deficiencies (Castell et al. 1972). By exchanging lipid for cellulose, Millikin (1983) found the best and most economical growth response for striped bass when the diet contained 47% protein and 12% lipid. Takeuchi et al. (1978a,b) found that a diet of 35% protein and 18% lipid was the best combination for rainbow trout.

Lipids are both more digestible by fish and a denser source of energy than carbohydrates, so the protein-sparing action of carbohydrate is considered inferior to that of lipids (Adron et al. 1976; Medale et al. 1991; Hilton and Atkinson 1982). Nevertheless, carbohydrates are generally less expensive than lipids, so studies have been made to determine the optimal combination of the two, a carbohydrate-to-lipid ratio (CHO:L). This is done by using isocaloric, isonitrogenous diets with varying amounts of oil and carbohydrate. Gatlin and Stickney (1982) found that channel catfish fingerlings grew well for all CHO:L ratios examined (1.84 to 7.72), which extended the range of 0.45 to 4.5 defined earlier by Garling and Wilson (1977). Williams and Robinson (1988) found the best weight gain and feed conversion in red drum when the CHO:L ratio was between 1.61 and 3.51. For Tilapia, El-Sayed and Garling (1988) found the best response to diets with CHO:L ratios of 0.81 to 8.76.

B. PROTEIN-ENERGY RATIOS

Several researchers have sought to determine the optimal protein-to-energy ratio of a variety of fishes. Since the energy content of a diet limits intake, the optimal P:E is determined by using isonitrogenous or isocaloric diets. Isonitrogenous diets can be used to determine the level of energy which limits intake (therefore

growth) at a given level of protein. Isocaloric diets can be used to determine the level of protein below which the given energy level will limit intake to such an extent as to reduce growth. Once the optimal P:E ratio is determined for a species, responses to various levels of protein and energy at the optimal P:E may be tested to determine the actual dietary protein requirement.

There are problems in determining the optimal P:E. First, what constitutes an "optimal" P:E ratio is dependant upon the intended use of the fish. For example, a diet at a certain P:E which produces large fish with substantial fat reserves may be considered optimal for stocking purposes, but not for commercial food production. Complicating this situation is the tendency of some researchers to measure dietary success based on weight gain alone. Without determining the proximate composition of the weight gained (protein, fat, moisture), the value of the data is limited.

Any errors in the estimation of EAA requirements or physiological fuel values can skew the true P:E ratio. The optimal P:E will be overestimated if there is a significant imbalance in the amino acid composition of the diet, since the concentration of the first limiting amino acid will determine the relative degree of excess of the other amino acids.

Anderson et al. (1991) illustrated the problem of using estimated physiological fuel values. For vegetable

feedstuffs, they found the actual digestible energy values for tilapia to be higher than those determined for rainbow trout and channel catfish. But for animal-derived feedstuffs, tilapia DE values were lower than corresponding trout values. The error involved in estimating fuel values is for the most part unavoidable at present since these values have been determined for only a few species.

Any factor which may affect requirements for protein or energy, such as temperature, salinity, or age, will affect the optimal P:E ratio. The comparison of P:E ratios determined by various investigators to understand these effects has been complicated by the type and digestibility of the dietary protein and energy sources used. Complicating the issue even further are the energy values estimated for the feedstuffs. Energy values can be expressed in several ways: gross energy (GE; the heat of combustion), digestible energy (DE; GE - energy lost as feces), or metabolizable energy (ME; {GE - [energy lost as feces, urine, and gill excretions]}). Comparisons of experiments which determine the optimal P:E of a fish are complicated by the fact that diets which are calculated to be isocaloric in DE, for example, may not be isocaloric if recalculated in terms of ME.

Fortunately there are some studies which have examined changes in the optimal P:E ratio due to changing growth parameters. Zeitoun et al. (1973) examined the effects of

salinity on rainbow trout and found that raising the salinity from 10 to 20 ppm increased the P:ME from 106 to 117. Zeitoun et al. (1974) repeated the salinity study using coho salmon. In this case, salinity had no effect on the P:ME ratio.

Anderson et al. (1981) fed nearly-isocaloric diets to age 0 and age 1 smallmouth and largemouth bass. They found no differences in the optimal P:GE between ages for either species. Likewise, Kaushik et al. (1989a) found the P:DE for Siberian sturgeon to be the same for both 90 g and 400 g fish. Mangalik (1986) found the optimal P:DE ratio of channel catfish to decrease slightly from 98 for 3 gram fish to 86 for 266 gram fish. This was because the requirements for both protein and energy decreased with age, but protein requirements decreased at a slightly higher rate than energy requirements.

The addition of anabolic agents to fish diets has resulted in improved growth for salmonids (Higgs et al. 1982). Ostrowski and Garling (1987) found that the addition of 17 α-methyltestosterone to rainbow trout diets resulted in an increased efficiency of protein utilization, thereby lowering the optimal P:ME from 120 to 100.

1. Warmwater fish

Garling and Wilson (1976a) fed channel catfish diets with various protein and energy levels resulting in a P:ME range of about 50 to 146. Based on protein deposition, a diet of 24% protein and a P:ME ratio of 87 was optimal. Chuapoehuk (1987) fed nearly isocaloric diets to walking catfish and found that the P:ME of 71 at 30% protein resulted in the best growth and survival of fry.

Daniels and Robinson (1986) reared juvenile red drum at two different temperatures and discovered this to have a distinct effect on the optimal P:GE ratio. At lower temperatures (22-26 C), a diet of 35% protein and a P:GE ratio of 86 resulted in the best growth and body composition. At higher temperatures (26-33 C), diets of 44% protein and P:GE ratios of 107 to 120 provided the best results.

Degani et al. (1987) fed large European eels nearly isocaloric diets ranging in protein content between 32.5% and 45.1%. Their results indicated that, based on weight gain and feed conversion, eels grew best with the P:GE ratio of 100.

Several studies have assessed the protein and energy requirements of the common carp. Schwarz and Kirchgessner (1988) tested diets of six different P and two different DE levels and found that the diet with a P:DE of 102 and 41% protein produced the highest protein deposition in 170 gram

carp. Takeuchi et al. (1989) found similar P:DE ratios of 89 to 100 for juvenile (5 to 7 gram) carp, and Watanabe et al. (1987) used isonitrogenous diets to determine a P:DE of 88 to be optimal for 5.8 to 256 gram fish. Bighead carp fry were found to grow best when fed a diet with a P:DE of 103 (Santiago and Reyes 1991).

An optimal P:ME ratio was determined for hybrid tilapia using combinations of two protein levels and six energy levels (Shiau and Huang 1990). Based on weight gain, feed conversion, and protein efficiency, when the P:ME ratio was 68, dietary protein could be lowered from 24% to 21%. Wang et al. (1985) and Siddiqui et al. (1988) examined the effects of varying protein levels in isocaloric or nearly isocaloric diets on the growth, feed conversion, and protein utilization of Nile tilapia. Wang et al. found that 3.5g fish grew best on the diet with a P:DE of 83. Siddiqui et al. found that 0.8g fry required 40% protein, resulting in a P:ME of 137. Fingerling fish (40g) required only 30% protein, for a P:ME of 101. Juvenile Sarotherodon mossambicus (1.8g) fed isocaloric diets with protein levels ranging from 0-56% had the best growth and net protein utilization when the dietary P:ME was 117 (Jauncey 1982). In contrast, 1.7g Tilapia zillii showed the highest protein deposition rates when the dietary P:ME ratio was 81 (Mazid et al. 1979).

Lim et al. (1979) used isocaloric casein-based diets to determine the optimal P:DE for milkfish fry. Based on growth and survival, a P:DE of 146 was considered best. Kanazawa et al. (1980) also used isocaloric casein diets to determine that the P:DE of about 108 was optimal for 2 gram puffer fish.

2. Coolwater fish

Studies involving the protein and energy requirements of yellow perch are either vague or have centered on absolute protein and energy levels without first determining an optimal P:E ratio. Calbert and Huh (1976) fed yellow perch practical diets with protein levels of 27%, 40%, and 50% and metabolizable energy levels, estimated from poultry values, of 3445, 3536, and 3214 kcal/kg, respectively. The authors found that there was "little" (experimental results were not statistically analyzed) difference in weight gain of fish on the different diets, but 12 gram fish fed the diet with 27% protein grew "better" than fish fed diets with 40% or 50% protein. It is unclear if this growth was based on weight or length gain, and no proximate analysis of fish was made. The P:ME value of the 27% protein diet was 78 as based on poultry ME values. Recalculating the metabolizable energy with rainbow trout values (NRC 1981), the optimal P:ME ratio for 12 gram fish was 72 verses 104 and 139.

Reinitz and Austin (1980) did not observe significant differences in the growth of yearling yellow perch fed open

formula U. S. Fisheries and Wildlife Service feeds with varying levels of protein (53.1 to 61.8%), fat (15.3 to 22.8%) and estimated ME (3736 to 4229 kcal/kg). The P:ME values ranged from 138 to 152. Whole body protein did not differ significantly among groups fed the different diets. Since fish eat to satisfy their energy needs, the protein, lipid, or energy levels used in their study may have been in excess of requirements. This would indicate the optimal P:ME ratio to be something less than 138. Success of the different diets could not be assessed on whole body fat content because body fat content is known to increase with increasing dietary lipid (Watanabe 1982), and dietary lipid contents were not constant.

Experiments to determine the optimal P/E for striped bass and walleye have produced similar results. Striped bass were found to have the highest feed efficiency, protein retention, whole-body protein, and most economical use of protein when fed a diet with 47% protein and 12% fat, yielding a P:DE ratio of 132 (Millikin 1983). Weight gain and body protein content were positively affected by dietary protein levels for walleye (Barrows 1988). A diet of 51% protein at the P:ME of 144 produced maximum protein synthesis and weight gain.

3. Coldwater fish

Rainbow trout are perhaps one of the most thoroughly studied of the cultured coldwater fishes. Several studies have addressed the question of protein and energy requirements for this fish. Lee and Putnam (1973) demonstrated that 5 gram rainbow trout fed a diet of 48% protein and a P:ME of 162 had the highest protein and lowest fat content while exhibiting the best percentage of calorie retention. Satia (1974) tested isocaloric diets of varying protein content on 7 gram rainbow trout and determined that a diet with a P:ME of 105 was optimal. Takeuchi et al. (1978a,b) determined that protein efficiency increased with fat levels up to about 18%, and that a diet with a P:DE of 100-130 was optimal for 2.5 to 15 gram fish. Ostrowski and Garling (1987) tested the effect of isocaloric diets with various levels of protein on 4 gram rainbow trout and found the optimal P:ME to be 120.

Based on an experiment with isonitrogenous diets, Kaushik et al. (1989a) predicted the optimal P:DE to be about 75 for 90 to 150 gram Siberian sturgeon.

MATERIALS AND METHODS

I. General

A. Amino Acid and Proximate Analysis of Yellow Perch

The amino acid and proximate compositions of five individual whole yellow perch from each of three groups was determined. Groupings were based on fish origin and age: 0+ fingerlings from ponds in southern lower Michigan ("pond fish"; mean weight 14.4 ± 3 (± standard error) g), 2+ fish raised in the MSU Aquaculture Lab ("lab fish"; 78.2 ± 6.6 g), and 5+ yellow perch from Saginaw Bay, Lake Huron ("Bay fish"; 130.4 ± 7.9 g). The amino acid and proximate composition were also determined for skinless fillets (mean weight 24.8 ± 8.9 g; whole fish weight 142 ± 42.7 g) and eggs (mean weight 10.6 ± 5.0 g; whole fish weight 171.4 ± 50.3g) from adult Bay fish, and the eggs from lab fish (mean weight 7.6 ± 3.2 g; whole fish weight 81 ± 5.3 g).

All fish used were females. Pond and Bay fish were collected in November, 1989. Lab fish had been delivered as fingerlings to the Michigan State University Aquaculture Laboratory from Coolwater Farms, Pickering, Ontario, Canada and Stoney Creek Trout Farm, Grant, Michigan in November,

1987. Lab fish were subsequently raised on a commercial trout feed (Starr 1989).

B. Feeding Trials

Five feeding experiments were conducted at the Michigan State University Aquaculture Laboratory. In Preliminary Experiment 1, a semipurified diet (Tables 2-4) and a commercial trout feed were each fed to triplicate tanks of yellow perch (16.2 + 0.7 g, total tank weight approximately 231 g) for 6 weeks to test the acceptability of semipurified diets for the growth of yellow perch. In Preliminary Experiment 2, a commercial trout feed, the semipurified diet used in Preliminary Experiment 1, and a combination of the two were fed to individual tanks of yellow perch (14.1 + 0.2 g, total tank weight approximately 207 g) for 7 days for the purpose of determining an efficient way to train yellow perch to accept a semipurified diet. The combination diet was a mixture of trout feed (T) and semipurified diet (S). Fish receiving the combination diet were fed 90% T:10% S on day one. Thereafter T was exchanged for S at a rate of 20% per day. On days 6 and 7 fish were fed 100% semipurified diet.

In Experiment 1, isonitrogenous semipurified diets (Table 5) containing 45% crude protein and either 450, 409, 375, or 346 Kcal estimated metabolizable energy (Smith in Ostrowski and Garling 1987) per 100 grams of dry diet were

Table 2. Composition of the semipurified diet used in Preliminary Experiment 1 to determine the acceptability of semipurified diets for the growth of yellow perch.

INGREDIENT	%DIET	ME[b]
Casein	32.42	145.9
Gelatin	10.81	48.7
Amino Acid Mix[c]	4.80	21.6
Cod Liver Oil	2.65	22.5
Soy Oil	9.35	79.5
Dextrin	17.77	56.9
Vitamin mix[d]	1.00	
Mineral mix[e]	5.66	
Cellulose	15.54	
Total ME		375.0
P:ME[a]		120.0

[a] Diet contains 45% protein based on 93.7% protein in a 67.5 : 22.5 : 10 mixture of casein:gelatin:amino acid mix (ICN Biomedicals).

[b] Total metabolizable energy (ME) per 100g of dry diet based on values estimated for rainbow trout (Ostrowski and Garling, 1987).

[c] Crystalline amino acid mix comprised of 20.4% L-histidine, 49.0% L-lysine, and 30.6% L-methionine.

[d] Vitamin mix according to NRC (1978) recommendations.

[e] Mineral mix according to NRC (1978) recommendations.

Table 3. Vitamin mixture used in semipurified fish diets (NRC 1978).

Vitamin	mg/g premix[a]
Choline-Cl	450.000
Niacin	100.000
Inositol	20.000
Ascorbic Acid	15.000
Vitamin K[b]	12.000
Calcium pantothenate	6.000
Pyridoxine	1.500
Riboflavin	1.500
Thiamin-HCL	1.500
Antioxidant[c]	1.000
Folacin (folic acid)	0.500
Biotin	0.150
Vitamin B_{12}	0.003
Vitamin	**IU/g**
Vitamin A	500
Vitamin D_3	200
Vitamin E	5

[a] These quantities added to alpha-cellulose to make one gram.
[b] Menadione dimethylpyrimidinol bisulfite.
[c] Butylated hydroxytoluene (BHT) and/or ethoxyquin.

Table 4. Mineral mixture used in semipurified fish diets (NRC 1978).

Mineral	g/Kg premix
$CaHPO_4-2H_2O$	366.046
$CaCO_3$	261.714
KH_2PO_4	176.834
NaCl	106.100
$MgSO_4$	53.050
KCl	17.683
$FeSO_4-7H_2O$	8.842
$MnSO_4-H_2O$	6.189
$ZnCO_3$	2.653
$CuSO_4-5H_2O$	0.531
KIO_3	0.177
$NaMoO_4-H_2O$	0.147
$CoCl_2$	0.030
Na_2SeO_3	0.004

Table 5. Composition of the isonitrogenous[1] semipurified diets used in Experiment 1 to determine the optimal protein-to-metabolizable energy ratio for yellow perch.

		P:ME		
INGREDIENT	**100**	**110**	**120**	**130**
Casein	32.42	32.42	32.42	32.42
Gelatin	10.81	10.81	10.81	10.81
Histidine	.98	.98	.98	.98
Lysine	2.35	2.35	2.35	2.35
Methionine	1.47	1.47	1.47	1.47
Dextrin	27.91	28.38	17.77	16.66
Cod Liver Oil	2.65	2.65	2.65	2.65
Soy Oil	14.35	9.35	9.35	6.35
Mineral Mix	5.66	5.66	5.66	5.66
Vitamin Mix	.55	.55	.55	.55
Choline Chloride	.45	.45	.45	.45
Carboxymethyl Cellulose	.40	4.93	15.54	19.65
Metabolizable Energy	450	409	375	346

[1] All diets contain 45% protein as a percentage of diet dry weight.

fed to triplicate groups of yellow perch (18.6 ± 0.6 g, total tank weight approximately 272 g total) for 10 weeks. In Experiment 2, isocaloric semipurified diets (Table 6) containing 375 Kcal ME /100 grams dry diet and either 22, 28, 34, or 40% protein were fed to triplicate groups of yellow perch (21.9 ± 1.1 g, total tank weight approximately 237 g) for 8 weeks. In Experiment 3, semipurified diets (Table 7) with a P:ME of 91 and containing either 38, 32, 26, or 20% protein were fed to triplicate groups of yellow perch (27.1 ± 0.9g, total tank weight approximately 222 g) for 11 weeks. In all cases Zeigler High Performance Trout Grower was the commercial trout feed used as a reference feed.

All feeding experiments were conducted in 110 liter flow-through aquaria with constant water-level siphon-type drains (Garling and Wilson 1976b). For both Preliminary Experiments, filtered 13 C well water was delivered to each tank at 0.5 liter/min. Dual submersible heaters in each tank maintained a temperature of 20 ± 1 C. For Experiments 1-3, heated and ambient water from a common well were mixed and aerated in a head tank and delivered to the aquaria at 20 ± 1 C at 0.5-1 liter/min. Loading and density were well below recommended maximums for yellow perch in a flow-through system (Glass 1991). Water temperature for Experiment 3 was decreased to 19 C after 8 weeks to abate mortalities associated with flagellated bacteria.

Table 6. Composition of the isocaloric[1] semipurified diets used in Experiment 2 to determine the optimal protein-to-energy ratio for yellow perch.

	P:ME			
INGREDIENT	**107**	**91**	**75**	**59**
Casein	28.82	24.50	20.17	15.85
Gelatin	9.61	8.17	6.72	5.28
Histidine	.87	.74	.61	.48
Lysine	2.09	1.78	1.46	1.15
Methionine	1.31	1.11	.91	.72
Cod Liver Oil	3.00	3.00	3.00	3.00
Soy Oil	11.00	11.00	11.00	11.00
Dextrin	20.00	29.06	38.13	47.00
Mineral Mix	5.66	5.66	5.66	5.66
Vitamin Mix	.55	.55	.55	.55
Choline Chloride	.45	.45	.45	.45
Alpha Cellulose	14.65	11.99	9.34	6.86
Carboxymethyl Cellulose	2.00	2.00	2.00	2.00
% PROTEIN	40	34	28	22

[1] All diets contain 375 Kcal ME / 100 grams dry diet.

Table 7. Composition of the semipurified diets used in Experiment 3 to determine the optimal level of dietary protein for yellow perch when fed diets formulated to have the optimal protein-to-metabolizable energy ratio of 91 mg protein/Kcal estimated metabolizable energy.

	PERCENT PROTEIN			
INGREDIENT	38	32	26	20
Casein	27.37	23.05	18.72	14.41
Gelatin	9.12	7.68	6.24	4.80
Histidine	0.83	0.70	0.57	0.44
Lysine	1.99	1.67	1.36	1.05
Methionine	1.24	1.05	0.85	0.65
Col liver oil	3.00	3.00	3.00	3.00
Soy oil	10.00	7.95	5.90	3.84
Dextrin	38.94	32.79	26.64	20.50
Mineral mix	5.66	5.66	5.66	5.66
Vitamin mix	0.55	0.55	0.55	0.55
Choline chloride	0.45	0.45	0.45	0.45
Alphacellulose	0.00	13.52	28.08	42.66
Carboxymethyl Cellulose	0.85	2.00	2.00	2.00
Metabolizable Energy	417.60	351.60	285.60	219.80

Since yellow perch are easily excited by movement around the glass aquaria, each aquarium was wrapped in thick (6 mils) black plastic. The windows were blackened and light was furnished by overhead fluorescent tubes about 6 feet above the water surface. The light fixtures were covered with thin (1 mil) black plastic to lower light intensity. A 16:8 light:dark photoperiod was maintained (Huh, et al., 1976).

Age 0+ yellow perch fingerlings were obtained each November and held in 1900 liter circular fiberglass tanks with center bottom drains. Well water was supplied at 13 C and fish were trained to accept a commercial trout feed. All fingerlings used were the progeny of Saginaw Bay fish which were spawned at the MSU Aquaculture Lab each Spring (Starr 1989). Swim-up fry were planted into ponds and the resulting fingerlings were harvested each Fall. Fish were graded according to length and randomly allocated to the experimental tanks.

II. Experimental Diet Preparation

Thirteen semipurified experimental diets were prepared with varying protein or metabolizable energy content (Tables 2-4 and 5-7). Types and amounts of dietary ingredients were based on previous work done with rainbow trout (Ostrowski and Garling 1987) and general recommendations for laboratory animals and coldwater fish (NRC 1978, 1981). The energy

values used were 4.5, 8.5, and 3.2 Kcal ME/g for protein, lipid, and carbohydrate, respectively. These values were derived from growth chamber experiments using rainbow trout fed casein-gelatin-dextrin diets (Smith, pers. comm., reported in Ostrowski and Garling 1987). The estimated essential amino acid composition of each diet was formulated to match the A/E ratio of yellow perch fillets determined in the Initial Biochemical Analysis (Table 8).

Dry ingredients were mixed thoroughly by hand. Cod liver oil and soy oil were mixed together in a beaker. The oils were slowly whisked into the dry ingredients using an industrial food mixer (Univex M-12B). Ingredients were blended for about ten minutes until homogeneous. Room-temperature water was slowly mixed in until the mixture became a fluffy dough.

The dough was extruded using the Univex grinder attachment. Die hole diameter was 3/16 inch. The resulting spaghetti-like product was dried on plastic screens in a forced-air oven without heat (25-30 C) for 8-24 hours, depending on ambient conditions. The dried strings were then placed in 2-pint plastic containers in a -40 F freezer until they became frozen and brittle. After freezing, the diets were ground in a Waring blender and passed through a series of U.S.A. Standard Testing Sieves. Particles between 1.4 and 3.35 mm were kept and re-frozen. Allotments of each feed were measured weekly and kept in the

Table 8. The A/E ratios of yellow perch muscle and dietary protein sources used to formulate experimental semipurified diets.

Amino acid	A/E[a]			
	75% casein: 25% gelatin mixture	Crystalline amino acid mix	Casein-gelatin mix: amino acid mix, 9:1	Fillet
Arg	129.7		116.8	113.2
His	44.8	204	60.7	62.3
Ile	93.0		83.7	93.6
Leu	152.4		137.2	148.4
Lys	142.7	490	177.4	179.9
Met + Cys	54.2	306[b]	79.4	78.4
Phe + Tyr	175.1		157.6	146.3
Thr	77.4		67.4	77.4
Trp	18.4		16.5	ND[c]
Val	114.9		103.4	98.5

[a] A/E concept of Arai (1981) = (essential amino acid content/total EAA content + cys + tyr) x 1000.
[b] Methionine only.
[c] ND = not determined

refrigerator. Percent dry matter was determined for each diet. A proximate analysis of diets was determined using standard A.O.A.C. (1975) methods.

III. Experimental Procedures

A. Conditioning fish to tanks and feeds

Fish were transferred to the experimental tanks for the purpose of acclimation at least ten days prior to beginning an experiment. Water temperature was increased about 2 C per day from the holding temperature of 13 C to the experimental temperature of 20 C.

As a result of Preliminary Experiment 2, fish in Experiments 1-3 were trained to accept a semipurified diet by replacing the commercial trout diet with semipurified diet at a rate of 20% per day. The semipurified diet used for this purpose was of different protein and energy content than the experimental diets. Fish readily accepted the experimental and control diets the first day of each experiment.

B. Weighing and Feeding Level

The wet weight of fish was determined every two weeks. Fish were dip netted into a bucket with 75 ppm tricaine methanesulfonate (MS 222) to reduce handling stress. Then fish were transferred to a pre-weighed water-filled bucket. Total weight of fish was calculated by subtracting the

initial weight of the bucket and water from the final weight of the bucket containing water and fish. Feed was withheld one day prior to weighing to avoid undigested material affecting the weights (Meyer and Garrett 1967). Feeding resumed the day after weighing.

Fish were fed in excess at a rate of 3% of their wet body weight/day (Calbert and Huh 1976). The daily ration was divided into three equal amounts fed approximately 4 hours apart. Since feed amounts were in excess at each feeding, fish were essentially fed to satiation three times daily. This method was necessary since the experimenter's ability to determine the point of satiation is subject to bias (M. Ducharme, pers. comm.) and confounded by the excitability of yellow perch by movement over the tanks and their feeding behavior. Yellow perch did not always feed actively, but continued to eat feed from the tank bottom for a prolonged period after feed was administered. Since fish were fed in excess, common growth parameters such as feed conversion, feed efficiency, and protein deposition could not be calculated. Feed amounts were adjusted every two weeks based on weight samples. Feed was fed on a dry matter basis. Feces and uneaten feed were siphoned daily from the aquaria 20 minutes following the first and third feedings.

IV. Data Analysis

A. Growth and Condition Parameters

Average fish weight was determined for each tank by measuring the total wet weight of fish per tank every two weeks. The total wet weight value was divided by the number of fish per tank for the average weight per fish.

Average weight increase per period was calculated by subtracting the average weight of a fish at the previous weighing from the average weight at the current weighing.

The relative daily gain (RDG) was calculated as:

$$RDG = ((WTf - WTi)/WTi)/14 \text{ days} \times 100,$$

where WTf = the final average weight of a fish in a tank at the end of the 14 day feeding period, and WTi = the average weight of a fish from a tank at the beginning of the 14 day feeding period.

Standard length was determined to the nearest millimeter at the beginning and end of each experiment, and total length change was determined by subtraction.

Hepatosomatic Index was calculated as:

$$HSI = (\text{grams wet liver} * 100)/(\text{grams wet fish}).$$

The gonadosomatic index was calculated using wet weights:

$$GSI = (\text{gonad weight}/\text{whole fish weight}) \times 100$$

Age of the Saginaw Bay perch used in the amino acid analysis was estimated according to Figure 3 reported in Diana and Salz (1990).

The weight and length of all mortalities were recorded for inclusion into weight estimates. Dead fish were not replaced.

B. Biochemical Analysis

All fish samples were initially frozen whole. To prepare the frozen fish for amino acid or proximate analysis, samples were defrosted slightly until malleable. Fish from which egg or carcass samples were taken were opened ventrally and the carcass separated from the ovaries and gut. While still partially frozen, whole fish and carcasses were hand ground in a #5 Chop-Rite meat grinder, then chopped in a Waring blender until of a peanut butter consistency. Fish from which fillets were to be removed were thawed completely but kept chilled. Fillets were minced by hand before blending as above, and eggs were crushed with a mortar and pestle. Pond fish were finely chopped by hand. All samples were refrozen until time of analysis.

The proximate analysis of tissues in the Initial Biochemical Analysis was conducted according to Harris (1970). Other proximate analyses were according to standard A.O.A.C. (1975) methods. Percent protein was calculated using the standard equation 6.25(Nitrogen content) = Protein. The amino acid composition of yellow perch tissues was determined according to Cohen et al. (undated).

Tryptophan levels were not determined since the initial step of the amino acid analysis, acid hydrolysis, destroys tryptophan.

For Experiments 1-3, proximate analysis was determined in triplicate on pooled samples of five fish per tank. Liver analysis for Experiment 1 was done in duplicate.

C. Statistical Analysis

All statistical methods used were according to Gill (1978). Amino acid and corresponding proximate composition and GSI were evaluated using a simple analysis of variance (ANOVA). Other proximate data, HSI and length were evaluated with a nested ANOVA. Weight gain was evaluated with a split-plot repeat measure univariate (ANOVA) and/or multivariate (MANOVA) design as the average grams gained per fish per period. The effect of diets on the rate of weight gain was analyzed using an analysis of covariance (ANCOVA). When significant treatment effects were found, diets were ranked using Dunnett, Bonferroni, and Tukey tests where appropriate. Proximate results were ranked using a Tukey test.

RESULTS

Amino Acid and Proximate Composition of Yellow Perch

1. Amino acids on a percent protein basis.

There were significant differences ($p<0.05$) in the amino acid composition of yellow perch whole bodies, eggs, and muscle (Table 9). Whole body essential amino acids were not significantly different regardless of fish origin (Table 9). However there were differences for the nonessential amino acids glutamic acid, serine, and alanine. The source or age of fish from which egg samples were taken appeared to have no effect on amino acid composition, as no significant differences were found for either essential or nonessential amino acids between the egg sets (Table 9). When compared to whole fish, the egg EAAs were greater than ($p<0.05$) or equal to the whole body EAAs except for arginine, histidine, and lysine.

Fillets were significantly higher than ($p<0.05$) or equal to whole body samples for all EAAs except threonine and valine (Table 9). Fillets and eggs were similar, but eggs were higher in threonine, valine, and methionine+cystine. The percentage of protein made up of

Table 9. Amino acid composition[1] of yellow perch tissues[2,3] collected from Saginaw Bay, a culture pond, and the MSU Fish Culture Lab in November 1989.

Amino acid	Bay Fillet	Bay whole	Pond whole	Lab whole	Bay eggs	Lab eggs
ARG	6.32^{ab}	6.66^{a}	6.50^{a}	6.66^{a}	5.48^{c}	5.81^{bc}
HIS	3.59^{a}	2.87^{b}	2.96^{b}	2.87^{b}	2.57^{c}	2.50^{c}
ILE	6.26^{a}	4.82^{cd}	4.52^{d}	4.48^{d}	5.83^{ab}	5.37^{bc}
LEU	8.29^{a}	7.66^{ab}	7.47^{b}	7.30^{b}	8.07^{a}	7.91^{a}
LYS	10.05^{a}	8.68^{b}	8.51^{b}	8.22^{b}	7.03^{c}	6.78^{c}
MET	3.33^{a}	2.80^{b}	2.94^{b}	2.74^{b}	2.31^{c}	2.42^{c}
CYS	1.05^{b}	0.72^{b}	0.70^{b}	0.67^{b}	2.01^{a}	2.62^{a}
PHE	4.47^{b}	4.66^{b}	4.61^{b}	4.57^{b}	5.49^{a}	5.50^{a}
TYR	3.70^{ab}	3.37^{bc}	3.13^{c}	3.10^{c}	3.97^{a}	3.77^{a}
THR	4.32^{b}	4.49^{b}	4.27^{b}	4.32^{b}	5.14^{a}	5.16^{a}
VAL	5.50^{b}	5.41^{b}	5.13^{b}	5.21^{b}	6.34^{a}	5.76^{ab}
ASP	10.06^{ab}	7.49^{c}	8.42^{bc}	7.15^{c}	11.10^{a}	10.84^{a}
GLU	15.31^{a}	13.46^{b}	14.02^{b}	12.90^{c}	13.30^{bc}	13.52^{b}
SER	3.63^{c}	3.89^{c}	3.85^{b}	3.91^{c}	5.15^{a}	4.73^{ab}
GLY	5.24^{c}	7.24^{ab}	8.74^{a}	9.79^{a}	4.98^{c}	6.29^{bc}
ALA	6.21^{d}	7.13^{b}	7.26^{ab}	7.40^{a}	6.38^{c}	6.56^{c}
PRO	3.37^{c}	5.97^{a}	5.30^{ab}	6.83^{a}	4.64^{bc}	4.79^{b}
H-PRO	0.35^{b}	1.50^{a}	1.66^{a}	1.88^{a}	0.00^{c}	0.00^{c}
EAA as % of protein	55.85^{b}	52.14^{a}	50.74^{a}	50.14^{a}	54.24^{b}	53.19^{ab}

1 On a percentage protein basis.
2 Row values with different superscripts are significantly different ($p<0.05$).
3 Sample size per measurement (n) = 5.

EAAs was slightly but significantly higher ($p<0.05$) for the fillets and Bay eggs than the whole fish and lab eggs (Table 9).

2. Essential amino acids as A/E ratios.

Since the proportion of total protein made up of EAAs was different for whole body samples than for fillet and egg, comparisons between EAAs change when expressed in A/E ratio terms.

Whole body samples differed when expressed in A/E ratio form (Table 10). Lab fish were higher in arginine, while pond fish were higher in histidine and methionine + cystine. Bay fish were intermediate in histidine but highest in isoleucine. Bay and pond fish equally exceeded lab fish for lysine. There were no significant differences for any other essential amino acids.

Differences were also found between egg sets. Bay eggs were significantly higher ($p<0.05$) in isoleucine, lysine, and valine but lower in arginine, methionine + cystine, and threonine. Other amino acid values did not differ significantly. Egg and whole body A/E values compared much the same way as they did on a percentage protein basis; the eggs equalled or exceeded the whole body for all EAAs except arginine, histidine, and lysine.

Fillet A/Es were equal to or greater than the whole body except for threonine and valine. Similarly, fillet

Table 10. The A/E ratios[1] of yellow perch tissues[2,3] collected from Saginaw Bay, a culture pond, and the MSU Fish Culture Lab in November 1989.

Amino acid	Bay Fillet	Bay whole	Pond whole	Lab whole	Bay eggs	Lab eggs
Arginine	111^{b}	128^{c}	128^{c}	133^{d}	101^{a}	108^{b}
Histidine	63^{e}	55^{b}	58^{d}	57^{c}	47^{a}	47^{a}
Isoleucine	94^{b}	92^{b}	89^{a}	89^{a}	107^{d}	101^{c}
Leucine	146^{a}	147^{a}	147^{a}	146^{a}	149^{a}	148^{a}
Lysine	177^{e}	167^{d}	168^{d}	164^{c}	130^{b}	126^{a}
Methionine + Cystine	78^{c}	68^{a}	72^{b}	68^{a}	80^{c}	94^{d}
Phenylalanine + Tyrosine	144^{a}	154^{a}	153^{a}	153^{a}	174^{a}	173^{a}
Threonine	76^{a}	86^{c}	84^{b}	86^{c}	95^{d}	96^{e}
Valine	97^{a}	104^{c}	101^{b}	104^{c}	117^{e}	107^{d}

[1] Essential amino acid ratio (A/E) = {(individual EAA content / total EAA content + Cys + Tyr) x 1000}.

[2] Row values not sharing the same superscript are significantly different ($p<0.05$).

[3] Sample size (n) = 5.

values exceeded or equalled all egg values except threonine, valine and methionine + cystine from lab eggs.

3. Proximate analysis

Whole body samples were not statistically different from each other for protein and ash (Table 11). Whole body fish of lab origin had significantly higher amounts of fat than the Bay fish. Pond fish were intermediate in this respect, not being statistically different from either Bay or lab fish in fat content. Lab fish were significantly higher in dry matter content than Bay and pond fish.

Eggs did not differ from each other for protein, ash, or dry matter (Table 11). However, lab eggs were significantly fattier than bay eggs.

As was expected, the fillets were significantly higher in protein than eggs or whole bodies.

The fish from which the Bay eggs were taken were significantly greater in weight than the lab fish (Table 11). However, the gonadosomatic indices did not differ significantly ($P>0.10$) for the lab and Bay fish (Table 11).

Preliminary Experiments

Preliminary Experiment 1 - Testing the acceptability of a semipurified diet for the growth of yellow perch.

After six weeks, weight gain and length increase of fish fed the control diet were significantly greater

Table 11. Proximate composition[1] and GSI[2] of yellow perch tissues[3,4] collected from Saginaw Bay, a culture pond, and the MSU Fish Culture Lab in November 1989.

Component	Bay Fillet	Bay whole	Pond whole	Lab whole	Bay eggs	Lab eggs
Protein	90.2^{a}	63.5^{b}	68.5^{ab}	54.9^{b}	64.6^{b}	51.6^{b}
Fat	1.3^{d}	8.4^{cd}	11.9^{bc}	21.0^{b}	15.9^{b}	32.6^{a}
Ash	5.5^{b}	19.3^{a}	16.1^{a}	17.1^{a}	4.4^{b}	5.0^{b}
Dry matter	20.8^{c}	25.5^{b}	25.5^{b}	28.2^{a}	29.7^{a}	29.0^{a}
Fish weight (g)	142.4^{a}	130.4^{ab}	14.4^{d}	78.2^{bc}	171.4^{a}	81.0^{c}
Gonad weight (g)					10.6^{a}	7.6^{a}
GSI					6.0^{a}	9.3^{a}

1 Expressed as a percentage of dry matter.
2 Gonadosomatic index = (gonad wet weight/whole fish wet weight) x 100.
3 Row values with different superscripts are significantly different ($p<0.05$).
4 Sample size (n) = 5.

($p<0.05$) than the fish fed the semipurified diet (Table 12). The relative daily gain (RDG) of control fish increased sharply between days 15 and 28, then decreased to near the original value by the end of the study (Figure 1).

The fish fed the semipurified diet lost weight in the initial period, then gained steadily throughout the study to end at the same ($p<0.01$) RDG as the control fish (Table 12 and Figure 1). The initial period of weight loss coincided with large quantities of feed left uneaten on the aquaria bottom.

There were no mortalities.

Preliminary Experiment 2 - Feed-transition study

The RDG of fish fed the combination diet strategy was not significantly different ($p<0.05$) than the RDG of fish fed the practical trout feed (Table 13). Fish fed only the semipurified diet lost weight (Figure 2). In addition, fish fed using the combination diet strategy consumed all the feed offered to them on days 6 and 7 when 100% semipurified diet was fed.

There were no mortalities.

Table 12. Comparison of growth results[1,2] of yellow perch fed a semipurified diet and a trout feed in Preliminary Experiment 1 to determine the acceptability of a semipurified diet for the growth of yellow perch.

Diet	Length (cm) increase	Average weight gain (g)	RDG[3] for final growth period
Control	1.8[a]	8.37[a]	0.657[a]
Semipurified	0.8[b]	1.76[b]	0.616[a]

[1] Column values with different superscripts are significantly different ($p<0.05$).

[2] Sample size (n) = 3.

[3] RDG = $\{[(Wt_f - Wt_i)/Wt_i]/\text{days in growth period}\} \times 100$.

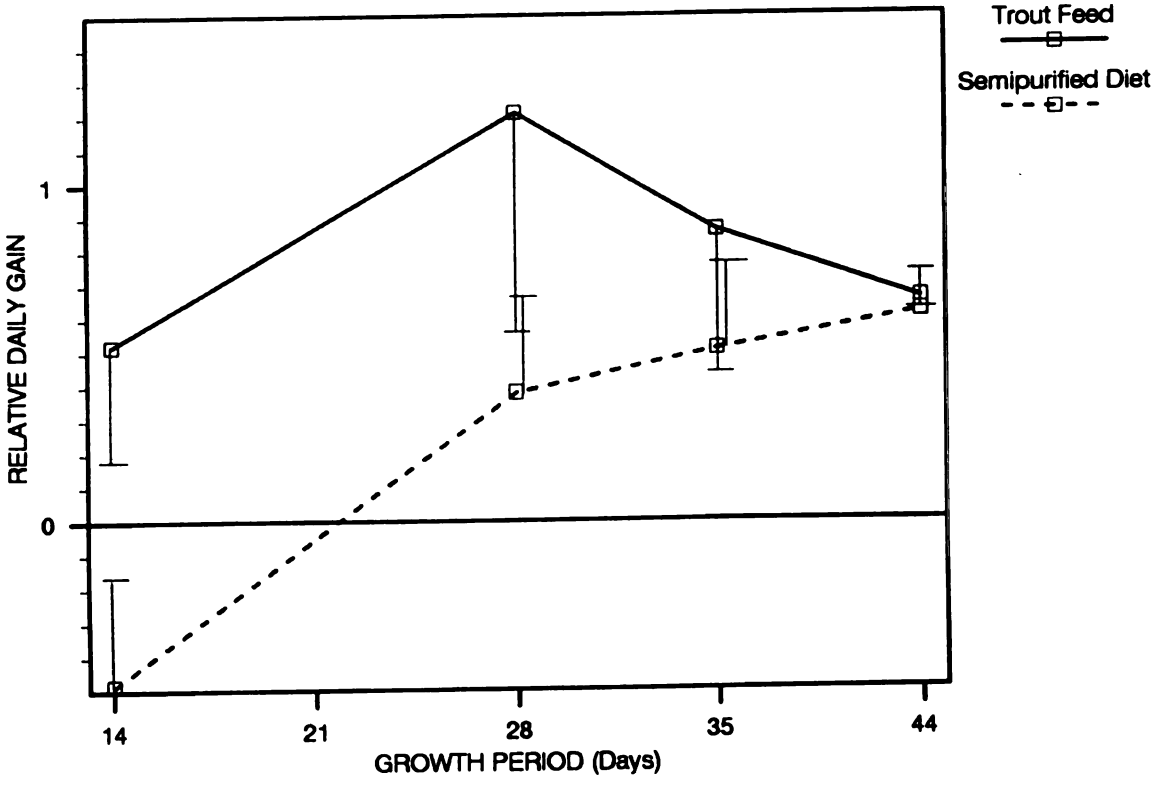

Figure 1. Relative daily gain of yellow perch fed a commercial trout feed and an experimental semipurified feed in Preliminary Experiment 1 to determine they acceptability of a semipurified diet for the growth of yellow perch.

Table 13. Growth results[1,2] of Preliminary Experiment 2 to test a feed-transition strategy for yellow perch.

WT (g)	TROUT	SEMIPURIFIED	COMBINATION
Initial	14.10	14.30	13.90
Final	15.10	13.70	14.90
% Gain / Fish	7.09	-4.20	7.19
Average Weight Gain	1.00[a]	-0.60[b]	1.00[a]
Relative Daily Gain	1.42[a]	-0.84[b]	1.44[a]

1 Row values with different superscripts are significantly different ($p<0.05$).

2 Sample size (n) = 1.

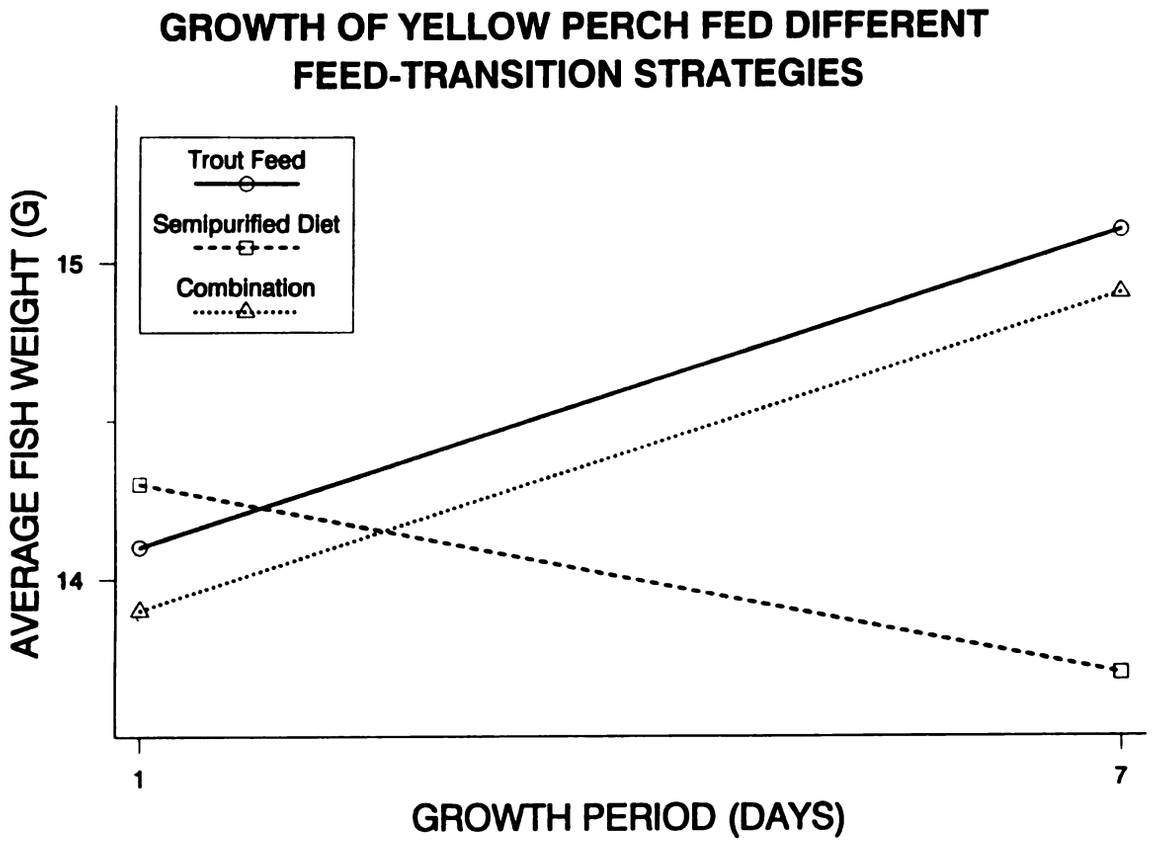

Figure 2. Changes in average weight of yellow perch over time when fed different feed-transition strategies in Preliminary Experiment 2.

Protein-to-Metabolizable Energy Experiments

Experiment 1 - Isonitrogenous diets to determine the optimal P:ME ratio for yellow perch.

Feeding the diet with a P:ME of 100 was discontinued after two weeks because diet stability was poor and adversely affected water quality. Poor diet stability was probably the result of the high (17%) oil content of the diet. Data for fish fed this diet were not included in the analysis.

A split-plot analysis of variance with repeated measurement revealed no diet x period interaction or diet effects (Figure 3). Period effects were indicated ($p<0.10$). Dietary treatments had no effect on total changes in length ($p>0.05$).

Neither whole body nor empty carcass protein, lipid, or moisture content were significantly affected by diet composition (Table 14). Whole body ash was not affected by diet, but fish fed the highest energy diet, P:ME 110, had significantly fattier livers ($p < 0.05$) than the other fish.

There were seven mortalities, 1 to 3 from each treatment (Table 14).

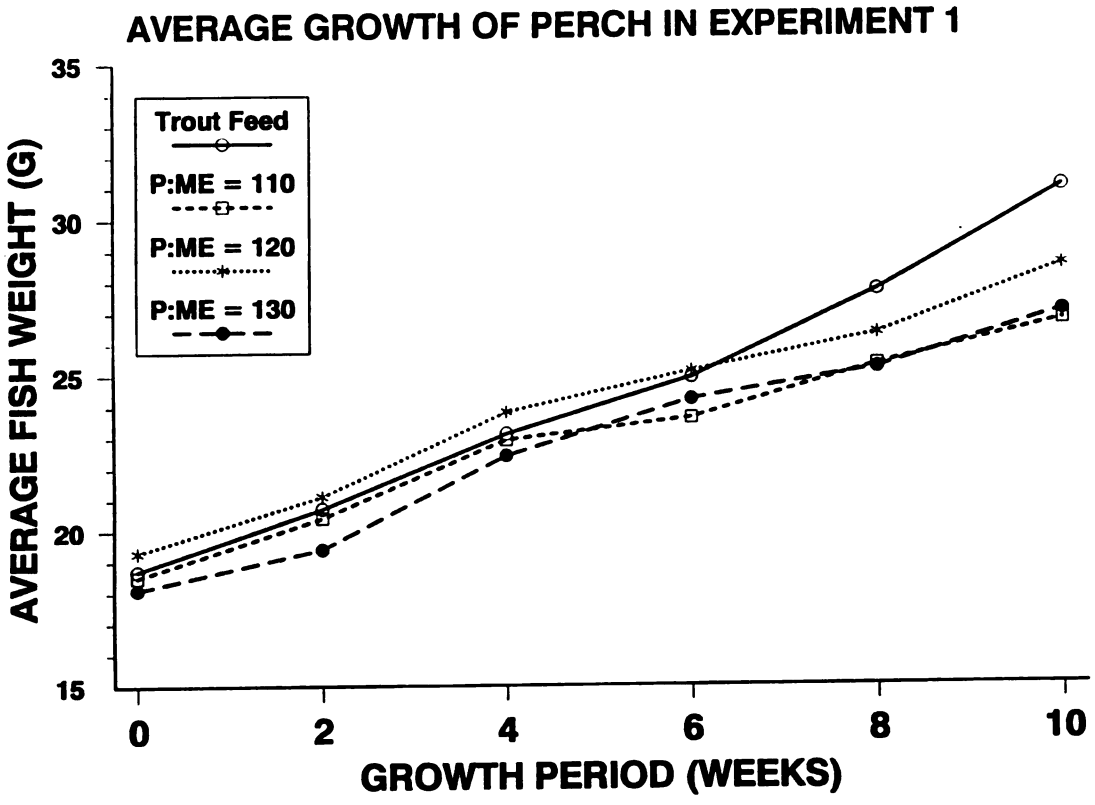

Figure 3. Changes in the average weight of yellow perch over time when fed isonitrogenous diets in Experiment 1 to determine the optimal protein-to-metabolizable energy ratio.

Table 14. Proximate composition[1], mortalities and average growth of yellow perch[2,3] fed isonitrogenous semipurified diets in Experiment 1 to determine the optimal protein-to-energy ratio for yellow perch.

	P:ME			
SAMPLE	**CONTROL**	**110**	**120**	**130**
PROTEIN:				
Empty Carcass	18.82[a]	19.15[a]	18.86[a]	19.05[a]
Whole Fish	17.29[a]	17.76[a]	17.79[a]	17.94[a]
FAT:				
Empty Carcass	3.30[a]	3.23[a]	3.38[a]	3.04[a]
Whole Fish	7.88[a]	7.52[a]	7.28[a]	7.23[a]
Liver	20.16[a]	54.72[b]	40.69[a]	43.48[a]
MOISTURE:				
Empty Carcass	73.68[a]	72.78[a]	73.39[a]	73.40[a]
Whole Fish	70.89[a]	70.53[a]	71.19[a]	70.91[a]
Liver	65.44[a]	56.27[a]	60.84[a]	61.96[a]
ASH: Whole fish	12.30[a]	16.10[a]	14.50[a]	17.40[a]
AVG GRAMS INCR/FISH:	12.30[a]	8.20[b]	8.60[b]	8.90[b]
Mortalities	1	3	1	1

[1] Expressed on a dry matter basis.
[2] Row values with different superscripts are significantly different ($P<0.05$).
[3] Sample size (n) = 3.

Experiment 2 - Isocaloric diets used to determine the optimal P:ME ratio for yellow perch.

A split-plot MANOVA with repeated measurement revealed no diet x period interaction, but period effects were significant ($p<0.003$, Figure 4). A difference in diets was suggested ($p<0.09$). A Bonferroni test shows that the total growth of fish fed the diet with a P:ME of 91 (diet 91) may have been significantly greater ($p<0.20$) than the growth of fish fed the other semipurified diets (Table 15). An analysis of covariance (ANCOVA) provided modest evidence that the rate of weight gain was affected by dietary treatment ($p<0.10$). A Tukey test showed that diet 91 had ($p<0.10$) a greater effect on the rate of weight gain than the other diets (Table 15). There were no differences in the final lengths of fish fed different diets ($p<0.05$).

The proximate composition of whole body protein, lipid, ash and dry matter were not affected by diet composition (Table 15). However fish fed diets with 22, 28, and 34% protein had significantly larger ($p<0.05$) HSIs than the fish fed the control and 40% protein diets. The livers of fish receiving the semipurified diets were lighter in color (whitish) than livers of the control fish.

This experiment was concluded after eight weeks due to disease-related mortalities (Table 15). Mortalities did not appear to be related to diet.

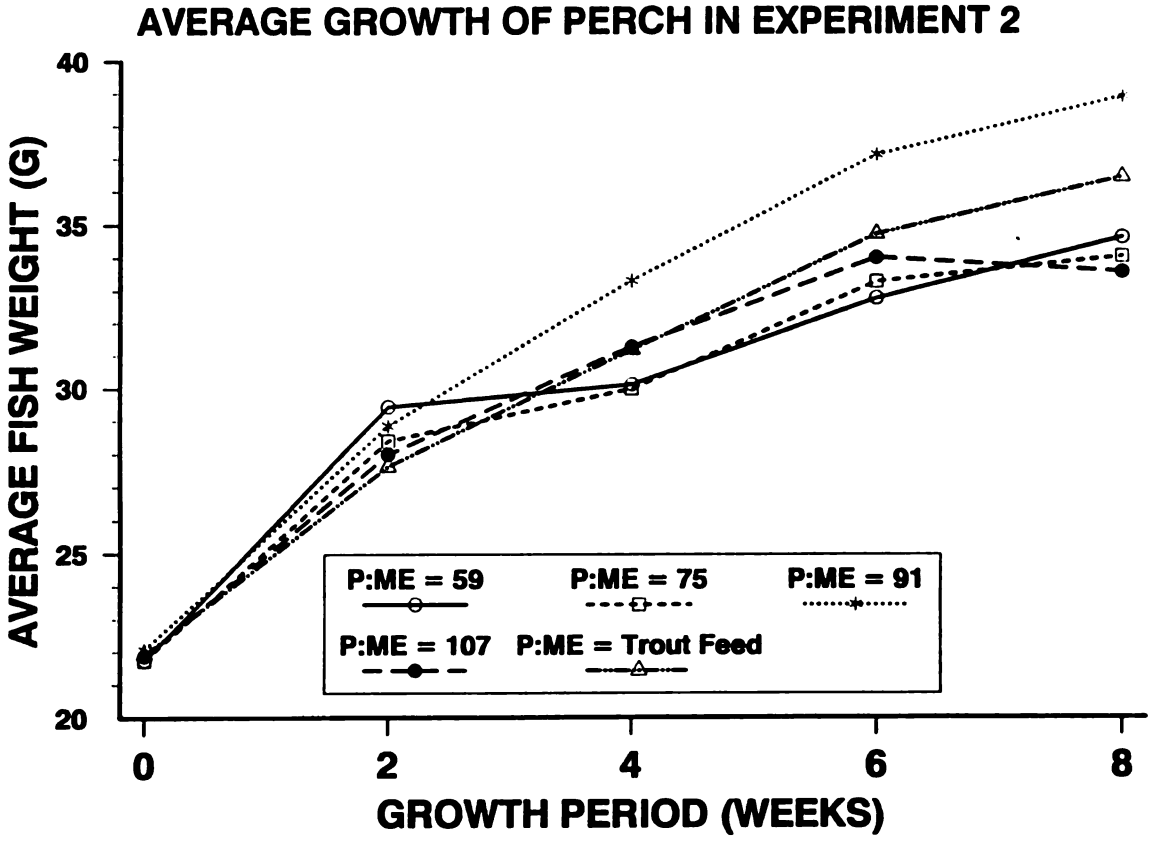

Figure 4. Changes in the average weight of yellow perch over time when fed isocaloric semipurified diets in Experiment 2 to determine the optimal protein-to-metabolizable energy ratio.

Table 15. Proximate composition[1,2], average growth, and mortalities of yellow perch[3,4] fed isocaloric semipurified diets in Experiment 2 to determine the optimal protein-to-energy ratio for yellow perch.

			P:ME			
SAMPLE	**BEFORE**	**CONTROL**	**59**	**75**	**91**	**107**
PROTEIN	16.78	16.33	15.64	16.51	16.41	16.53
LIPID	8.03	8.01	9.01	8.53	8.82	8.17
ASH	17.20	12.70	16.90	16.00	14.20	14.20
DRY MATTER	30.44	29.76	30.69	29.96	30.00	30.13
AVG GRAMS INCR/FISH		14.51^{ab}	13.64^{b}	12.29^{bc}	16.66^{a}	10.97^{c}
MORTALITIES		1	3	0	3	2
HSI		8.32^{c}	16.07^{a}	12.26^{b}	13.76^{ab}	9.41^{c}
RATE OF WT GAIN		AB	AB	B	A	AB

1 Whole fish.
2 On a dry matter basis.
3 Row values with different superscripts are significantly different ($p<0.05$). There were no significant differences for protein, lipid, ash, or dry matter content of fish fed the different diets.
4 Sample size (n) = 3.

Experiment 3 - Diets with the P:ME of 91 and varying protein levels used to determine the optimum protein level.

A split-plot MANOVA with repeated measurement revealed no differences in treatments and no period x treatment effects (Figure 5). Period effects were significant ($p<0.007$). However, based on an analysis of covariance, dietary treatments had an effect the rate of weight gain ($p<0.05$). A Tukey test showed that the diet containing 20% protein may have had a smaller effect on the rate of weight gain than the other semipurified diets ($p<0.20$; Table 16).

No differences were found for whole body protein, fat, ash, or dry matter content (Table 16). There were no differences in length increases of fish fed the different diets.

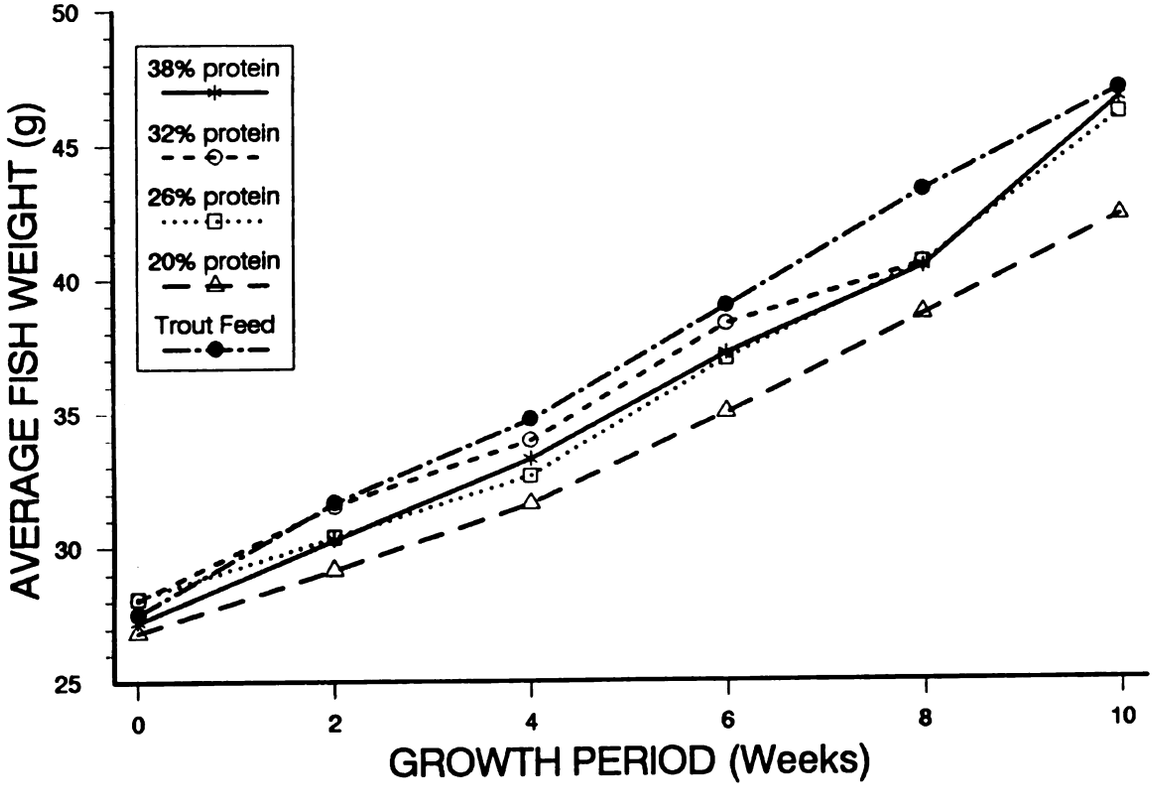

Figure 5. Changes in the average weight of yellow perch with time when fed semipurified diets in Experiment 3 to determine the optimal level of dietary protein when diets were formulated to have the optimal protein-to- metabolizable energy ratio of 91 mg protein/Kg estimated metabolizable energy.

Table 16. Proximate composition[1,2], average growth, and mortalities of yellow perch[3,4] fed semipurified diets in Experiment 3 to determine the optimal level of dietary protein for yellow perch when fed diets formulated to have the optimal protein-to-metabolizable energy ratio of 91 mg protein / Kcal estimated metabolizable energy.

Diet:	38	32	26	20	Control
Protein	50.08	56.70	48.35	49.44	51.27
Fat	25.97	22.28	27.59	21.34	20.59
Ash	29.80	30.30	29.10	31.60	26.40
Dry matter	29.89	30.34	30.97	29.68	29.32
Avg grams incr/fish	17.13	15.04	15.75	15.37	19.42
Mortalities	4	2	1	0	0

1 Whole fish.
2 On a dry matter basis.
3 There were no significant differences for protein, lipid, ash, dry matter, or growth.
4 Sample size (n) = 3.

DISCUSSION

Amino Acid and Proximate Composition of Yellow Perch Tissues

1. Amino acids on a percent protein basis.

The amino acid profiles determined for yellow perch egg, whole body and muscle were similar to those determined for other fishes (Tables 17-19). The findings of no differences between yellow perch whole body EAAs, in spite of differences in the age and size of the fish, was also observed for channel catfish by Wilson and Poe (1985) and carp by Schwarz and Kirchgessner (1988). However, adult dolphin fish appeared to require more arginine, histidine, and tryptophan than juvenile dolphin fish (Ostrowski and Divakaran 1989).

As with the whole body amino acid content, no differences in the amino acid content of the Bay and lab eggs were found, even though the lab fish had a higher GSI than expected for their age and the time of year (Diana and Salz 1990). Kaitaranta et al. (1980) also found that the amino acid content of rainbow trout and Baltic herring roe did not change with maturity. This suggested that the dietary and environmental conditions under which the eggs

Table 17. Essential amino acid composition of the eggs of several fishes.

Amino acid	Walleye[1]	Rainbow trout[2]	Channel catfish[3]	Atlantic salmon[4]	Atlantic silversides[5]	Yellow perch[6]
Arg	5.70	6.00	5.47	6.40	6.50	5.48
His	2.90	2.70	2.29	2.70	4.70	2.57
Ile	6.40	5.20	5.24	6.40	5.00	5.83
Leu	8.30	8.50	9.95	10.30	7.70	8.07
Lys	7.80	6.70	7.83	8.80	7.90	7.03
Met	3.10	2.20	3.51	2.70	3.90	2.31
Cys	ND[7]	4.20	ND	1.70	0.50	2.01
Phe	4.80	5.80	3.95	5.30	3.60	5.49
Tyr	4.10	4.60	3.99	5.90	4.60	3.97
Thr	5.20	4.70	6.10	5.90	5.20	5.14
Trp	1.20	0.90	ND	0.98	ND	ND
Val	7.10	5.50	6.07	6.20	7.20	6.34

1 Ketola 1982
2 Kaitaranta et al. 1980
3 Wilson and Poe 1985
4 Cowey et al. 1962
5 Schauer et al. 1979
6 Eggs from Saginaw Bay fish, Initial Biochemical Analysis.
7 ND = not determined or reported.

Table 18. The amino acid composition of the whole bodies of several fishes.

Amino acid	Rainbow trout[1]	Cherry salmon[2]	Channel catfish[3]	Fathead minnow[4]	Yellow perch[5]
Ala	6.57	6.15	6.31	6.56	7.13
Arg	6.41	6.01	6.67	6.37	6.66
Asp	9.94	9.62	9.74	9.25	7.49
Cys	0.80	1.30	0.86	0.73	0.72
Glu	14.22	14.91	14.39	14.39	13.46
Gly	7.76	7.31	8.14	7.53	7.24
His	2.96	2.32	2.17	2.53	2.87
Ile	4.34	3.84	4.29	4.19	4.82
Leu	7.59	7.31	7.40	7.78	7.66
Lys	8.49	8.54	8.51	8.88	8.68
Met	2.88	3.04	2.92	3.01	2.80
Phe	4.38	4.49	4.14	4.55	4.66
Pro	4.89	4.20	6.02	4.80	5.97
Ser	4.66	4.34	4.89	5.74	3.89
Thr	4.76	4.49	4.41	5.15	4.49
Trp	0.93	0.80	0.78	1.11	ND
Tyr	3.38	3.47	3.28	3.09	3.37
Val	5.09	4.70	5.15	4.36	5.41
H-pro	ND[6]	0.87	ND	ND	1.50

1 Wilson and Cowey 1985
2 Ogata et al. 1983
3 Wilson and Poe 1985
4 Gatlin 1987
5 Saginaw Bay fish, Initial Biochemical Analysis.
6 ND = not determined or reported.

Table 19. Essential amino acid composition[1] of the muscle of several fishes.

Amino acid	Yellow perch[2]	European perch[3,4]	Pike[5]	Cod[6]	Eel[7]	Dolphin fish[8]
Arg	6.3	1.1	7.4	6.4	6.6	9.3
His	3.6	0.6	2.1	3.3	4.5	6.0
Ile	6.3	1.0	5.3	4.7	4.7	4.9
Leu	8.3	1.6	8.2	8.9	8.2	6.6
Lys	10.1	2.0	9.2	9.9	9.7	7.0
Met	3.3	0.6	3.2	2.0	2.8	3.9
Cys	1.1	0.2	0.8	1.4	ND	1.4
Phe	4.5	0.8	4.2	4.5	3.7	4.4
Tyr	3.7	0.6	2.8	3.9	3.2	3.9
Thr	4.3	0.9	5.0	5.0	4.4	4.7
Trp	ND[9]	0.2	0.9	1.3	ND	0.9
Val	5.5	1.0	5.6	5.5	5.2	5.0

1 On a percent protein basis.
2 Initial Biochemical Analysis
3 Scherz and Kloos 1981
4 Expressed as mole percent
5 Wunsche and Steffens 1968
6 Connell and Howgate 1959
7 Sidwell and Ambrose 1974
8 Ostrowski and Divakaran 1989
9 ND = not determined or not reported.

developed did not affect the protein quality of the eggs.

Contrasts between whole body and egg EAA content are similar to those observed for salmonids (Ogata et al. 1983; Arai 1981) and channel catfish (Wilson and Poe 1985). A notable difference is the high level of the NEAA cystine in yellow perch eggs as compared to the whole body. This difference was not found for salmonids (Arai 1981; Ogata et al. 1983) or catfish (Wilson and Poe 1985; Tables 17 and 18).

The EAA composition of yellow perch fillets is very similar to the muscle EAA composition of other fishes except the dolphin fish (Table 19). The most notable difference between yellow perch and other fishes is the relatively high content of isoleucine in yellow perch fillets.

The small amount of hydroxyproline found in the fillets (Table 9) was probably the result of small rib fragments left in the fillets. It is unlikely that this hydroxyproline was free in the muscle itself, since proline is peptide-bound in procollagen (at the site of deposition, i.e. rib bones) before it is hydroxylated (Hunt and Groff 1990). In humans, dietary hydroxyproline is not incorporated into collagen. Instead, free proline is the source of proline and hydroxyproline used in collagen synthesis (Stetten and Schoenheimer 1944; Stetten 1949).

2. Essential amino acids as A/E ratios.

The A/E ratios of different fishes are similar, but can differ by up to 5% for a given amino acid (Tables 20 - 22). Yellow perch whole body A/E ratios closely resemble those of the channel catfish (Table 20), but yellow perch eggs are more similar to salmon eggs (Table 21). The muscle A/E values of yellow perch and European perch are very similar, differing at the most by 1.7% for phenylalanine + tyrosine (Table 22).

3. Proximate analysis.

The proximate composition of fish eggs varies greatly between species and seasons (Cantoni et al. 1975; Vuorela et al. 1979; Medford and Mackay 1978; Kaitaranta and Ackman 1981; Craig 1977). However the composition of the Bay and lab perch eggs resembled reported values for yellow and European perch eggs collected in November (Craig 1977; Tanasichuk and Mackay 1989; Table 23). The proximate composition of Bay and lab whole bodies was also similar to reported values for wild and cultured yellow perch, respectively (Reinitz and Austin 1980; Malison et al. 1988; Tanasichuk and Mackay 1989; Table 24).

The differences in the lipid content of wild and cultured yellow perch whole fish and eggs may have reflected dietary and habitat differences. The commercial trout feed that the lab perch were fed was quite dense in

Table 20. Whole body A/E ratios for several fishes.

Amino acid	Yellow perch[1]	Channel catfish[2]	Golden shiner[3]	Cherry salmon[4]	Dolphin fish[5]
ARG	130	132	124	119	162
HIS	57	43	51	46	68
ISO	90	85	80	76	90
LEU	147	146	148	145	116
LYS	166	168	171	170	122
MET + CYS	70	75	80	86	86
PHE + TYR	153	147	147	158	169
THR	85	87	96	89	82
TRP	ND[6]	15	18	16	12
VAL	103	102	86	93	92

1 Average values for Bay, lab and pond whole fish from the Initial Biochemical Analysis
2 Wilson and Poe 1985
3 Gatlin 1987
4 Ogata et al. 1983
5 Ostrowski and Divakaran 1989
6 ND = not determined

Table 21. Egg A/E[1] ratios for several fishes.

Amino acid	Yellow perch[2]	Coho salmon[3]	Cherry salmon[4]	Channel catfish[5]	Dolphin fish[6]
ARG	105	103	101	101	134
HIS	47	47	45	42	58
ILE	104	92	91	144	134
LEU	149	153	156	183	121
LYS	128	143	144	96	92
MET+CYS	87	83	80	65	99
PHE+TYR	174	156	185	146	166
THR	96	97	79	112	86
TRP	ND[7]	14	13	--	12
VAL	112	112	108	112	98

1 Essential amino acid ratio (A/E) = {(Individual essential amino acid content (EAA) / total EAA content + Cys + Tyr) x 1000}.
2 Average of values for Bay and lab eggs from the Initial Biochemical Analysis
3 Arai 1981
4 Ogata et al. 1983
5 Wilson and Poe 1985
6 Ostrowski and Divakaran 1989
7 ND = not determined

Table 22. Comparison of muscle A/E[1] ratios for several fishes.

Amino acid	Yellow perch[2]	Adult dolphin fish[3]	Juvenile dolphin fish[3]	European perch[4]	Cod[5]
Arg	111	163	142	108	113
His	63	101	76	57	58
Ile	110	86	92	97	83
Leu	146	116	127	148	157
Lys	177	122	133	188	174
Met + Cys	76	70	80	72	56
Phe + Tyr	144	154	147	127	148
Thr	76	82	92	86	88
Trp	ND[6]	16	14	19	23
Val	97	88	96	98	97

1 Essential animo acid index (A/E) = {(Essential amino acid content (EAA) / total EAA content + Cys + Tyr) x 1000}.
2 Initial Biochemical Analysis
3 Ostrowski and Divakaran 1989
4 Scherz and Kloos 1981
5 Connell and Howgate 1959
6 ND = not determined

Table 23. Proximate composition of perch eggs[1] collected in November.

	Lab[2]	Bay[2]	European perch[3]	Yellow perch4
Protein	51.6	64.6	51.6	54.0
Lipid	32.6	15.9	22.5	27.0
Ash	5.0	4.4	ND[5]	ND
Dry matter	29.0	29.7	27.5	22.5

1 Expressed on a dry matter basis for sample size (n) = 5.
2 Lab and Bay yellow perch eggs were collected from fish from the MSU Aquaculture Lab and Saginaw Bay, respectively, in November 1989.
3 Craig 1977.
4 Tanasichuk and Mackay 1989
5 ND = not determined or reported.

Table 24. Proximate composition[1] of cultured and wild whole yellow perch collected in November.

	Cultured perch			Wild perch	
	reported[2]	reported[3]	lab[4]	Bay[4]	reported[5]
Protein	67.1	63.3	54.9	63.5	64.7
Lipid	18.1	21.7	21.0	8.4	9.7
Ash	13.9	14.1	17.1	19.3	ND[6]
Dry matter	26.1	27.9	28.2	25.5	24.6

1 Expressed on a dry matter basis, sample size for Lab and Bay treatments (n) = 5.
2 Reinitz and Austin 1980.
3 Malison et al. 1988.
4 Lab and Bay treatments were collected from the MSU Aquacualture Lab and Saginaw Bay, respectively, in November 1989.
5 Tanasichuk and Mackay 1989.
6 ND = not reported.

energy-available nutrients (45% protein, 12%fat, 5035 Kcal GE/g) and yellow perch fed it appeared to have a higher percentage of body cavity fat than wild perch (Starr 1989; Table 24).

In contrast to the energy-rich diet fed to the lab fish, the Bay fish likely developed under conditions of low food availability (Diana and Salz 1990). It may be argued that the differences in body fat levels were a natural result of the age differences between the 5+ Bay fish and the 2+ lab fish. Craig (1977) has found that stored energy is inversely related to age in the European perch. But even 2+ yellow perch from a eutrophic Canadian lake did not contain the high body and egg fat levels of our lab fish (Tanasichuk and Mackay 1989; Tables 23 and 24).

The artificial, year-round growing conditions in the lab may have also affected the body composition and rate of gonad development of the lab fish. The fat content of yellow and European perch eggs peaked in February at 7 to 8% of gonad wet weight (Tanasichuk and Mackay 1989; Craig 1977). This closely resembled the November value of 9.5% of gonad wet weight for the lab eggs. Further, Diana and Salz (1990) have found the November GSI of age 3+ Saginaw Bay yellow perch to be about 4.5 times greater than the November GSI of 2+ perch. Since the GSIs of the 2+ lab fish and 5+ Bay fish were not significantly different (Table 11), some different rate of maturation in the lab-raised fish was

suggested. Finally, female yellow perch raised in other laboratories under artificial light regimes have been observed to lay eggs off-season, with no associated spawning activity in the males (K. Dabrowski, School of Natural Resources, Ohio State University, pers. comm.)

The proximate composition of fish muscle has been shown to vary greatly between fishes (Table 25). However, the composition of yellow perch fillets did resemble the edible portion of European perch.

Preliminary Experiments

Preliminary Experiment 1 - Testing the acceptability of a semipurified diet for the growth of yellow perch.

A prerequisite for the study of the dietary requirements of fishes is the ability of the investigator to closely control dietary components. Consequently, the use of diets composed of purified or semipurified ingredients is common in fish nutrition research. Preliminary Experiment 1 was done to insure that yellow perch would grow on the type of semipurified diet we planned to use in subsequent experiments to determine the optimal dietary protein-to-metabolizable energy ratio for yellow perch. An experimental semipurified diet was formulated to match the estimated protein and energy content of the commercial trout feed used as a control in all of the feeding experiments.

Table 25. Proximate composition[1] of the muscle of yellow perch and other fishes.

	Yellow perch[2]	European perch3	Croaker[4]	Mullett[4]	Thread herring[4]	Siberian sturgeon[5]
Protein	90.2	89.8	93.5	77.0	84.6	78.5
Lipid	1.3	3.9	3.0	23.2	9.5	20.6
Ash	5.5	0.2	5.0	5.8	7.1	ND[6]
Dry matter	20.8	20.5	20.1	26.5	25.3	20.5

1 Expressed on a dry matter basis
2 Initial Biochemical Analysis
3 Scherz and Kloos 1981
4 Sidwell and Ambrose 1974
5 Medela et al. 1991
6 ND = not determined or reported.

Since fish fed both diets showed the same RDG for the last period of the experiment, we speculated that the basic semipurified diet formulation was adequate for yellow perch growth studies. Because the initial period of weight loss of fish fed the semipurified diet coincided with large quantities of feed left uneaten on the aquaria bottom, it was also concluded that yellow perch require a feed-transition period.

The reason for the delayed acceptance of the semipurified diet is unclear. Diet color, texture, and palatability are all possible considerations for differences in diet acceptance. The color of the experimental diet was yellow compared to the brown color of the trout feed. It was assumed that the yellow color was acceptable since larval yellow perch and fingerling walleye are known to select prey colored in contrast to their environment (Hinshaw 1985; Masterson and Garling 1986). In our experiments, the aquaria had black sides and brown bottoms, so the yellow diet was in contrast with the color of the tank surroundings.

The hardness of the control and semipurified diets were not determined, but Starr (1989) found that the hardness of commercial pelleted feeds had no effect on the feed acceptance of fingerling yellow perch.

Although the principal reason for including cod liver oil into the semipurified diets was to provide essential

fatty acids, it was also intended to increase the palatability of the diets. However, it is likely that the experimental diet was different in flavor than the trout feed since the protein source of the trout feed was primarily fish meal.

The acceptability of this semipurified diet for yellow perch growth relied partly on whether or not yellow perch could utilize the combination of casein, gelatin, and crystalline amino acids as efficiently as the complex proteins in the control feed. Diet acceptability was also dependent upon how well the amino acids were balanced to meet yellow perch requirements. Evidently the protein sources and amino acid balance were adequate (at least over the short-term experiment) since the perch grew well once they began eating the diet. Other fishes have been reared successfully on diets supplemented with crystalline amino acids. For example, Rumsey and Ketola (1975) showed that Atlantic salmon fed a casein diet supplemented with crystalline AA to simulate the AA profile of isolated fish protein had an efficiency of protein deposition equal to that of fish on an isolated fish protein diet. Carp and channel catfish can utilize purified amino acid test diets only after the dietary pH had been neutralized (Nose et al. 1974; Wilson et al. 1977).

In spite of the good growth of fish on the semipurified diet in the latter half of the experiment, it is still

possible that the essential amino acids in the experimental diet were not well balanced. This would not have been detected in the experiment if total dietary protein was in excess and there were no severe excesses or deficiencies in any single essential amino acid (Harper et al. 1970). This was an important consideration since this work was based on the premise that the commercial trout feed (and the experimental diet formulated to match it) were indeed excessive in protein content.

Preliminary Experiment 2 - Feed-transition study

Since fish fed using the combination diet strategy had the same weight gain as fish fed the trout feed, it was concluded that a combination feeding strategy was the best way to train yellow perch to accept a semipurified diet. Since changes in the feeding regimes of terrestrial livestock are made gradually to avoid feed rejection (Church and Pond 1988), it is not unreasonable to conclude that aquatic species would benefit from similar treatment.

This was an informal experiment. To have been statistically sound, each feeding strategy should have been fed to triplicate tanks (or more) of fish. Also, the experiment should have included a trout feed treatment with decreasing percentages like the trout feed portion of the combination diet. If fish fed this treatment had a smaller weight increase than the fish fed the combination diet, we

could have concluded with more certainty that the fish on the combination diet continued their growth by gradually switching to the semipurified diet rather than merely picking out the trout feed particles. Of course, this may be a mute point since fish on the combination diet did consume all feed offered to them on days 6 and 7 (100% semipurified diet). Consequently the combination method was used in Experiments 1 through 3.

Protein-to-Metabolizable Energy Experiments

Experiment 1 - Isonitrogenous diets used to determine the optimal P:ME ratio for yellow perch.

The results of this experiment indicate that dietary protein was excessive in relation to dietary energy; even fish fed the diet with the highest energy level (409 Kcal ME/100 g dry diet) were able to ingest enough protein to match the growth of fish fed diets with fewer calories. This agrees with the findings of Calbert and Huh (1976) and Reinitz and Austin (1980), who found no differences in the growth of yellow perch fed practical diets with P:ME ratios ranging from 104 to 152 (40 to 61.8% dietary protein).

Livers from the fish fed the diet containing a P:ME of 110 (diet 110) were significantly higher in fat than for the other diets (Table 14). Because diet 110 had the same protein and fat content as diet 120, the higher dextrin content of diet 110 is the only remaining dietary

explanation of the high liver lipids. Since the liver is the primary site of lipogenesis in salmon and catfish (Lin et al. 1977; Likimani and Wilson 1982), the excess carbohydrate was probably being converted to fat. The feeding of high-carbohydrate diets to rainbow trout also resulted in elevated liver fat (Austreng et al. 1977).

The finding of no significant differences in the proximate composition of fish fed the different diets seems incongruous with the growth and liver composition data. Even if all the diets had excessive protein relative to energy, those diets with higher levels of dextrin and oil would be expected to produce fattier fish (Watanabe 1982). At a given level of dietary protein, an increase in dietary energy usually results in decreased whole body protein and moisture, and increased whole body lipid. This is true whether increases in dietary energy were the result of increasing dietary fat alone (channel catfish: Garling and Wilson 1976; walleye: Barrows et al. 1988) or combined increases in dietary fat and carbohydrate (rainbow trout: Takeuchi et al. 1978a, Lee and Putnam 1973).

Since yellow perch are slow-growing fish (0.15 grams/day in Experiment 1 vs. 0.4 g/d for rainbow trout; Ostrowski and Garling 1987), it is possible that the experiment was not long enough for differences in whole body composition to become apparent. After a 10 week experiment, Millikin (1983) found no changes in the proximate

composition of striped bass (maximum growth of 0.12 g/d) fed isonitrogenous diets with increasing levels of energy.

It is curious that the growth of perch on the control diet was so much better than the growth of fish on the semipurified diets during the 4th and 5th periods (Figure 3). There may have been some nutritional deficiency or unbalance in the semipurified diets. High amounts of carbohydrate have been shown to inhibit the growth of the omnivorous red sea bream when included above 30% of the diet (Furuichi and Yone 1980). Austreng et al. (1977) found that levels of dietary carbohydrate up to 35% of ME had no effect after 24 weeks on dressed carcass weights of rainbow trout, but suspected that such carbohydrate-rich diets may interfere with the efficiency of protein digestion. The highest dextrin level used in Experiment 1 was 23% of dietary ME, or 28% dry matter in the diet with a P:ME of 110.

The results of Experiment 1 were used as a basis for formulating the diets used in Experiment 2. Since it was found that 409 Kcal ME / 100 g dry diet was not high enough to critically limit protein intake, Experiment 2 diets were formulated to be isocaloric at 375 Kcal ME / 100g dry diet with protein concentrations ranging from 22% to 40% (Table 6).

Experiment 2 - Isocaloric diets used to determine the optimal P:ME for yellow perch.

Although there appears to be a distinct difference between the average weight increase of fish fed the different diets (Table 15), the high variability of responses among tanks of fish receiving the same diet resulted in a large error term. This in turn confounded the statistical separation of the diets. The analysis was also complicated by missing values resulting from mortalities. The missing value problem was circumvented by using the ANCOVA, which does not require balanced data.

Our finding of better growth of fish fed the diet with a P:ME of 91 ("diet 91") is similar to the findings of Calbert and Huh (1976). They found that 12 gram yellow perch grew better on a practical diet with a P:ME (recalculated with trout ME values; NRC 1981) of 72 versus 104 and 139. Their diet also contained about the same level of metabolizable energy as diet 91 (3750 Kcal/kg dry diet). This is consistent with the our earlier interpretation of Reinitz and Austin (1980, see "Protein-Energy Ratios"), that the optimal P:ME ratio for yellow perch should be less than 138. Other fishes of a similar size fed diets with a P:E ratio close to 91 are the omnivorous channel catfish (P:DE = 92, Mangalik 1986), carp (P:DE = 88, Watanabe et al. 1987) and Nile tilapia (P:ME = 101, Siddiqui et al. 1988).

The finding of no significant differences for the proximate content of fish at the end of the experiment was not surprising based on the results of Experiment 1. The enlarged, whitish livers of the fish on diets with 22, 28, and 34% protein indicate that dietary carbohydrate may have been excessive in those diets. Enlarged livers, mostly due to glycogen accumulation, are associated with high levels of carbohydrate in the diets of carp (Palmer and Ryman 1972), sturgeon (Medela et al. 1991), and trout (Walton 1986). There is some evidence that increased glycogen can impair the liver functions of trout (Hilton and Dixon 1982). However, the yellow perch fed diet 91 (34% protein) had a high HSI and also exhibited the best growth.

Since this was a preliminary investigation into the protein and energy requirements of yellow perch, the modest statistical evidence that diet 91 resulted in better total growth and a higher rate of growth was accepted. Experiment 3 diets were therefore formulated with the optimal P:ME ratio of 91.

Experiment 3 - Diets with the optimal P:ME of 91 and varying protein levels used to determine the optimal protein level.

Since the growth of fish fed the experimental diets differed little or not at all (Table 16), it is possible that the protein content of yellow perch diets could be

lowered to 26% if the P:ME ratio remained at 91. Calbert and Huh (1976) also concluded that 12 gram fish grew better when fed a practical diet with 27% protein (estimated P:ME = 78) than when fed diets with 40 or 50% protein. However their conclusion was not tested statistically. As with the P:ME ratio, the apparent low protein requirement of yellow perch in this study closely resembles the protein requirements of the omnivorous channel catfish (Garling and Wilson 1976) and Nile tilapia (Siddiqui et al. 1988). Piscivorous fishes such as the walleye and rainbow trout generally have higher optimal P:E ratios and higher total protein requirements (Barrows et al. 1988; Lee and Putnam 1973).

SUMMARY

The essential amino acid composition of yellow perch fillets, whole bodies and eggs were very different. The age and origin of the whole fish and the fish from which the eggs were taken had little or no effect on the amino acid content, but a great effect on the whole body fat content. The proximate composition of Lab and Bay fish resembled reported values for other cultured and wild perch, respectively. The EAA profiles of yellow perch muscle, egg and whole body were similar to those of other fishes.

The results of the growth experiments showed that yellow perch could grow well for at least 10 weeks on a semipurified diet. Fish which were previously fed practical trout feeds required a five-day feed-transition period before they would fully accept the semipurified diet. Reasons for refusal of the semipurified diets may have been differences in feed palatability, texture, or color.

Eight and 10 week growth experiments were not long enough for body composition changes to become apparent in the yellow perch. This is possibly because yellow perch do not grow as rapidly as other commonly cultured fishes.

Dietary dextrin levels above about 20% result in enlarged and/or fatty livers.

Based on Experiments 1 through 3, the optimal P:ME ratio for yellow perch under experimental conditions was 91 mg protein/Kcal estimated metabolizable energy of dry diet. When yellow perch were fed diets with a P:ME of 91, dietary protein could be reduced to 26%. These figures closely resemble values reported for channel catfish (Mangalik 1986; Garling and Wilson), carp (Watanabe et al. 1987), and Nile tilapia (Siddiqui et al. 1988).

CONCLUSIONS

Experiment 3 showed that yellow perch could grow as well on the 26% protein diet as on diets with higher protein levels. This indicates that the essential amino acid composition of the semipurified diets, based on the A/E ratios of yellow perch muscle, was well balanced. Although muscle amino acid content was only determined for the Saginaw Bay fish, the finding that all three groups of whole yellow perch were identical in EAA composition and very similar in A/E ratios suggested that the EAA requirements of juvenile (14 gram) and adult (130 gram) perch were essentially the same. Therefore, the EAA formulation used in these experiments could be used to formulate feeds for both juvenile and adult yellow perch.

The digestive tract physiology and metabolic capacity of larval and adult fish are very different (Blaxter 1974; Dabrowski and Culver 1991). Because of this and the significant differences that were found between the AA compositions of whole yellow perch and their eggs, it is likely that the amino acid needs of perch larvae are quite different from those of juveniles and adults. Further investigation into the EAA requirements of larvae is warranted. Practical starter diets for perch fry are unavailable, and this has been identified as an important bottleneck in intensive yellow perch culture (Starr 1989).

Even though the two groups of eggs examined in the Initial Biochemical Analysis developed under very different environmental conditions, they did not differ in either essential or non-essential amino acid content. This would indicate that high protein commercial trout feeds are adequate to meet the requirements for yellow perch egg development; if the commercial diet had been unsatisfactory, retarded growth in favor of egg development would have been expected in the lab fish. The viability of our lab-raised eggs was unknown. However, several commercial yellow perch culturists successfully maintain their broodstocks on commercial salmonid diets similar to the ones we fed to our lab fish (D. Smith, Freshwater Farms of Ohio and J. Malison, University of Wisconsin-Madison, pers. comm.). Future study of the nutritional requirements of perch broodfish will be in order as regional yellow perch stocks are isolated and selected for their desirability as broodstock (Dr. D. Garling, Dept. Fisheries and Wildlife, Michigan State Univ., pers. comm.)

The results of Experiment 3 also indicate that the current use of high protein trout feeds by yellow perch culturists may not be warranted. High protein trout feeds, like the one used as a control in these experiments, contain at least 45% protein, 12% fat, and gross energy of 5035 Kcal/Kg. We determined in Experiment 3 that yellow perch fed the semipurified diet with 26% protein, 8.9% fat, and

gross energy of 3411 Kcal/Kg grew as well as fish fed the trout feed and the other semipurified diets. The 26% protein semipurified diet more closely resembles commercial channel catfish grow-out diets in composition (approximately 32% protein and 3200 Kcal/Kg; Lovell 1989) than trout feeds. Therefore it is recommended that the performance of yellow perch raised on practical trout and catfish feeds be tested under intensive culture conditions and over an extended period of time.

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