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Utilization of Dietary Carbohydrates and Lipids by Salmonids Sexually Sterilized with 17alpha-Methyltestosterone

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UTILIZATION OF DIETARY CARBOHYDRATES
AND LIPIDS BY SALMONIDS
SEXUALLY STERILIZED WITH 17 α -METHYLTESTOSTERONE

BY
BRUCE S. AHERN

A THESIS SUBMITTED IN PARTIAL FULLFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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IN
FISHERIES AQUACULTURE AND PATHOLOGY

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1986

MASTER OF SCIENCE THESIS

OF

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ABSTRACT

Two, twelve week feeding trials were conducted to determine the performance of juvenile and yearling rainbow trout (*Salmo gairdneri*) which were fed high-fat or high-carbohydrate diets. In each feeding trial and for each diet group, 50% of the fish used had been previously treated with 17 α -methyl-testosterone, given orally at the onset of feeding. These were called treated fish. Also, for each group, 50% were untreated rainbow trout (controls), kept and fed under identical conditions as treated fish. All fish were held in 100% flow-through systems and fed twice or three times daily. Feed requirement was determined as a percentage of the total fish weight for each tank, according to water temperature and average size of fish calculated at each growth check. Three replicates were used in the first feeding trial and two in the second. Growth measurements were made every three weeks. Performance was determined through feed conversion efficiency, actual weight gain, actual length gain, relative weight gain and condition factor. Plasma glucose and ammonia levels, and visceral fat levels were measured to obtain an estimation of the effects of diet and treatment on these parameters.

Two diets were formulated in the first trial, one high in lipid (24.1%) with no carbohydrate added and the other

lower in lipid (15%) and high in sucrose (23.8%) as a digestible carbohydrate. In the second trial three diets were formulated: one high in fat (24.1% lipid), one high in sucrose (25.8%) and low in lipid (14.1%), and one high in molasses (42.0%) and low in lipid. All diets were iso-nitrogenous and iso-caloric. The objective in these studies was to determine if a correlation existed between performance of treated or control rainbow trout and diet fed. In the first feeding trial, fish fed the sucrose diet performed better than those fed the high-fat diet. Treated fish on the sucrose diet out-performed all other groups with respect to growth parameters, though not significantly so in all instances. A positive correlation was found between treatment with methyl-testosterone and low plasma glucose and low visceral fat levels in fish fed these diets. In the second feeding trial a molasses (an alternate carbohydrate source) diet, a sucrose diet and a high-fat diet were fed to treated and control fish. Fish in general performed better when fed the two diets high in digestible carbohydrate. Molasses in the fish diet had no adverse affect on growth and results indicated that this sucrose source might potentially be used as a feed additive for fish. Treated fish fed the high-carbohydrate diets in the second trial did not demonstrate the superior performance which was evidenced in the first trial. Results which were obtained from

these feeding trials are discussed and possible implications for the aquaculture industry are put forth.

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TABLE OF CONTENTS

	<u>page</u>
Abstract	ii
Acknowledgements	v
Table of Contents	vii
List of Tables	viii
List of Figures	ix
Introduction	1
Literature Review	6
Materials and Methods	23
Results	36
Discussion and Summary	74
Literature Cited	89
Appendix I - Freeze Branding	99
Appendix II - Analysis of Variance for all Tests	106

LIST OF TABLES

<u>Table</u>	<u>Page</u>
I. Dietary Ingredients-Trial #1	32
II. Energy Content of Diets (Kcal/Kg)-Trial #1 ..	33
III. Dietary Ingredients-Trial #2	34
IV. Energy Content of Diets (Kcal/Kg)-Trial #2 ..	35
V. The effect of treatment with 17a-methyl- testosterone on the sexual development of rainbow trout.....	37
VI. Mean growth parameters for treated and control rainbow trout after feeding for 12 weeks with high-fat and sucrose diets- Trial #1.....	41
VII. Plasma glucose and visceral fat levels of treated and control rainbow trout after feeding for 12 weeks with high-fat and high sucrose diets-Trial #1.....	54
VIII. Mean growth parameters for treated and control rainbow trout after feeding for 9 weeks with high-fat, sucrose and molasses diets-Trial #2.....	59
IX. Plasma glucose and visceral fat levels of treated and control rainbow trout after feeding for 9 weeks with high-fat, sucrose and molasses diets-Trial #2.....	65

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Overall effect (Trials #1&2) on rainbow trout sexual development of treatment with methyl-testosterone early in life.....	38
2. Actual gain (grams) of treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks-Trial #1.....	42
3. Feed conversion (g fed/g gained) and protein conversion (%) of treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks-Trial #1.....	45
4. Relative gain (gain/initial wt) of treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks-Trial #1.....	47
5. Treatment and feeds interaction effect on actual weight gain and feed conversion levels in treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks-Trial #1.....	49
6. Plasma glucose and visceral fat levels of treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks-Trial #1.....	55
7. Actual gain (grams) of treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks Trial #2.....	60
8. Feed conversion (g fed/g gained) and protein conversion (%) of treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks- Trial #2.....	62
9. Relative gain (gain/initial wt) of treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks-Trial #2.....	66
10. Treatment and feed interaction effect on actual weight gain and feed conversion in treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks-Trial #2.....	68

11. Plasma glucose and visceral fat levels of treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks- Trial #271
12. Photo of yearling rainbow trout, with recognizable mark from freeze-branding apparatus, taken six weeks after branding 104

INTRODUCTION

Because the natural diet of wild trout is composed mainly of protein, researchers and aquaculturists have long entertained the belief that salmonids and, to a lesser extent, other cultured species require a high level of dietary protein in order to obtain maximum growth and optimum feed conversion (Scherbina and Tryamkina, 1974; Plakas et al., 1980; Robinson et al., 1980). Early investigators stated that protein levels needed to achieve maximum growth and feed conversion were as high as 40% of the diet or greater (DeLong et al., 1959; Chance et al., 1964). In nature, salmonids utilize dietary protein not only for growth (muscle protein synthesis and nitrogen retention) but also to help meet part of the fish's energy needs. In an aquaculture situation, it seems, other components might be incorporated into fish diets which could "spare" dietary protein and fulfill energy (maintenance and production) demands of the fish. Fish culturists are constantly monitoring new developments in the fish food industry, trying to obtain less expensive feeds which give adequate growth results and feed conversion efficiencies. The addition of lipids in higher percentages to fish diets has shown benefits with respect

to sparing dietary protein (Kellems and Sinnhuber, 1982). However, quality lipids which may be used in fish feeds are unavailable in some areas of the world and therefore of little practical value. The use of sucrose, a carbohydrate(CHO) easily digested by salmonids and other fish, when incorporated into fish diets should provide a "sparing" action on dietary protein. Sucrose is also readily available in most parts of the world. The use of molasses in fish feeds could also provide a means for formulating a less expensive diet.

Sucrose, a relatively inexpensive sugar, is 85% digestible by salmonids (Halver, 1972) and hence may be a good source of energy for meeting the daily caloric requirements of the fish. Sucrose, a disaccharide made up of fructose and glucose, is more easily digested by salmonids than other complex CHOs. Fructose may be taken up at the cellular level and enter directly into the glycolytic pathway without the use of insulin (Stryer, 1981). Blackstrap molasses, a sugar refinery by-product, might also be a practical source of sucrose and is readily available in many areas. Molasses, which contains 50-60% sucrose, also has the advantage of acting as a good binder in diet formulations.

For a long period of time, diet manipulation has been utilized in fish culture as a way to improve growth rates and feed conversion and to increase the market size of fish. Within the last decade researchers have increasingly

turned to the use of hormones and their possible application in fish culture as growth promoters. Both natural (growth hormone, prolactin, thyroid and steroid hormones) and synthetic (synthetic steroids, testosterone and estradiols) hormones have been used in studies ranging from inducing smoltification in anadromous salmonids to sex reversal and sterilization of salmonids for improvement of growth performance and use in genetic research (Donaldson and Hunter, 1982; Higgs et al., 1982; Smith, 1983). Steroid hormones have been used in both sex control of salmonids (Donaldson and Hunter, 1981; Billard et al., 1981) and as anabolic agents promoting growth (Hirose and Hibiya, 1968; Higgs et al., 1982). 17 α -methyltestosterone (17 α -MT), or 17 α -Methyl-4-androsten-17 β -ol-3-one, a synthetic steroid hormone, has been used to enhance gonadal development in grey mullet (Weber and Lee, 1985), produce sterility in salmonids (Donaldson and Hunter, 1982), and cause sex reversal in tilapia (Owusu-Frimpong and Nijjhar, 1980; Macintosh et al., 1985), carp (Roa et al., 1983; Jensen et al., 1983) and Atlantic salmon (Johnstone and Youngson, 1984). Along with these androgenic (sex-reversal and sterility) properties, this hormone has also been shown to be useful as an anabolic agent in fish culture. Dietary incorporation of 17 α -MT promoted growth and improved feed conversion in the American eel (Degani and Gallagher, 1985), carp (Lone and Matty, 1979) and salmonids (Higgs et al., 1977; Fagerlund et al., 1979). Sterile salmonids,

produced with high dietary levels of 17 α -MT given upon onset of feeding, initially show reduced growth when compared with controls. But as the fish mature, the sterile fish have greater growth and feed conversion efficiency than controls. Sterilized fish need not utilize energy for sexual maturation and instead may put this energy into muscle tissue production and growth (Higgs et al., 1982; Smith, 1983). Interestingly, the 17 α -MT-treated fish show improved growth performance over their control counterparts not only during this sexual maturation period but throughout the entire adult life of the fish (Donaldson and Hunter, 1982; Schreck and Li, 1983). A possible explanation of this phenomenon may be that treatment with high levels of 17 α -MT early in life somehow disrupts part of the biosynthetic pathways which play a role in metabolism of the fish. Alternatively, this might suggest that these hormones, present in higher levels in untreated fish, while influencing sexual maturation and development, could also suppress the natural growth potential of these fish.

The objectives of this study were two-fold. The first was to develop a practical diet which provided an inexpensive, utilizable CHO source for use in salmonid (and possibly other fish) culture. The purpose here was to determine if CHO provided in the proper form in salmonid diets stimulated growth and affected feed conversion efficiency. This research wished to determine if both

sucrose and molasses are feasible additives to any fish diet formulation if provided in correct amounts.

The second objective of this work was to study the growth response of salmonids which have been treated with high levels (50mg/Kg of diet) of 17a-MT at a young age, to high-fat and high-CHO diets. The performance of treated/sterile fish was compared with non-treated fish given these high-CHO and high-lipid diets. Results of these studies and implications for the fish feed and aquaculture industries will be discussed.

LITERATURE REVIEW

The findings of early researchers led to the development of commercial fish feeds which are very high (>40%) in crude protein and low in digestible carbohydrates (CHO) (DeLong et al., 1959; Chance et al., 1964; Shcherbina and Tryamkina, 1974; Kanid'yev and Sklyarov, 1979; Brown et al., 1985). It has been observed, though, that the quantity and quality of indispensable amino acids available in the diet is more important than high amounts of crude protein. (Shanks et al., 1962; Dupree and Halver, 1970; Plakas et al., 1980; Robinson et al., 1980). Therefore, the absolute amount of crude protein might be reduced in diets if adequate levels of essential amino acids were included in the diet of each cultured species. However, commercial pelleted diets continue to be made with high crude protein levels along with low or high levels of undigestible CHO (Rangens/Zeigler fish feeds, 1984; Martins salmonid feeds, 1984; Stinson Trout Line feeds, 1984).

DsSilva and Perera (1985), using young *Tilapia nilotica*, found that growth on diets containing only 28-30% protein was consistently better than higher protein diets, while feed conversion efficiency was decreased at protein levels above 30%. Machiels and Henken (1985), working with the African catfish (*Clarias gariepinus*), recommended that

an optimum protein/energy ratio be pursued when formulating diets. Pieper and Pfeffer (1980a) showed a positive relationship between high energy (sucrose)/lower protein formulations when varying protein and energy levels in rainbow trout (*Salmo gairdneri*) diets. They showed improved protein efficiency ratio (PER) after decreasing the protein content and increasing the proportion of sucrose in the diet. Hence, sucrose had a protein "sparing" action in the diet.

Because the natural diets of fish are composed not only of protein but also lipids, researchers have investigated the use of lipids in salmonids and other fish diets as an energy source. In carp (*Cyprinus carpio*), Nagai and Ikeda (1973), when comparing the practicality of using lipids versus CHO as a dietary energy source, found the rate of metabolism for lipids was twice as fast as that for complex CHO. Since Castell et al., (1972) identified the essential fatty acids, much work has been done varying the lipid and essential fatty acid levels in salmonid fish diets. Up to 24% salmon oil produced improvement in growth and feed conversion when provided in rainbow trout diets (Lee and Putnam, 1973; Reinitz et al., 1978). Ellis and Smith (1984), in studying digestibility and essentiality in fish, demonstrated the quality of the fatty acid content of fish oils and other oils which may be used in fish diets. The value of menhaden oil in diets of Florida pompano

(*Trachinotus carolinus*) was studied and an optimum dietary level of 8% menhaden oil was obtained for this fish (Williams et al., 1985). Above and below this optimum dietary level, feed conversion was less efficient. Lipids which are less expensive and of lower quality than these highly unsaturated oils though, yield poor results when used in fish diets. Incorporation of beef tallow into the diet of tilapia resulted in poor growth and poor utilization of this lipid (Stickney and McGeachin, 1984). In a study using 25-45% herring oil fed with gelatin bound proteins, Kellems and Sinnhuber (1982) stated that increased amounts of oil in the diet had a positive high relation to feed conversion efficiencies. However, these authors also noted that the overall desirability of the fish, as determined by a taste panel, was lower for those fed diets with high levels of herring oil. The use of lipid levels greater than 25% in trout diets leads to increased deposition of body fat (Kellems and Sinnhuber, 1982). It is evident that the use of high quality lipids in fish feeds might be necessary to a limited extent in order to provide essential fatty acids. The addition of high quantities of quality lipids (fish oils, seed oils) to fish diets though, may not be feasible due to taste intolerance, inordinate deposition of body fats or, as may be the case in many areas, high cost or inavailability of quality lipid ingredients.

The inclusion of CHO in fish diets has, for a long period of time been neglected or used simply as a filler. A number of researchers have reported that fish in general and salmonids in particular were poor utilizers of CHO (Austreng et al., 1977; Hilton et al., 1982; Anderson et al., 1984). More recently studies involving incorporation of different carbohydrates into fish diets has given room for optimism. Spannhof and Plantikow (1983) showed that starch content of rainbow trout feed and feed digestibility are negatively correlated. The same study demonstrated that dextrin, a starch product with an advanced degree of hydrolysis, improved digestion when included in the diet. These authors concluded that crude starch in trout diets reduces amylase activity in the intestinal juices and that crude starch contents of about 20% inhibits CHO absorption. Therefore, the use of crude starch and complex CHOs in salmonid diets yields poor performance at best. Edwards et al (1977) found that prospects for selectively breeding strains of rainbow trout which are better able to utilize carbohydrate are not promising. Austreng et al (1977) reported similar results and added further that reduced conversion efficiency of protein and energy, and inferior fish growth on high CHO diets might make the use of large amounts of CHO in trout diets unfeasible. Presumably then, the only way to incorporate high levels of CHO in fish diets is to provide

it in forms which are utilizable by fish or by hormonal manipulations which will allow for better absorption and digestion.

Nagai and Ikeda (1973) suggest the inferiority of carbohydrate as an energy source in carp and also demonstrated impaired glucose utilization in these fish. However, Anderson et al (1984) showed improved growth in the juvenile tilapia (*Oreochromis niloticus*) as the level of glucose, sucrose, dextrin or starch was increased from 0-40% of the diet. Glucose at higher levels in the diet spared less protein energy than dextrin or sucrose. At levels above 10% sucrose produced greater protein retention than glucose, indicating that this disaccharide has greater potential for sparing protein in complete rations. According to Hilton and Atkinson (1982), weight gain was significantly reduced in trout reared on diets highest (21%) in the available CHO, cerelose (alpha-glucose), as compared to lower dietary levels of this synthetic sugar. These authors concluded that trout have a limited ability to adapt to increased dietary CHO, and that a level in excess of 14% of the diet is not efficiently utilized. Hilton et al (1982) recommended that the maximum tolerable level of cerelose in salmonid diets appears to be dependent upon the protein, lipid, and overall energy content of the diet, and that higher levels of cerelose (up to 25%) in the diet may be efficiently utilized. In a study involving the

digestibility of starch by rainbow trout, Bergot and Breque (1983) concluded that starch becomes a valuable source of energy when its digestibility is enhanced by gelatinization. Crude native corn starch (non-gelatinized) in diets gave poor results. In another study, a 30% glucose diet promoted the best weight gain, feed conversion and protein efficiency when compared with starch diets and a 15% glucose diet (Bergot, 1979a). The results of this study, in contrast to some mentioned earlier, indicate that trout can tolerate a higher level ($\geq 30\%$) of glucose in their diet.

Other studies involving increasing proportions of sucrose and gelatinized maize starch in diets for rainbow trout have shown that there may be possibilities for CHO use in salmonid diets. Pieper and Pfeffer (1980a), in a study on the comparative efficiency of utilization of gross energy using different CHOs, proteins and lipid sources, found that in rainbow trout, sucrose was the most efficiently used energy source, with sunflower oil, gelatinized starch and glucose following in descending order. From this study it was concluded that glucose and gelatinized starch may be used energetically in trout as efficiently as sunflower oil and may be included in diets for the purpose of sparing dietary protein. In fact, it was suggested that CHOs, as compared with fat, showed a certain superiority with respect to sparing dietary

protein. These CHO's (sucrose and gelatinized maize) could be included in diets at levels up to 30%. The fact that sucrose and gelatinized maize were utilized more efficiently than glucose suggests that digestion is not a limiting factor for utilization of these CHO's at these levels. This may also reflect some negative physiological effects of a sudden large flux of glucose into the fish's metabolic system.

The same authors in another, similar study (Pieper and Pfeffer, 1980b) showed an improvement in food conversion when sucrose content was above 36% of the diet and protein level below 40%. Protein efficiency ratio (PER) was also improved by increasing the proportion of sucrose in the diet. The study gave further evidence that both sucrose and gelatinized maize starch may be used in trout diets to provide a relatively large percentage of the metabolizable energy needed and can spare dietary protein. This spared dietary protein in turn can be used in tissue building processes. Pieper and Pfeffer (1980b) further state that the use of these CHO's as substitutes on an energetic basis does not produce a great increase in body fat content, unlike the use of lipids, which do cause a proportionate increase in fish body fat. This may be of great interest from a consumer point of view. To this date, no use of other, sucrose-containing carbohydrates such as molasses or brewers solids has been reported, but

these have potential for use in fish diet formulations in the aquaculture industry.

Salmonids, because they are carnivores, cannot efficiently utilize most dietary CHO additives. This is due to poor digestion of crude and complex CHOs, high circulating levels of cortisol and low levels of insulin (Strange et al., 1978; Wagner and McKeown, 1982; Specker and Schreck, 1982; Bry, 1982; Ablett et al., 1983). The biological role of cortisol in fish and other vertebrates is to promote gluconeogenesis and enhance catabolic activity (Palmer, 1966; Storer, 1967; Hendricks et al., 1984). High cortisol levels reduce the ability of the fish to effectively utilize and metabolize CHOs due to reduced insulin levels (Butler et al., 1969; Pickford et al., 1970; Strange et al., 1978; Leach and Taylor, 1982; Bry, 1982; Ablett et al., 1983; Carneiro and Amaral, 1983). By reducing blood plasma cortisol levels, insulin may increase, allowing for better utilization of blood glucose and dietary glucose sources for energy needs (Tashima and Cahill, 1968; Ottalenghi et al., 1982; Furuichi and Yone, 1982a; Furuichi and Yone, 1982b). Patent (1970), working with the elasmobranch dogfish (*Squalus acanthias*) showed insulin to be a potent hypoglycemic agent while cortisol and corticosterone produced hyperglycemia when injected. This work also indicated that cortisol was an effective gluconeogenic agent in the shark.

Cortisol affects growth by acting on skeletal muscle causing an increase in protein catabolism and decreased protein synthesis, which in turn increases amino acid availability to the liver for gluconeogenesis (Hendricks et al., 1984). These authors showed that young bulls grow faster than older heifers due to lower circulating levels of cortisol. Palmer (1966) demonstrated net loss of weight after 24 hours, in normally feeding rats which had received a single injection of cortisol. Daily rhythm of weight gain was altered. It seems clear that high cortisol levels, which is the case in fish, has a potent negative effect on nitrogen retention, protein synthesis and CHO utilization and metabolism. In a study on gluconeogenesis in rainbow trout (*S. gairdneri*), it was shown that hyperglycemia was due to gluconeogenesis, confirming the key role of this process in trout (Morata et al., 1982). Bry (1982) gave evidence for a post feeding peak in plasma cortisol levels in well adapted rainbow trout. This suggests that cortisol plays a key role in digestion and metabolic processes, specifically CHO and protein metabolism. A reduction in circulating plasma cortisol levels might improve CHO (glucose) uptake and metabolism through increased circulating insulin levels. This would also reduce the gluconeogenic effects of this hormone, improve nitrogen retention, and increase tissue protein synthesis. Cortisol, a glucocorticoid, promotes

gluconeogenesis and catabolic processes and inhibits anabolic ones, whereas insulin acts to promote anabolic processes and inhibit catabolism in muscle, liver and adipose tissue. Insulin also stimulates glycolysis, increases the rate of protein and glycogen synthesis, and promotes the entry of glucose, some other sugars and amino acids into muscle and fat cells (Stryer, 1981).

Cortisol is produced in the adrenal cortex, cholesterol being the principal precursor of all the adrenal cortical hormones, including testosterone (Stryer, 1981; Allaben, 1982). Cortisol synthesis follows one pathway in immature individuals and another in mature individuals, with common precursors occurring in both pathways. Corticosterone occupies a separate pathway altogether, after the precursor cholesterol is reached. Testosterone has some common precursors and follows an identical route for a time with the cortisol pathway utilized by adults (Allaben, 1982). It has been shown that high circulating levels of testosterone in the blood coincides with low levels of cortisol in the catfish, *Heteropneustes fossilis* (Sundararaj et al., 1982; Lamba et al., 1983). These authors also showed that during preparatory and prespawning periods, cortisol increased dramatically in these fish, and that highest levels of plasma cortisol occur in recrudescing fish.

Fish which had been hormonally sterilized would not

be subjected to these pre- and post-spawning cortisol highs and would, therefore, not be subjected to cortisol during these periods. All energy could be diverted to maintenance and anabolic processes, instead of spawning preparation and recovery. In rats, high testosterone levels reduce the production of cortisol binding globulin (CBG) and hence, reduce the amount of free cortisol released into the bloodstream (Gala and Wesphal, 1965; Allaben, 1982;). Perhaps the high levels of 17 alpha-methyltestosterone (17aMT) which causes sterility when fed to young fish might also have some effect (permanently) on production of CBG, or a similar cortisol binder in fish, and reduce the circulating levels of cortisol in the blood. It is also possible that a high influx of testosterone (or 17aMT) in young trout for such a long period of time (900° days) might somehow damage or destroy the biosynthetic pathways which produce the glucocorticoids, hence reducing the amount of cortisol produced for the lifetime of the fish.

Methyl-testosterone (MT) has been used in aquaculture research for various reasons and has produced different results, depending upon dosage and mode of application. Synthetic testosteronees were first used in fish research for androgenic purposes or genetic sex manipulations. 17aMT has been shown to enhance spermatogenesis in the steelhead trout (Sower et al., 1983) and spermatogenesis in the grey mullet (Weber and Lee, 1985).

Through treatment with this hormone by implantation or oral administration in the diet at low levels (3.0-5.0 mg/Kg feed), sex reversal has been demonstrated in catfish (Goudie et al., 1983), tilapia (Owusu-Frimpong and Nijjhar, 1981; Macintosh et al., 1985), carp (Jensen et al., 1983; Rao et al., 1983) and salmonids (Donaldson and Hunter, 1982; Schreck and Li, 1983). This treatment produces sex-inverted male (previously females) fish which can then be mated to normal females to produce all female progeny. This has been achieved in Atlantic salmon (Johnstone and Youngson, 1984). This technique may be implemented by fish farmers to produce monosex cultures needed for specific uses. Sterility has been produced in salmonids by feeding higher levels of 17 α MT (40-60 mg/Kg) in the diet (Billard et al., 1981; Billard et al., 1982; Donaldson and Hunter, 1982; Solar et al., 1983; Schreck and Li, 1983). These sterile fish, after maturity, become easier to handle and prove to be more hardy than untreated fish (Smith, 1983). They also show improved growth rates over untreated fish of the same age and stock (Donaldson and Hunter, 1982; Higgs et al., 1982).

Androgens in general, and testosterone (natural and synthetic) in particular, act as anabolic agents to increase nitrogen retention and utilize protein and amino acids from the diet to build muscle tissue (Hirose and Hibiya, 1968; Higgs et al., 1977; Stryer, 1981;

Dasmahapatra and Medda, 1982; Lone and Matty, 1982). In the rat, subcutaneous injections of testosterone produced an increase in protein and amino acid synthesis and increased overall weight as compared to controls (DeLoecker, 1964). The same effect was demonstrated in rabbits after surgical implantation of testosterone by Grigsby et al (1976). Testosterone improved gain and feed efficiency and increased muscle synthesis rates.

Injections of testosterone propionate to the catfish (*Heteropneustes fossilis*) produced an anabolic effect on CHO metabolism. This was indicated by an increase in liver glycogen, resulting from its increased synthesis and/or less breakdown, in both male and female fish (Dasmahapatra and Medda, 1982). In the rainbow trout, Hirose and Hibiya (1968) produced a limited anabolic growth response through intra-muscular injection of 4-chlorotestosterone, though a more potent response was produced in the goldfish. Lone and Matty (1982) have shown increased growth rates and more efficient feed conversions with oral administration of 11-ketotestosterone to carp (*Cyprinus carpio*) at levels of 1.0-10.0 mg/Kg of diet, as compared to carp not fed this steroid. But 17alpha-methyl testosterone (17aMT) has been more widely used as an anabolic agent to promote growth in fish than any other of the synthetic anabolic steroids.

Again working with carp, Lone and Matty (1980),

after feeding for 90 days with different levels of 17aMT in the diet (1.0-10.0 mg/Kg), showed a positive growth response. They also reported a significant increase in total protein and RNA, and in the protein/DNA ratio in liver, kidney, brain and muscle tissue. These authors believe that 17aMT induces growth by acting probably in three different ways: (1) increased food conversion, (2) activation or secretion of other endogenous anabolic hormones, and (3) direct effect of 17aMT on gene expression in muscle cells. The belief here is that feed conversion efficiency is improved due to an increased ability of the fish to utilize and metabolize CHOs. In the American eel (*Anguilla rostrata*), dietary 17aMT at low levels (1.0 mg/Kg) significantly increased mean weight and improved feed conversion compared with controls (Degani and Gallagher, 1985). Using higher levels of 17aMT (10.0 mg/Kg diet), the same authors produced an increase in mean body weight in adult eels.

Higgs et al (1977) reported enhanced growth in yearling coho salmon (*Oncorhynchus kisutch*) with administration of 17aMT alone in the diet (1 mg/Kg) and also when 17aMT was given in combination with bovine growth hormone and L-thyroxine. In a study varying dietary protein and lipid in conjunction with 17aMT supplementation (1ppm), 17aMT significantly enhanced growth of juvenile coho salmon regardless of diet composition. Groups fed

17aMT diets also showed an increased protein efficiency ratio (PER) (Fagerlund et al., 1983). These authors demonstrated enhancement of thyroid activity in fish fed 17aMT-treated diets, as did Lone and Matty (1980) in carp. This may have been due to reduced circulating levels of cortisol. Specker and Schreck (1982), working with smoltification in coho salmon, reported that during early smolt periods when cortisol levels are lowest, thyroxine levels are at their peak. Spieler and Noeske (1984) also demonstrated that concentration peaks of cortisol and thyroxine were inversely related when they worked with photoperiod and feeding schedule in goldfish. Hunt and Eales (1979) showed that testosterone propionate has a marked effect on increasing thyroid activity and the production of thyroid hormones, T_3 and T_4 . Then, it appears there might be indirect evidence that incorporation of 17aMT in salmonid diets might somehow reduce circulating cortisol levels, thereby stimulating growth through the effect of these endogeneous anabolic hormones and through better feed CHO utilization. 17aMT was proven as a growth promoter in a coho salmon hatchery, and feed utilization was improved in fry fed 1mg 17aMT/Kg diet for a seven month period (Fagerlund et al., 1979). Improvement in growth and feed conversion has also been demonstrated in rainbow trout and Atlantic salmon with incorporation of 17aMT into diets (Higgs et al., 1982).

The use of 17aMT in fish culture operations is a highly feasible one because this synthetic hormone is relatively inexpensive, only small amounts are required, they are used successfully in sex control studies, and benefits with respect to weight gain and feed conversion are substantial. It has also been shown that this hormone is safe to use and is eliminated (cleared) from tissues in a short period of time after application has ceased. In work done with coho salmon, ten days after steroid withdrawal from diets, concentrations were 1ng/g or lower in blood and 16 other tissues (Fagerlund and McBride, 1978). In tilapia and rainbow trout juveniles, 99% of radioactively labeled 17aMT was eliminated from all tissues within 100 hours of omission of the hormone from the diet, after feeding at a level of 40mg/Kg of diet (Johnstone et al., 1983). Thus, fish fed 17aMT at an early age to eliminate sex characteristics would contain no residues of the hormone when reaching market size. Those given 17aMT as adult fish to produce anabolic effects would first have to be "cleared" of 17aMT for a short time before marketing could occur. At this time 17aMT has not been officially sanctioned for use by the U.S.D.A. in hatchery and fish culture operations, but this may change soon. Steroids and antibiotics have long been used in the beef, swine and poultry industries and it is clear that 17aMT use would be a boon to all aquaculturists.

The present research was conducted to study the performance of rainbow trout, treated with high levels of 17aMT at a young age, on a high-carbohydrate diet versus a high-lipid diet. The reasoning here is that fish which have been sterilized with high levels of 17aMT at an early age will respond better to a diet which is high in digestible CHO. These sterilized fish have consistently out-performed control fish of the same age under identical conditions as the fish mature. It is thought that perhaps the high dose of 17aMT has precipitated an inability of the fish to produce high levels of cortisol throughout its lifetime, thereby enhancing the ability of the fish to utilize dietary CHO and allow for increased anabolic effects of thyroid hormones also. Reduced cortisol levels could also increase the role of insulin in salmonids, leading to better glucose uptake and increased glycolysis for the production of energy. The benefits of low cost, highly digestible CHOs in salmonid diets will be discussed in the context of all other pertinent factors.

MATERIALS AND METHODS

Experimental Animals

Rainbow trout (*Salmo gairdneri*), directly after hatching, were fed diets containing 50mg of 17-methyl testosterone (17 α MT) per kilogram of feed for 900° days (water temperature in °C x number of days) to induce sterilization (Donaldson and Hunter, 1982). These fish were held in a large tank with a 90% water reuse system at the East Farm Aquaculture Center (EFAC) of the University of Rhode Island until needed for experimental purposes. At the age of six months for the first feeding trial, and ten months for the second feeding trial these sterilized fish were then transferred, in appropriate numbers, to smaller bioassay tanks which operated with a 100% water flow-through system. At this time, untreated (control) rainbow trout, which had been held in an identical situation to the treated fish, were also transferred to the experimental tanks. Sterilized (17 α MT-treated) and control (non-treated) fish were of the same spawning group and therefore the same age at the time of the experiment. All experimental fish were held in a 100% water flow-through

system throughout both feeding trials. All fish were spawned, hatched and raised at EFAC and were fed identical diets until the experiments began. Treated fish were distinguished from control fish by clipping of the adipose fin of treated fish prior to placing in experimental tanks for both feeding trials.

At the termination of both feeding trials, fish were sacrificed and a gross visual inspection of sexual organs (gonads) was made. Determination of the effect of 17aMT treatment (given in feed to young fish at the onset of feeding) was made in comparison to control fish of the same age and described according to certain criteria. The three descriptive categories were: 1.) sterile- no evidence of gonadal material, or gonadal material so rudimentary that males and females could not be differentiated; 2.) underdeveloped male or female-gonads were underdeveloped for the age of the fish (as compared to controls), difficult to differentiate between males and females, gonads very rudimentary and probably non-functional; and 3.) fully developed male or female-gonads large and fully developed, sexes easily differentiated and identified, eggs easily seen and milt often present. A sample of treated fish (35) and control fish (15) of the same spawning group as those used in both feeding trials were examined histologically to determine the effects of treatment at the cellular level.

Experimental Design

After transferring fish from larger tanks to smaller experimental tanks and prior to the start of both feeding trials, fish were allowed to acclimate to new surroundings for 10 to 14 days. Six tanks (200 litre capacity) were used for each feeding trial. For the first trial, 20 rainbow trout (*S. gairdneri*) were placed in each tank, 10 of which were treated fish and 10 control fish. This was done to eliminate any tank effect during the course of the trial. Throughout the first trial, which lasted 12 weeks, tanks 1-3 were fed a diet high in fat while tanks 4-6 were fed a diet high in sucrose (as a digestible carbohydrate). Feed for each tank was weighed out daily and fed according to the required percentage of body weight based on fish size and water temperatures. Feeding levels ranged between 1.5 and 3.0% of the live weight daily. Fish were fed twice per day at low feeding levels and 3 times per day at high feeding levels. At three week intervals during the trial fish were anesthetized (with MS-222), weighed, and measured and average gains were calculated for each group (17aMT-treated and control) and for each tank. During the first feeding trial water temperatures ranged from 6°C to 12°C.

On the days of the sixth and ninth week length and weight measurements, one treated fish and one control fish from each tank was bled. Fish were fed four hours prior to

bleeding. Fish were returned to appropriate tanks after bleeding. Blood was taken from the cardinal vein (caudal area) with a syringe coated with heparin. Plasma was obtained for each fish through centrifugation and separation from cells. Values for plasma glucose and ammonia were obtained through Sigma diagnostic procedures (# 15-UV for glucose, # 170-UV for ammonia) for the quantitative enzymatic determination in plasma with the use of a B&L spectrophotometer (Spectronic 70). Results were compared for each tank and each fish. Upon termination (12 weeks), each fish was weighed, measured, bled with heparinized syringes and sacrificed. Feed conversions were calculated at specified intervals and at termination. Fish were starved for more than 12 hours prior to bleeding. Blood was taken and plasma obtained with the same technique as was used earlier in the trial. After sacrificing fish, visceral fat of each fish was weighed for comparisons between groups and the sex of each fish was determined in order to judge the effect of the 17aMT treatment administered earlier in life. Blood glucose and blood ammonia was then determined for each fish in each tank using Sigma diagnostic procedures (#s 15-UV and 170-UV) and spectrophotometer as before. For the first feeding trial fish were not marked individually and therefore individual differences for actual growth and relative growth (gain/initial weight) could not be determined. Only total

values and average values for each group and each tank could be analyzed. Data was analyzed using Analysis of Variance (ANOVA) and mean separation techniques (Least Significant Difference- LSD) at .05 level of significance.

For the second feeding trial, 24 rainbow trout were placed in each tank, 12 of which were treated fish and 12 of which were control fish. AT the time of initial measurements, each fish was marked using a freeze-branding technique (Mighell, 1969; Sorenson et al., 1983) to allow for individual differences to be obtained and individual statistical analysis techniques to be used. Four brand orientations and three locations on the fish were used, giving 12 recognizable brands per tank. Taking into account that treated fish were distinguishable by their severed adipose fin, this facilitated the use of 24 different brands (one per fish) for each tank. The branding mechanism was easily built and the brands applied after fish were anesthetized (see appendix I).

In the second feeding trial, a molasses diet was incorporated, with molasses providing the digestible CHO as opposed to sucrose. In this trial two tanks were fed a high fat diet (tanks 1 and 4), two tanks were fed a high sucrose diet (tanks 2 and 5) and two tanks a high molasses diet (tanks 3 and 6). As before, food for each tank was weighed out daily and fed according to the required percentage of body weight based on fish size and water

temperature. Feeding levels ranged between 1.5 and 3.0% of the live weight daily and fish were fed two or three times daily. During this trial length and weight determinations were made every three weeks from the start to finish. Fish were anesthetized, weighed and measured and returned to respective tanks as soon as possible. Brands were identified at this time in order to show gains of individual fish. Feed conversion was calculated and feeding percentage readjusted for each tank. During the second feeding trial water temperatures ranged from 8°C and 14°C.

During the sixth and ninth week length and weight measurements, blood was taken from two fish (one treated, one control) per tank and plasma obtained and analyzed for glucose and ammonia as was done in the first trial. Results were again compared for each tank and each diet. As with the first trial, upon termination (12 weeks) fish were weighed, measured, bled from the caudal vein with heparinized syringes, and sacrificed. Feed conversions, for the last growth period and overall, were calculated. Analysis of growth data and calculation of overall feed conversions for the three feeds and treated versus control fish was done at nine weeks for the second trial. This was done because after nine weeks fish became anorexic (poor appetite) and sick, with a few deaths occurring. Visceral fat was weighed for each fish to compare results between

diets and treatments and fish were sexed to determine the effect of 17aMT treatment given to the fish earlier. Plasma glucose and ammonia was once again determined and results used for statistical comparisons. Because of individual fish records, growth data could be analyzed more completely by computing actual and relative growth rates (gain/initial weight) for each fish. This and other data was analyzed through ANOVA and LSD methods.

Feed Formulations

Feeds were prepared using a ground herring fish meal "cake" product (supplied by Stinson Canning Co., Bath, Maine, 1984). This herring cake was pre-treated with preservatives and an anti-oxidant (santoquin) prior to use. Part (1/2 to 2/3) of this initial ground herring was washed with commercial grade hexane in order to extract fat from the product. The hexane was then decanted and the remaining product allowed to desolventize at ambient temperature, hence reducing the fat content of the ground herring. These high and low-fat ground fish products were then used as a base to which other ingredients were added. In the first feeding trial ground fish, casein, fish oil, alpha-cellulose (as a filler), water and gelatin (as a binder) were the ingredients used in both diets, in varying amounts. Granulated sugar was used as a source of sucrose

in the high sucrose diet (Table I). To both formulations vitamin C (as ascorbic acid) and thiamine (HCL) were included. All components of the diets were mixed (1Kg/batch) with a Hobart automatic mixer (Model # c-100-T) for 10-15 minutes, a period long enough to blend all ingredients evenly. The mixture was then extruded through an appropriate sized die to create the desired pellet size, and fed at the required level. Diets were calculated and formulated to be isocaloric and isonitrogenous (Table II).

In the second feeding trial a diet with a high molasses content was compared with the high fat and high sucrose diets. To facilitate the formulation of a molasses diet a ground fish base product was needed which was very high in protein content and low in fat and moisture content. This ground fish product was made by grinding up whole herring and treating with propionic and acetic acids (as preservatives) and an anti-oxidant (santoquin) to prevent oxidation of fats. The resulting high-fat fish meal was then mechanically pressed and air dried to reduce moisture, followed by washing with hexane several times to extract much of the fat. This produced the high protein, low fat and low moisture fish meal base to be used in the molasses diet formulations. In the second feeding trial, all of the diets contained ground fish meal, casein, fish oil and a binder (carboxy-methyl cellulose). To the high fat and sucrose diets water was also added, and

alpha-cellulose was used as a filler in the molasses diet. Granulated sugar in the sucrose diet and "blackstrap" molasses in the molasses diet were added in an amount to provide an equal number of calories in both diets. All dietary ingredients (Table III) were mixed and extruded into feeding pellets of the appropriate size, as in trial #1. All diets were isonitrogenous and isocaloric (Table IV).

Table I - DIETARY INGREDIENTS - Trial #1

	<u>High-Fat Diet</u>	<u>Sucrose Diet</u>
<u>Ingredients</u>	<u>gm/Kg diet</u>	<u>gm/Kg diet</u>
herring meal*	493.5	459.0
casein	81.2	81.2
gelatin	30.0	30.0
herring oil	111.1	92.0
sucrose	----	235.0
a-cellulose	100.0	50.0
water	184.2	52.8

total	1000	1000

protein	40.0%	40.0%
lipid	24.1%	15.0%
carbohydrate		
(sucrose)	0.0%	23.5%
a-cellulose	10.0%	5.0%
water	18.4%	5.3%
other**	7.5%	11.2%

* = herring meal cake (HMC) compositions (given below)

<u>ingredients</u>	<u>high-fat HMC</u>	<u>low-fat HMC</u>
protein	60.8%	64.4%
fat	26.4%	12.7%
moisture	11.4%	14.3%
ash	1.4%	7.6%

**--includes water, ash, etc. from herring meal

Table II - ENERGY CONTENT OF DIETS (Kcal/Kg)
Trial #1

	gm/Kg of diet fed	grams available to fish	energy/ gram (Kcal)	total energy obtained (Kcal)
<u>High-Fat Diet</u>				
protein	400.0	400.0	3.9	1560.0
fat	241.0	241.0	9.0	<u>2170.0</u>
total				3730.0
<u>Sucrose Diet</u>				
protein	400.0	400.0	3.9	1560.0
fat	150.0	150.0	9.0	1350.0
sucrose	235.0	200.0*	4.1	<u>820.0</u>
total				3730.0

* = sucrose is only 85% digestible by salmonids

Table III - DIETARY INGREDIENTS - Trial #2

<u>Diets-></u>	High-Fat	Sucrose	Molasses
<u>Ingredients (gm/Kg diet)</u>			
herring meal ⁴	500.0	466.5	450.0
casein	149.0	149.0	50.0
herring oil	170.1	100.5	53.1
sucrose	-----	258.2	-----
molasses ¹	-----	-----	420.0
binder ²	10.0	10.0	10.0
a-cellulose	-----	-----	16.9
water	170.9	15.8	-----
total	1000	1000	1000
protein	35.0%	35.0%	35.0%
lipid	24.1%	14.1%	14.1%
carbohydrate			
(sucrose)	-----	25.8%	25.2%
a-cellulose	-----	-----	1.7%
water	36.6%	20.3%	15.4%
other ³	4.3%	4.8%	8.6%

¹=molasses contains ~60.0% sucrose, 5.0% protein and 26.0% moisture.

²=the binder, carboxy-methyl-cellulose, is added dry, directly to each diet.

³=includes water, ash, etc. not accounted for.

⁴ = compositions of herring meal cake used in:

ingredients	high-fat diet	sucrose diet	molasses diet
protein	43.2%	46.3%	63.1%
fat	14.2%	8.7%	19.6%
moisture	39.0%	40.0%	10.0%
ash (~)	3.6%	5.0%	7.4%

Table IV ENERGY CONTENT OF DIETS (Kcal/Kg)
Trial #2

	gm/Kg of diet fed	grams available to fish	energy/ gram (Kcal)	total energy obtained (Kcal
<u>High Fat Diet</u>				
protein	350.0	350.0	3.9	1365.0
fat	241.1	241.1	9.0	<u>2170.0</u>
total				3535.0
<u>Sucrose Diet</u>				
protein	350.0	350.0	3.9	1365.0
fat	141.1	141.1	9.0	1270.0
sucrose	258.2	219.5*	4.1	<u>900.0</u>
total				3535.0
<u>Molasses Diet</u>				
protein	350.0	350.0	3.9	1365.0
fat	141.1	141.1	9.0	1270.0
sucrose	252.0**	214.2*	4.1	<u>878.3</u>
total				3513.3

* = sucrose is only 85% digestible by salmonids

** = molasses contains about 60% sucrose;

420 grams of molasses @ 60% sucrose = 252g sucrose

RESULTS

Effects of Methyl-Testosterone Treatment

The effects of MT treatment on experimental fish, as described in the methods and materials section, are shown in Table V for fish from both feeding trials. In the first trial, 31.2% of the fish were described as sterile, 53.0% as underdeveloped males and 12.3% as underdeveloped females while only 3.5% had fully developed gonads of either sex. In the second trial the trend continued to show a pronounced effect of MT treatment on sexual development, with 10.0% of the fish considered sterile and 83.0% of the fish showing under-developed gonads. Only 7.0% of treated fish had fully developed gonads which were easily identified. All control fish (100%) were fully developed in both trials and were sexed with ease. Male:female sex ratio in control fish favored the female 1:1.5, while in treated fish which were identified the sex ratio was 3:1. Total effects of treatment (Table V) are shown graphically in Figure 1. Overall, 20.6% of treated fish were judged to be sterile, 45.2% underdeveloped males, 27.8% underdeveloped females, 4.8% developed males and 1.6% developed females. Histological examination of treated (35#) and control (15#) fish gonads from fish of the same spawning group as those used in both trials gave results

Table V - The Effect of Treatment With 17a-methyl-testosterone on the Sexual Development of Rainbow Trout (numbers given are % of total number of fish)

	** Sterile	Undev-M	Undev-F	Full M/F	M:F Ratio
<hr/>					
Trial #1					
Treat	31.2%	53.0%	12.3%	3.5%	5:1
Contr	---	---	---	100.0%	1:2.2
Trial #2					
Treat	10.0%	38.4%	44.7%	7.0%	1:1
Contr	---	---	---	100.0%	1:1.1
<hr/>					
Total					
Treat	20.6%	45.7%	28.5%	5.25%	3:1
Contr	---	---	---	100.0%	1:1.5

Sterile = no evidence of gonadal material, cannot be identified

Undev-M = underdeveloped male-testis rudimentary and difficult to identify

Undev-F = underdeveloped female-egg follicle rudimentary, difficult to identify

Full M/F = fully developed male or female-sexes easily differentiated

M:F Ratio = male : female ratio

Figure 1 Overall effect (Trial #s 1&2) on rainbow trout sexual development of treatment with 17 α -methyltestosterone early in life (shown as a percentage of total population).

STERILE = no gonadal material present; cannot be identified grossly.

UD = underdeveloped male or female; difficult to identify.

FD = fully developed male or female; easily identified.

which generally agreed with those obtained from gross visual inspection. All treated rainbow trout which were examined were at only initial stages of development or contained only connective tissue and/or primordial germ cells. The only fully-developed, functional males were found in the control fish which were examined. Females at several stages of development were found in both treated and control groups.

Trial I--Sucrose vs. High Fat Diets

Actual mean weight gain, mean length gain and feed, conversion were calculated for each diet and for treated and control fish within each diet. These are shown in Table VI and increase in mean weight gain over time is demonstrated in Figure 2. The difference in weight gain was found to be significantly different between feeds and for the interaction between feeds and treatment. The sucrose diet had gains significantly ($P < 0.05$) better than did the high fat diet. Statistical analysis also showed that the interaction of treated fish with the sucrose diet gave significantly ($P < 0.05$) better growth than either control or treated fish on high fat diets. Treated fish showed improved performance on the sucrose diet, though not significantly better than sucrose controls. Overall, the treatment alone had no significant effect on growth at

Table VI - Mean growth parameters for treated and control rainbow trout after feeding for 12 weeks with high-fat and sucrose diets - Trial #1

	initial wt/ final wt (grams)	actual gain (grams)	relative gain ¹	actual length gain (cm)	feed conversion (protein) ²
<u>High-Fat Diet</u>					
Treated	17.2/ 77.6	60.4+	3.51+	7.03	1.50++ (26.7%)
Control	19.8/ 90.6	70.7+	3.56+	7.57	1.28++ (31.2%)
Total (mean)	18.6/ 84.4	65.8*	3.54	7.30*	1.39* (29.0%)
<u>Sucrose Diet</u>					
Treated	20.5/ 102.5	82.0+	4.01+	8.02	1.24++ (32.3%)
Control	22.4/ 100.5	78.2	3.51+	7.97	1.28++ (31.3%)
Total (mean)	21.4/ 101.5	80.1*	3.74	8.00*	1.26* (31.8%)

¹ = relative gain = gain (grams) / initial weight (grams)

² = protein conversion = % protein efficiency

* = fish fed sucrose diet had significantly better ($P < 0.05$) actual weight gain, length gain and feed conversion than did those fed high-fat diet

+ = treated fish on the sucrose diet had significantly improved ($P < 0.05$) actual weight gain over treated and control fish fed the high-fat diet; sucrose fed treated fish had significantly improved ($P < 0.05$) relative weight gain over all other groups

++ = treated fish fed the high-fat diet had the poorest feed conversion; the remaining groups were not significantly different ($P < 0.05$)

Figure 2 Actual gain (grams) of treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks. Trial #1.

$P < 0.05$. There was a significant difference ($P < 0.05$) in mean length gain only between the two diets, with the sucrose diet giving better linear growth. The feed conversion data shown in Table VI demonstrates that fish on the sucrose diet had significantly improved ($P < 0.05$) overall feed conversions than those fed the high fat diet. An interaction effect is also in evidence, with treated fish doing better on the sugar diet than on the high fat diet ($P < 0.05$). Treated fish fed the sugar diet also had better feed conversions than control fish fed the sugar diet, though not significantly so. Figure 3 graphically illustrates mean feed conversion values for all groups. Protein conversion efficiencies were naturally also better in treated fish on the sugar diet because all of the diets were iso-nitrogenous (equal protein levels).

Relative growth or gain (gain/initial weight), shown in Table VI, was also determined for treated and control fish on each diet. This was done to account for varying initial (starting) weights of each animal and how this might affect growth in each group. Relative gain (gain/initial weight) calculations show that there were significant differences between diets (sucrose diet was superior) and between treated and control fish (treated was superior) at $P < 0.05$ level of significance. The effect of interaction between treatment and diets was significant ($P < 0.05$) and showed that treated fish on the sucrose diet

Figure 3 Feed conversion (g. fed/g. gained) and protein conversion (%) of treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks. Trial #1.

Figure 4 Relative gain (gain/initial wt) of treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks. Trial #1.

Figure 5 Treatment and feeds interaction effect on actual weight gain and feed conversion levels in treated and control rainbow trout fed high-fat and sucrose diets for 12 weeks. Trial #1.

grew better (taking into account initial size) than did treated fish on the high fat diet. Treated/sucrose fish also gained better than control fish on both sugar and high fat diets. Figure 4 shows relative growth over time for each group. Figure 5 shows graphically the interaction effects of some of the growth parameters. Condition factor was calculated for each group based on mean data and was not found to be significantly different between diets or treatments.

Individual measurements were used in determining visceral fat levels (% body weight) and blood plasma glucose differences between groups. Results indicated that the blood glucose of treated fish at termination was significantly lower ($P < 0.05$) than that of control fish in both the high fat and sucrose diet groups. The mean blood glucose value for treated fish was 65.6 mg glucose/ml plasma, while that of control fish was 82.7 mg/ml plasma. For all treatment/feed interactions, means for plasma glucose were significantly different from one another at $P < 0.05$ level of significance. visceral fat of treated fish was significantly lower ($P < 0.05$) than that of control fish in the first feeding trial. Means calculated from individual measurements of fish visceral fat showed that treated fish had a lower visceral fat weight:body weight ratio (0.012:1 or 1.2% of body weight) than did control fish (0.017:1 or 1.7% of body weight). There was no

significant difference (up to $P < 0.10$) between visceral fat levels (%) of fish fed sugar and high fat diets. With respect to interaction effects, using mean values of visceral fat, none of the treatment/feed combinations were significantly different from one another. Mean values for blood plasma glucose and visceral fat (% body weight) are tabulated in Table VII and illustrated graphically in Figure 6. No correlation was found between blood plasma ammonia levels and blood plasma glucose levels or between plasma ammonia levels and treatment or diets. There was no significant difference between fish blood ammonia on different diets, treatment or any interaction thereof.

Trial II-Sucrose vs Molasses vs High Fat Diets

Actual weight gain and length gain were determined for each fish and mean values were calculated along with feed conversion for both treated and control fish on each diet. Growth data and feed conversions for the second trial were calculated after nine weeks of feeding because of poor performance of fish in four of the six experimental tanks from weeks 10-12. This is due, it was believed, to a contaminant in two of the experimental diets. After nine weeks of growth, both sucrose and molasses diets gave significantly ($P < 0.05$) better weight gain than the high fat

Table VII - Plasma glucose and visceral fat levels of treated and control rainbow trout after feeding for 12 weeks with high-fat and sucrose diets - Trial #1

	(mg/100ml) <u>Plasma Glucose</u>	(% body weight) <u>Viseral Fat</u>
<u>High Fat Diet</u>		
Treated	53.81	1.22
Control	57.75	1.70
Total (mean)	55.78*	1.46+
<u>Sucrose Diet</u>		
Treated	77.39	1.18
Control	107.69	1.66
Total (mean)	92.54*	1.42+
Treatment mean	65.60**	1.20++
Control mean	82.70	1.68

* = means for plasma glucose are significantly different ($P < 0.05$) between diets

** = plasma glucose for treated fish is significantly lower ($P < 0.05$) than that for control fish

+ = means for visceral fat % are not significantly different ($P < 0.05$) between diets

++ = visceral fat % for treated fish is significantly lower ($P < 0.05$) than that for control fish

diet. Sucrose and molasses diets were not significantly different from one another with respect to actual weight gain. The treatment with MT had no significant effect on actual weight gain in any of the diets. All diets were significantly different ($P < 0.05$) from one another with respect to mean overall length gain. But treatment had no significant effect on length gain, nor did interaction of treatment and different feeds affect length gain. Feed conversions of the molasses and sucrose diets were much better and significantly different ($P < 0.05$) from those obtained for the high fat diet. Values for actual mean weight gain, mean length gain and feed conversions are shown in Table VIII. Figure 7 illustrates weight gain over time for control and treated fish within each diet. Feed conversion data is represented in Figure 8.

Mean relative gain (weight gain/initial weight) for treated and control groups fed the three diets was calculated in the second trial by averaging individual relative gains for each fish (Table VIII). After nine weeks of feeding, there was a significant difference ($P < 0.05$) in relative growth between both feeds and treated versus control groups. Interaction of the two variables (feed and treatment) was also found to have a significant effect on relative gain at $P < 0.01$. The diet high in fat gave the poorest average relative gain (ave=1.16), while the molasses diet gave the best results (ave=1.53). The

sucrose diet (ave=1.44) did not do as well as the molasses diet but showed a significantly better response than the high fat diet. The molasses diet fish had significantly better ($P<0.05$) relative growth than either of the other two diets. Mean separation tests (LSD) showed that treated fish fed the molasses diet had better relative growth than any of the other interaction means. Treated fish on the sucrose diet had significantly better relative growth than treated fish on a high fat diet, as was the case with control/sucrose versus control/ high fat fish. Overall, treated fish on a diet high in digestible CHO's exhibited better relative gains than any other group (Table VIII). Figure 9 illustrates the effect of relative growth over time for treated and control fish on each diet.

Interaction effects of some growth parameters are shown in Figure 10. Calculation of fish condition factor yielded no significant differences between diets or treatments.

Mean values for blood plasma glucose and visceral fat levels (% body weight) were determined for both treated and control fish fed each diet. Statistical analysis of mean plasma glucose levels showed that there was a significant difference between the different feeds ($P<0.05$) and mean separation tests (LSD) showed that both the sucrose and molasses diets were significantly higher in glucose than the high fat diet. The sucrose and molasses diets were not significantly different from one another in plasma glucose

Table VIII - Mean growth parameters for treated and control rainbow trout after feeding for 9 weeks with high-fat, sucrose and molasses diets - Trial #2

	initial wt/ final wt ¹ (grams)	actual gain (grams)	relative gain ²	actual length gain (cm)	feed conversion (protein) ³
<u>High Fat Diet</u>					
Treated	56.8/ 121.6	65.3	1.16+	5.33	1.425 (32.0%)
Control	52.1/ 111.2	59.1	1.15	5.23	1.56 (29.3%)
Total (mean)	54.5/ 116.4	62.2*	1.16**	5.28**	1.49++ (30.7%)
<u>Sucrose Diet</u>					
Treated	57.6/ 139.9	82.3	1.44+	5.83	1.17 (39.0%)
Control	55.1/ 133.0	78.3	1.44	5.95	1.23 (37.2%)
Total (mean)	56.3/ 136.6	80.3*	1.44**	5.89**	1.20++ (38.1%)
<u>Molasses Diet</u>					
Treated	52.5/ 134.0	81.55	1.61+	6.22	1.19 (38.0%)
Control	56.9/ 138.4	81.51	1.45	5.85	1.18 (37.2%)
Total (mean)	54.7/ 136.2	81.53*	1.53**	6.04**	1.183++ (37.6%)

¹ = final weight at 9 weeks

² = relative gain = gain (grams)/initial weight (grams)

³ = protein conversion = % protein efficiency

* = sucrose and molasses diets gained significantly better ($P < 0.05$) than did the high-fat diet

** = all diets were significantly different ($P < 0.05$) from the other two diets in length gain and relative weight gain (molasses and sucrose were superior)

+= treated fish fed molasses showed significantly improved relative gain over all other groups; sucrose > high-fat

++= molasses and sucrose diets gave significantly better ($P > 0.05$) feed conversions than the high-fat diet

1. FAT/TREATED
 2. FAT/CTRL
 3. SUCROSE/TR
 4. SUCROSE/CTR
 5. MOLASSES/TR
 6. MOLASSES/CTRL

Figure 7 Actual gain (grams) of treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks. Trial #2.

Figure 8 Feed conversion (g. fed/g. gained) and protein conversion (%) of treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks. Trial #2.

values. Blood plasma glucose values of treated fish were not significantly different from those of control fish. Measurement of visceral fat as a percentage of body weight resulted in significant differences ($P < 0.05$) between feeds with only the sucrose diet being significantly lower in % visceral fat than the other two diets. There were no significant differences in visceral fat levels between treated and control groups, but treated fish did have lower mean levels of visceral fat (1.021%) than did non-treated fish (1.0286%). Average results for visceral fat % and blood plasma glucose levels are shown in Table IX and in Figure 11. Ammonia levels in blood plasma showed no correlation with any other factors, with no significant differences in levels between treatment or diets.

In a related feeding study, using three commercial pelleted diets which contained varied levels of carbohydrate, treated and control rainbow trout were individually marked using a freeze-branding technique and fed for 12 weeks under stringent controls (Smith and Ahern, 1985). Results from the study, agreeing with some of those obtained from this thesis research, showed that treated fish outperformed controls in all parameters measured and did the best on the diets highest in carbohydrates.

Table IX - Plasma glucose and visceral fat levels of treated and control rainbow trout after feeding for 9 weeks with high-fat, sucrose and molasses diets - Trial #2

	(mg/100ml) <u>Plasma Glucose</u>	(% body weight) <u>Visceral Fat</u>
<u>High Fat Diet</u>		
Treated	68.78	1.13
Control	58.95	1.03
Total (mean)	63.87*	1.08+
<u>Sucrose Diet</u>		
Treated	96.07	0.85
Control	89.10	1.01
Total (mean)	92.59*	0.93+
<u>Molasses Diet</u>		
Treated	100.79	1.08
Control	103.82	1.05
Total (mean)	102.30*	1.07+
Treatment mean	88.55**	1.02++
Control mean	83.96**	1.03++

* = plasma glucose significantly lower ($P < 0.05$) for high fat diet as compared to other two diets

** = plasma glucose is not significantly different ($P < 0.05$) between treatments

+ = visceral fat is significantly lower ($P < 0.05$) for sucrose diet as compared to other two diets

++ = visceral fat is not significantly different ($P < 0.05$) between treatments

Figure 9 Relative gain (gain/initial wt) of treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks. Trial #2.

Figure 10 Treatment and feeds interaction effect on actual weight gain and feed conversion in treated and control rainbow trout fed high-fat, sucrose and molasses diets for 9 weeks. Trial #2.

Feed Formulation Results

All feeds were produced by identical methodology (see Methods and Materials section). Physical properties of all feed pellets were adequate for use in fish culture systems. The sucrose and molasses diets showed the better physical characteristics, dropping slowly through the water column and eventually settling on the bottom of the tank. All diets were palatable to the fish, as they fed vigorously at feeding time, eating even pellets which had settled to the bottom of the tank. The high fat diet was the least desirable of the three diets, with a portion often floating on the water surface. Observation indicated that this portion was also eventually eaten. The high fat diet produced the greatest amount of physical solids waste while the sucrose diet produced the least. The molasses diet was intermediate in solids production and produced a water discoloration immediately after feeding. This cleared though, within 1/2 hour after feeding and had no noticeable affect on performance of the diet. Solid waste production seemed directly related to the amount of undigestible CHO (filler) in the diet; a diet with no filler produced no waste. Proper addition of anti-oxidants and preservatives extended the shelf life of each product (feed) over the duration of each experiment.

DISCUSSION

Administration of alpha-methyltestosterone at a dose of 50.0 mg/Kg of diet at the onset of feeding to rainbow trout appears to produce the desired result of sexual sterility to these fish. Almost one year after removal of MT from the diet of the fish, gross (and histological) examination of gonads revealed that the great majority (66.3%,) of treated fish were considered either sterile or underdeveloped (and non-functional) males. Gonads seen in the majority of treated fish were much less developed than those of controls. The sex ratio of those fish positively identified male or female heavily favored the male (3:1- Table V). Other investigators have obtained comparable results in various *Salmo* species (Jalabert et al., 1975; Johnstone et al., 1978; Harbin et al., 1980; Donaldson and Hunter, 1982; Schreck and Li, 1983). Control fish, on the other hand, were 100% identifiable as males or females and had a more balanced sex ratio (1:1.5) as compared to treated fish, and favored the female. This may indicate sex reversal from female to male when complete sterility did not occur with MT treatment. Also in agreement with previous research (Higgs et al., 1982; Rao et al., 1983; Smith, 1983), it appeared that overall, treated fish performed better in both feeding studies as compared to

controls, long after elimination of MT from the diet. In a more detailed study on the long term effects of MT treatment in fish, it is suggested that other measurements might be made, such as circulating serum testosterone and plasma gonadotropin (Gth) levels and calculation of gonadosomatic index differences between treated and control groups (Billard et al., 1981; Sundararaj et al., 1982). These parameters of sexual reproductive potential would give a good indication of how well the MT treatment worked. In a study related to the feeding trials of the present study using three commercial pelleted diets fed to treated and control fish, results indicated the superiority of treated fish over controls for all growth measurements.

The use of glucose, sucrose and more complex CHO's in salmonid diets has given mixed results. Investigators have demonstrated that more complex CHO's such as starch are poorly digested by salmonids (Edwards et al., 1977; Spannhof and Plantikow, 1983), resulting in poor growth and feed conversion. Other researchers though, have shown that restriction of intake and gelatinization of starch allows for better digestion and hence better results (Pieper and Pfeffer, 1980 b; Bergot and Breque, 1983). The inclusion of the disaccharide sucrose in salmonid diets, as the present trial indicates, can yield increased growth and improved feed conversion results. In developing countries sucrose is less costly than in the U.S., where the price is

artificially high due to government subsidization, and the use of this sugar as a fish feed additive may preclude such costly techniques as cooking or gelatinization to make the CHO more digestible. A molecule of sucrose, upon digestion, yields one glucose molecule and one fructose molecule. Hence 50% of the CHO supplied in the diet is digested to fructose which is readily taken up at the cellular level and entered into glycolysis without the use of insulin, which is usually at minimal levels in fishes. The glucose supplied by the sucrose in the diet requires the aid of insulin for utilization. Treated (MT, sexually sterile) fish were included in each trial to determine the effect of treatment on glucose utilization in these fish, and how this would influence growth.

Addition of glucose to salmonid diets has given conflicting conclusions. Bergot (1979a) stated that maximal growth and feed conversion can be achieved at glucose levels as high as 30% of the diet. He also stated, though, that a depressive effect on food intake can occur at high dietary glucose levels. In the present study no such anorexic effect was observed in either the high-sucrose or high-molasses diets; aggressive feeding occurred throughout the feeding periods. Hilton et al (1982) suggest that the maximum tolerable level of glucose in salmonid diets appears to be dependent on protein, lipid and overall energy content of the diet, and may be in

excess of 25% of the diet for rainbow trout. Another study indicated that rainbow trout have a limited ability to adapt to increased dietary CHO, and levels in excess of 14% of the diet is not efficiently utilized (Hilton and Atkinson, 1982). From the data and results presented in this paper, it is clear that in all cases utilization of gross energy, provided in equal amounts in all diets, was much better in fish fed high-CHO (sucrose and molasses) diets as opposed to high-fat diets. This agrees with studies by Pieper and Pfeffer (1980a) which showed increased utilization of dietary gross energy in rainbow trout fed a high-sucrose diet over those fed diets high in glucose, gelatinized starch, and even sunflower oil. These authors believe that sucrose and other digestible dietary CHOs show a certain superiority with respect to sparing of dietary protein as compared to lipids. They also showed that digestion is not a limiting factor for these CHOs in trout, because they were more efficiently utilized than glucose. By feeding sucrose as opposed to glucose directly, a sudden flux of glucose into the fish's metabolic system may be prevented, hence avoiding the negative physiological effects of such a flux. Rainbow trout fed a 30% glucose diet (Bergot, 1979b) quickly developed (within 6 hours) a pronounced glycemia after feeding.

In the first feeding trial (sucrose vs high-fat),

though equal amounts of metabolizable energy were provided in each diet, the high-sucrose diet outperformed the high-fat diet in all respects. Fish (treated and control combined) fed the high-sucrose diet showed significantly improved actual weight, relative weight gain (gain/initial weight), length gain and feed conversions (Table VI; Figures 2,3,4). In sucrose fed fish, both actual weight gain and relative weight gain were significantly enhanced. Relative gain was the highest for treated fish on the sucrose diet, perhaps indicating better CHO utilization in this group. Means for overall feed conversion and protein conversion efficiencies were much improved in sucrose fed fish (1.25) than those fish fed the high-fat diet (1.39).

Though plasma glucose levels were significantly higher in sucrose fed trout (Table VII, Figure 6) as compared to high-fat fed trout, levels were not abnormally high (ave~92.5 mg/100ml plasma), an indication that good digestion and rapid assimilation of dietary sucrose occurred. Normal plasma glucose levels in salmonids a few hours after feeding is between 80-100 mg per 100ml of plasma. Measurements of visceral fat (% of body weight-Table VII, Figure 6) yielded no significant differences between the two diets, indicating that dietary sucrose was used by the fish to fulfill daily energy requirements and was therefore not stored as visceral fat. Visual inspection of fish livers was made at necropsy and

no distinct gross differences were observed between fish fed the two diets. The incidence of low visceral fat levels in sucrose fed fish may signify that sucrose is being used energetically as efficiently or better than herring oil in supplying metabolizable energy to the fish. Sucrose may be more efficient in sparing dietary protein, which in turn could be used for tissue building processes instead of merely as a highly expensive dietary source of energy. Pieper and Pfeffer (1980a) arrived at identical conclusions in their work with rainbow trout. These results, and considerations of monetary aspects such as the high cost of quality oils in some areas, should lead to an increased use of highly digestible CHO's in salmonid and other cultured fish diets. Because the two diets were isonitrogenous, fish fed the sucrose diet naturally had more efficient protein conversions.

In the second feeding trial, where molasses was incorporated as an alternate digestible CHO source, both of the high CHO diets demonstrated significantly increased actual weight gain and actual length gain than the high fat diet after nine weeks of feeding rainbow trout (Table VIII, Figure 7). Calculation of relative gain (Figure 9) for this feeding trial indicated that both of the high-CHO diets had significantly improved gain than the high-fat diet. The molasses diet also showed higher relative gain than the sucrose diet in this regard, due to the superior

growth of treated fish on the molasses diet. Therefore with respect to relative gain, molasses (1.53) > sucrose (1.44) > high-fat (1.16). Relative gain was used as an indicator of growth in order to account for variant initial weights of treated and control fish at the start of the experiment and rule out any advantage larger fish might have.

Over the nine week period of the second trial feed conversion efficiencies and hence, protein conversion efficiencies, were superior in the high-CHO diets (Table VIII, Figure 8). Overall feed conversion of the high fat diet was 1.47 while those of the high-sucrose and molasses diets were 1.20 and 1.18 respectively. These differences may be due to increased utilization of the CHOs in the sugar and molasses diets by treated fish. These results coincide with the high actual and relative weight gains of the fish fed these diets. Calculation of condition factor for the three diet groups yielded no significant differences, indicating that length:weight ratio was not influenced by diet. Therefore visual appearance and market value of fish would not be affected by feeding a high sucrose or high molasses diet. Blood glucose and visceral fat levels were also calculated for the second trial (Table IX, Figure 11). Visceral fat levels were the highest for those fish fed the high-fat diet, indicating that CHO utilized in the diet was not laid down as visceral fat but

was used as metabolizable energy to meet the day to day energy needs of the fish. In agreement with the first feeding trial, fish fed the high-CHO diets had higher plasma glucose levels than those fed the high-fat diets, though values were not abnormally high (~97mg/100ml of plasma). This once again indicates increased utilization of dietary CHO after digestion occurs.

Results from the two feeding trials presented here almost conclusively show that fish diets high in digestible and utilizable CHOs such as sucrose and molasses give growth performance better than a high lipid (fish oil) diet of comparable caloric quantity and quality. Herring oil, the dietary lipid used in this study, has been used successfully in high caloric amounts in sparing dietary protein (Kellems and Sinnhuber, 1982) but this feed additive may be prohibitively costly or unavailable in many areas. The present study agrees with other research (Pieper and Pfeffer, 1980a and 1980b) in demonstrating the superiority of sucrose (molasses is 50-60% sucrose) over lipids as a protein-sparing dietary energy source. Molasses appears to show the same effects in this regard, as results from the second feeding trial would indicate. Incorporation of molasses into fish diets has not been researched prior to this work and therefore literature reviewing this aspect is unavailable.

Sucrose has been demonstrated to be a superior feed

additive over glucose and starch also in the tilapia, *Oreochromis niloticus* (Anderson et al., 1984). These results along with those given in other studies using rainbow trout (Pieper and Pfeffer, 1980a) indicate that, contrary to Edwards et al (1977), sucrose is more efficiently absorbed and utilized than glucose in these important aquacultural species. The data presented in this thesis research tends to correlate and complement the view that sucrose is indeed a superior feed additive for providing dietary calories to the fish. Molasses, containing only 50-60% sucrose, must be added in higher amounts to fish diets to provide equal caloric value. But this waste product of the sugar refining industry is relatively inexpensive in most areas and is palatable to the fish, making it also practical for aquacultural use. The use of these quality CHO ingredients along with the cheaper, high quality cooked or uncooked protein mixes which Robinson et al (1985) investigated, might provide the aquaculture industry with an inexpensive and high performance fish feed.

The availability and cost practicality of sucrose, molasses and other highly digestible CHOs such as brewers solids, makes the possibilities for use of these products as fish feed ingredients near limitless. The evidence submitted here and in other studies has shown the advantages of using such additives in any fish diet

formulation. Diets using these ingredients are easily prepared, can produce a good feeding pellet, and will provide physical properties superior even to high-fat preparations. Incorporation of these products produces a feed which is highly palatable to the fish and gives more than adequate results as compared to conventional pelleted feeds used today.

The effect of MT treatment on fish used in these feeding trials appeared to elicit the desired response to the different diets. In all cases growth parameters indicated that treated fish did much better on a high-CHO diet than on a high fat diet. In the first feeding trial, MT-treated fish had much poorer mean feed conversions when fed the high-fat diet (1.50) than when fed the sucrose diet (1.24) and also did better than control fish fed either the high-fat or sucrose diets (1.28). The same was true with respect to actual weight gain in the first trial. Treated fish fed the sucrose diet showed significantly better gains than any other group. Plasma glucose levels were significantly lower in treated fish on both diets, which suggests improved glucose uptake at the cellular level. Percentage of visceral fat was significantly lower for treated trout (~1.20%) than for control fish (~1.68%), another indication that the treatment given earlier in the fish's life has had some effect on dietary CHO utilization, and the laydown of visceral fat. The first feeding trial

clearly indicated an advantage of using MT-treated trout over non-treated trout when feeding a digestible carbohydrate diet.

In the second feeding trial, results did not show such a clear-cut advantage of treated fish over untreated but, generally growth parameters were better for MT-treated fish than controls. Feed conversions were slightly improved overall (though not significantly so) and actual weight gain was increased for treated fish fed all of the different diets (also not significant). With respect to relative weight gain, treated fish fed the molasses diet showed significantly improved performance over all other groups. This agreed with results obtained for relative gain in the first trial, using sucrose as the sole CHO source. Contamination of a feed ingredient may have negated the effect in the sucrose fed fish in the second feeding trial. The overall mean showed that, in general, treated fish had significantly better relative weight gain than control fish when both were fed a high-CHO diet (Table VIII). Analysis of visceral fat and plasma glucose data gave no significant difference between the treatments, although the lowest visceral fat levels occurred in the treated group on the sucrose diet. Plasma glucose levels remained within normal range for all groups tested. Condition factor was not significantly different between treated and control groups showing that neither treatment

nor diet, as was shown before, would have an effect on the overall health and visual appearance of the fish.

In a related feeding study using commercial feeds with treated and control fish, there was evidence that treated fish performed better than controls with respect to growth and feed conversion on the diets which were highest in carbohydrates (Smith and Ahern, 1985). MT induced sexually-sterilized fish had improved actual and relative weight gains than controls on all three of the commercial diets used. This was especially evident on the diet highest in CHO. Treated fish also had lower levels of visceral fat (as % of body weight) than control fish fed the same diets. Explanations of why treated (sexually-sterile) fish perform better on a high-CHO diet have been alluded to in the introduction and literature review of this thesis. Treatment with MT at the onset of feeding in young fish may have affected the normal endocrinological controls in these fish. Along with possible blockage of normal testosterone production in these fish, preventing sexual maturity, it is possible that normal cortisol production is somehow disrupted at an early age through feedback control or other mechanisms. Cortisol and testosterone follow the same biosynthetic pathway for a time before breaking off into separate pathways at later steps of synthesis. If a common intermediate of both end products is inhibited, then production of both products might be affected. Reduced

circulating cortisol levels throughout the life of the fish would allow other, anabolic type hormones, such as thyroxin, insulin and somatomedins to have greater influence. This would in turn initiate increased protein synthesis and hence, better growth. Also, of course, fish which had been sexually sterilized would continue to grow during the period a normal fish would put energy into gonadal tissue development. Therefore a larger, healthier fish is produced in a shorter time span.

It is clear that further research into this area must be done before any positive conclusions can be drawn about how the mechanisms work which allow for better growth of treated fish over those which have not been treated. What can be concluded from this study is that in most cases under the same conditions, MT-treated (sterile) fish will out-perform control fish on a diet high in digestible CHO. This study also shows that a high-sucrose or high-molasses diet will produce an enhanced growth response than a high fat diet of comparable energy value. It also dispells the belief that salmonids require, and only do well on a high-protein diet to the exclusion of all else. Lower levels of crude protein which contain adequate amounts of essential amino acids may certainly be used in conjunction with higher levels of digestible carbohydrates. Also, the use of freeze branding in the second trial of this study has shown this method of marking rainbow trout to be a

valuable tool in obtaining individual statistics for analysis of experimental data (see Appendix I). Feeding studies and other research using fish, which extend over a 12-20 week period, may be analyzed individually to account for differences due to diet and/or treatment. With proper technique, this method provides clear brands for at least 16 weeks and would preclude infection and disease problems encountered in conventional tagging methods.

In future such MT-treated versus control type studies, it is suggested that experimental design takes into account several aspects which have been overlooked in this study. Perhaps, because treated fish have been shown to be more docile and less aggressive than controls of the same age and spawning group (Smith, 1983), as part of the study, treated fish should be maintained apart from controls in order to determine how this aggression aspect would affect growth performance. It is also recommended that, as has been done in other carbohydrate feeding studies, liver weight and hepatic lipid and glycogen levels should be measured, in order to determine the effect of diet on this organ with respect to CHO utilization. In order to determine positively blood parameters which control CHO utilization in treated or control fish, perhaps plasma cortisol, testosterone, thyroxin and/or gonadotropin (GTH) levels should be measured. Muscle tissue or whole body analysis of fish after termination of each trial could be

done to determine the effect of each diet on quality and quantity of body flesh. Dressed carcass weight could also be determined at termination as a measurement of feed and protein conversion. One may conclude that although good evidence of the superiority of diets high in digestible carbohydrate, and better growth performance of treated fish has been demonstrated in this study, further research into the nutritional and hormonal aspects of fish culture must be continued. To help meet the increasing demands for high-protein foods in the modern world at minimal cost, these nutritional and hormonal applications may be instituted in fish culture operations.

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Appendix I - The use of a freeze-branding mechanism as
a means of marking rainbow trout for later
individual identification.

In a preliminary feeding trial which was not included in this thesis report, brook trout (*Salvelinus fontinalis*) were individually marked with plastic, numbered tags at the start of the trial. Tags were manually imbedded just ventral to the dorsal fin into the dorsal musculature using a tagging gun. The needle of this gun was injected under the skin and into the muscle and anchored there through a twisting action of the gun. Within three weeks of the tagging high mortality began to occur. The area around the tags became infected with bacteria (*Aeromonas spp.*) and ulceration and necrosis of the tissue resulted. Tags often fell out, and by the end of the feeding trial at least 50% mortality occurred in each tank. Primary bacterial infection was often followed by secondary bacterial or fungal (*Saprolegnia spp.*)

infection. Fish were often anorexic and showed poor growth. Due to the initial stress of the tagging technique and the open site for infection where the tag is inserted, fish pathogens may easily infect the fish and cause high mortality. This method of individually marking fish to obtain better statistical analyses is certainly unsatisfactory. A less stressful, safer and more effective method of marking must be used to insure more dependable results with no mortality problems.

In the second feeding trial of the present thesis research, a freeze-branding procedure using liquid nitrogen was utilized to individually mark all of the fish in each tank. The freeze branding apparatus was easily constructed and inexpensive to make. The outside shell of the device was simply a cylindrical cardboard container, inside of which was placed a brass reservoir to hold the liquid nitrogen. Between the outside cardboard shell and the inside brass reservoir was placed about 5 cm of polyurethane material for insulation. The polyurethane was cut to fit tightly and all cracks were filled with a polyurethane spray foam. The diameter of the outside cardboard container was 20.5 cm and the brass reservoir was 10.0 cm in diameter, constructed of 0.25 cm solid brass material and molded into a cylindrical form with a cap soldered on at the bottom. The reservoir was about 13.0 cm in

depth and could hold more than 1.0 litre of liquid nitrogen. Between the bottom of the reservoir and the bottom of the cardboard container about 3.0 cm of polyurethane was placed for insulation. The total height of the apparatus (cardboard container) was 24.0 cm.

To the brass reservoir, about $2/3$ of the way down from the top, a solid brass rod (0.8cm diameter) was soldered to the inside wall of one side and extended through the interior of the reservoir and through the opposite wall and the insulation beyond it. This rod was extended outside the cardboard container about 10.0 cm to give ample space for manipulation of hands and fish when branding. To the end was soldered a solid copper "T" which was used as the brand. This "T" was 0.8 cm across the top and 0.7 cm from top to bottom. During the branding operation, the section of the rod inside the reservoir (~10.0cm) was completely immersed in liquid nitrogen. The holding capacity of the brass reservoir (>1.0 litre) allowed for effective branding of more than 140 fish. The whole apparatus was mounted on a plywood platform making it portable and safe to use.

At the start of the second feeding trial used for this thesis, rainbow trout were individually branded for later identification using the following procedure.

One litre of liquid nitrogen was placed in the brass reservoir and capped with about 3.5 cm of polyurethane insulation, on top of which was placed the cardboard cover, also lined with 2.5 cm of polyurethane insulation. This cooled the brass rod and copper brand to minus 190 degrees. Fish were anesthetized with MS-222 and individually pressed against the cooled brand for 1-3 seconds and immediately placed back into fresh water. The brand was cleaned with a wire brush after every 2 or 3 applications to prevent fouling and to insure a clear mark. The brand was applied in four different orientations and at three different locations on the fish (front of dorsal-left side, back of dorsal-left side and front of dorsal-right side) thereby giving 12 different brands per tank. Taking into account the fact that treated fish had the adipose fin cut off, 24 different fish could be identified. If more identifications were needed other, different locations on the fish could be utilized. If need be, the brand could be made removable and different brand designs could be attached and used. Brands were easily read at each growth check and at termination so that individual fish could be identified. This allowed for determination of growth parameters and other measurements for individual fish so that statistical analysis might be improved. No mortality occurred

throughout the trial due to branding and less stress is incurred by the fish from this procedure as compared to the previously mentioned tagging methods. The skin is not broken and the wound is quickly healed over while still allowing for the brand to be easily read. This apparatus and procedure provides a feasible and safe method for identification of salmonid fishes for experimental purposes. This system might also be utilized for other species of fish which have small scales or for scaleless species such as catfish. Figure 12 shows a recognizable brand on a rainbow trout 6 weeks after the brand was applied.

Figure 12 Photo of yearling rainbow trout with recognizable brand from freeze branding apparatus. Photo taken six weeks after brand was administered.

Appendix II - Analysis of Variance for all Parameters

Analysis of variance for actual weight gain of rainbow trout over a 12 week period - Trial #1

source	df	SS	MS	F value	@ 0.05	@0.01
total	11	1008				
reps	2	147.5	73.8	9.9	sign	N.S. ³
feeds	1	636.4	636.4	85.7	sign	sign
TvsC ¹	1	32.2	32.2	4.3	N.S.	N.S.
inter- action ²	1	147.5	147.6	19.9	sign	sign
error	6	44.6	7.4			

Analysis of variance for actual length gain of rainbow trout over a 12 week period - Trial #1.

source	df	SS	MS	F value	@ 0.05	@ 0.01
total	11	2.7				
reps	2	0.3	0.15	1.67	N.S.	N.S.
feeds	1	1.5	1.45	15.95	sign	sign
TvsC*	1	0.2	0.17	1.88	N.S.	N.S.
inter- action	1	0.3	0.26	2.87	N.S.	N.S.
error	6	0.5	0.09			

¹ = treatment vs. control = treatment effect

² = interaction effect of treatment and feeds (additive)

³ = N.S. = not significant

Analysis of variance for relative gain of rainbow trout over a 12 week period - Trial #1.

source	df	SS	MS	F value	@ 0.05	@0.01
total	11	0.71				
reps	2	0.01	0.005	0.2	N.S.	N.S. ³
feeds	1	0.15	0.152	5.4	N.S.	N.S.
TvsC ¹	1	0.15	0.153	5.4	N.S.	N.S.
inter- action ²	1	0.23	0.229	8.1	sign	N.S.
error	6	0.17	0.028			

Analysis of variance for feed conversion of rainbow trout over a 12 week period - Trial #1.

source	df	SS	MS	F value	@ 0.05	@ 0.01
total	11	0.16				
reps	2	0.00	0.001	0.31	N.S.	N.S.
feeds	1	0.06	0.06	15.94	sign	sign
TvsC*	1	0.03	0.03	7.00	sign	N.S.
inter- action	1	0.05	0.05	14.44	sign	sign
error	6	0.02	0.004			

¹ = treatment vs. control = treatment effect

² = interaction effect of treatment and feeds (additive)

³ = N.S. = not significant

Analysis of variance for plasma glucose levels of rainbow trout at termination of experiment - Trial #1

source	df	SS	MS	F value	@ 0.05	@0.01
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		(times) (10 ¹⁰)				
total	119	1097				
reps	2	21.9	1099.6	34.9	sign	sign
feeds	1	405.4	40542.0	1288.3	sign	sign
TvsC ¹	1	87.95	8795.1	283.7	sign	sign
inter- action ²	1	545.5	54546.9	1704.6	sign	sign
error	114	35.9	31.5			
<hr/>						

Analysis of variance for visceral fat level (%) in rainbow trout after a 12 week feeding trial - Trial #1.

source	df	SS	MS	F value	@ 0.05	@ 0.01
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total	119	19.74				
reps	2	0.72	0.36	8.01	sign	sign
feeds	1	0.05	0.05	1.16	N.S.	N.S. ³
TvsC*	1	6.90	6.90	153.7	sign	sign
inter- action	1	0.0001	0.0001	----	N.S.	N.S.
error	114	5.12	0.04			
<hr/>						

¹ = treatment vs. control = treatment effect

² = interaction effect of treatment and feeds (additive)

³ = N.S. = not significant

Analysis of variance for actual weight gain of rainbow trout over a 12 week period - Trial #2.

source	df	SS	MS	F value	@ 0.05	@0.01
(times 10)						
total	143	3934				
reps	1	167.7	1676.9	18.97	sign	sign
feeds	2	1121.3	5606.3	29.77	sign	sign
TvsC ¹	1	41.7	416.8	2.21	N.S.	N.S. ³
inter-action ²	2	23.5	117.7	0.62	N.S.	N.S.
error	137	2580	188.3			

Analysis of variance for actual length gain of rainbow trout over a 9 week period - Trial #2

source	df	SS	MS	F value	@ 0.05	@ 0.01
total	143	71.09				
reps	1	1.56	1.56	4.08	sign	sign
feeds	2	15.21	7.61	19.86	sign	sign
TvsC*	1	0.47	0.47	1.22	N.S.	N.S.
inter-action	2	1.38	0.69	1.80	N.S.	N.S.
error	137	52.4	0.38			

¹ = treatment vs. control = treatment effect

² = interaction effect of treatment and feeds (additive)

³ = N.S. = not significant

Analysis of variance for relative gain of rainbow trout over a 12 week period - Trial #2.

source	df	SS	MS	F value	@ 0.05	@0.01
total	143	12.9				
reps	1	6.78	6.776	351.1	sign	sign
feeds	2	3.64	1.820	118.9	sign	sign
TvsC ¹	1	0.11	0.111	7.28	sign	sign
inter- action ²	2	0.22	0.108	7.05	sign	sign
error	137	2.12	0.015			

Analysis of variance for feed conversion for rainbow trout over a 9 week period - Trial #2

source	df	SS	MS	F value	@ 0.05	@ 0.01
total	11	0.37				
reps	1	0.08	0.077	12.14	sign	N.S. ³
feeds	2	0.24	0.121	19.19	sign	sign
TvsC*	1	0.01	0.011	1.71	N.S.	N.S.
inter- action	2	0.01	0.006	0.89	N.S.	N.S.
error	4	0.03	0.006			

¹ = treatment vs. control = treatment effect

² = interaction effect of treatment and feeds (additive)

³ = N.S. = not significant

Analysis of variance for plasma glucose levels of rainbow trout at termination of experiment - Trial #2.

source	df	SS	MS	F value	@ 0.05	@0.01
		(times) (10 ¹⁰)				
total	143	1306				
reps	1	21.3	2126.8	3.30	sign	N.S. ³
feeds	2	383.5	19176.8	29.77	sign	sign
TvsC ¹	1	7.58	758.1	1.18	N.S.	N.S.
inter- action ²	2	10.9	547.7	0.85	N.S.	N.S.
error	137	883	644.2			

Analysis of variance for visceral fat level (%) in rainbow trout after a 9 week feeding trial - Trial #2

source	df	SS	MS	F value	@ 0.05	@ 0.01
total	143	21.26				
reps	1	0.09	0.095	0.67	N.S.	N.S.
feeds	2	0.68	0.34	2.41	N.S.	N.S.
TvsC*	1	0.002	0.002	----	N.S.	N.S.
inter- action	2	1.11	0.20	1.47	N.S.	N.S.
error	137	19.3	0.14			

¹ = treatment vs. control = treatment effect

² = interaction effect of treatment and feeds (additive)

³ = N.S. = not significant

Analysis of variance for condition factor of rainbow trout fed three diets for 9 weeks - Trial #2.

source	df	SS	MS	F value	@ 0.05	@0.01
total	143	.002				
reps	1	-----*	-----	0.43	N.S.	N.S. ³
feeds	2	-----	-----	0.31	N.S.	N.S.
TvsC ¹	1	-----	-----	0.00	N.S.	N.S.
inter- action ²	2	-----	-----	0.14	N.S.	N.S.
error	137	.002	-----			

¹ = treatment vs. control = treatment effect

² = interaction effect of treatment and feeds (additive)

³ = N.S. = not significant

* = insignificant values