

**STUDIES ON THE EFFECT OF STEROID HORMONES ON THE
GROWTH AND BIOCHEMICAL COMPOSITION
OF THE MULLET**

Liza Darsia (Hamilton)

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CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON THE EFFECT OF STERIOD HORMONES ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF THE MULLET LIZA PARSIA (HAMILTON)" is the bonafide record of the work carried out by JADHAV BHASKAR LAXMAN under my guidance and supervision and that no part thereof has been presented for any other Degree.



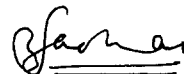
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DECLARATION

I hereby declare that this thesis entitled "STUDIES ON THE EFFECT OF STEROID HORMONES ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF THE MULLET LIZA PARSIA (HAMILTON)" has not previously formed the basis of the award of any degree, diploma, associateship, fellowship ~~of~~ other similar titles or recognition.

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(JADHAV BHASKAR LAXMAN)

C O N T E N T S

	Page Nos.
Preface	i - vi
Acknowledgement	vii - viii
List of Tables, Figures and Plates	ix - xiii
CHAPTER I	
INTRODUCTION AND REVIEW OF LITERATURE	1 - 37
CHAPTER II	
MATERIALS AND METHODS	
(a) Feeding experiments	38 - 50
(b) Injection experiments	50 - 54
CHAPTER III	
RESULTS	
(1) 17 α - methyltestosterone (MT)	55 - 62
(2) Diethylstilbestrol	62 - 64
(3) 17 β - Estradiol	64 - 69
(4) Interaction of MT and thyroid hormone (T3)	69 - 79
(5) Protein - sparing action of 17 - methyltestosterone	79 - 87
(6) Injections of MT and estrone	87 - 90
CHAPTER IV	
DISCUSSION	
(i) 17 α - methyltestosterone	91 - 102

(ii) Diethylstilbestrol	102 - 106
(iii) 17B - Estradiol	106 - 109
(iv) Interaction of thyroid hormone (T3) and MT	109 - 117
(v) Effects of MT on dietary levels of protein	117 - 122
(vi) Injection experiment	
(a) Injections of MT	122 - 123
(b) Injections of Estrone	123 - 123
SUMMARY	124 - 130
REFERENCES	xiv - xxxviii

PREFACE

Aquaculture is emerging as a successful bio-industry, both in developed and developing nations of the world, to augment protein rich food production. The phenomenal growth in human population and the growing threat of depletion of wild stock of commercially important aquatic species, due to excessive exploitation during the past few decades, have prompted Governments to plan and execute programmes to produce more fish by developing hitherto unutilized or partially utilized waste-lands and water resources through farming of species useful to man.

Pisciculture is an ancient practice in many countries and the ^{culture} technology has undergone marked changes. At present we have a wide range of ^{fish culture} technologies - extensive, semi-intensive, intensive and supra-intensive - suitable for traditional fish farmers to high-tech bio-industries. Finfishes contribute a major share (60.6%) to the world aquaculture production of 10.6 million metric tonnes.

In India, although fresh water fish culture with major and minor carps is being practiced for several centuries, brackish water fish-culture is a relatively new area and the farmed fishes include mullets, cichlids, and milkfish.

The grey mullets of the family Mugilidae are an international group of fish and constitute 0.1 - 0.3% of the total marine landings of India; and the production of mullets from Kerala backwaters forms about 11% of its total landings (Jhingran, 1983). Day (1988), identified 26 species of mullets from Indian waters of which 3 were reported to enter fresh water, and another 19 estuaries. Among the mullets Liza parsia (Hamilton) is of considerable importance to India, but second only to Mugil cephalus.

According to Jhingran (1982) Liza parsia is one of the common grey mullets in the Cochin Backwaters and supports a thriving fishery in estuaries and backwaters of India. It is also abundant in West Bengal, Madras, Vishakapatnam, Palk Bay, Gulf of Mannar and Andaman Islands. Outside India, this species is restricted to the Indo-Pacific region where it is fairly distributed along the coast of Pakistan, Sri Lanka, Hong Kong, Australia, Indonesia etc.

Mulletts are highly rated food fishes, and are suitable for large-scale farming in brackish water impoundments in monoculture and polyculture systems. The gold-spot mullet Liza parsia is a highly eurynaline fish and holds immense potential as a candidate species for coastal aquaculture in India. The fish has the ability to utilize food from the

lowest trophic level and is amenable for artificial propagation, thus opening up opportunities for scientific farming of the species to maximise production from an unit area, by the adoption of proper management practices and judicious use of operational inputs, within a reasonable period of time. Among the operational inputs feeds, by and large, is the major component in semi-intensive and intensive aquaculture systems and the cost of production is considerably affected by the cost and efficiency of feed.

The constant efforts to develop low cost feeds of higher efficiency has stimulated continued search for more suitable combinations of known nutrients and for new additives which will increase the feed efficiency, rate of growth and reduce the period of rearing. In this context the use of some chemicals and biological substances have come to light, which when added to the diets promote faster growth and increase the food conversion efficiency. These selected additives are referred to as growth promoting agents. A variety of substances fall into this category which are primarily administered to the animal as pellet implants, as injections, through water media, or through the diets (Donaldson et al., 1979). However, oral administration of growth promoting agents through the diets offers the most convenient and

practical approach (Higgs et al., 1982). The materials generally used as growth promoting agents are antibiotics, hormones, tissue preparations, plant extracts and some chemicals depending upon the recipient animals. Of the growth promoters mentioned above, steroid hormones have been found to be effective anabolic agents for many fish species (Higgs et al., 1982). However, with the exception of Mugil auratus (Bonnet, 1970) there is no information on the use of steroid hormones in any of the mullet species. It is against this background that the present study was undertaken.

Studies on the effect of steroid hormones on the growth and body composition of the mullet Liza parsia (Hamilton) is a comprehensive attempt to find out the efficacy of few steroid hormones such as 17 α -methyltestosterone, diethylstilbestrol, 17 β -estradiol, as growth promoting agents in the diets of Liza parsia. Histological changes in the ovary of the fish associated with the injections of an androgen 17 α -methyltestosterone and an estrogen, estrone have also been studied with a view to induce maturation of the species under partly controlled conditions.

The thesis is divided into four chapters

Chapter I

Presents the introduction with a review of the literature on the subject.

Chapter II

Deals with the materials and methods employed in the design, conduct and evaluation of experiments which is sub-divided into two parts (i) dealing with the feeding experiments and (ii) with the injection experiments.

Chapter III

Deals with the results of the experiments and treated under six sub-titles, viz., (i) 17α -methyltestosterone, (ii) diethylstilbestrol (iii) 17β -estradiol, (iv) individual and combinations of thyroid hormone (T3) and 17α -methyltestosterone (MT), (v) protein sparing action of MT and (vi) injections of MT and estrone

Chapter IV

Presents the discussion

A summary follows the discussion

All the literature cited in the text have been pooled and presented in alphabetical order after the summary.

The present study has helped in finding out an efficient growth promoting substance for the fry of Liza parsia. 17α -methyltestosterone at the dosages of 2 mg/kg diet is the most effective anabolic agent for Liza parsia. The study also shows that by incorporating 2 mg MT/kg of diet the protein level in the diet could be reduced from 35 to 30%, thereby a significant saving in the cost of feed could be obtained. Further, the anabolic effect of MT helps to reduce the rearing period during the production of fingerlings from the fry stage.

Although 17β - estradiol had shown moderate anabolic effect, diethylstilbestrol did not show any anabolic effect in Liza parsia. None of the individual dose of T3 as well as combinations of MT and T3 produced better growth over 2 mg MT. Therefore use of thyroid hormone in the diets of Liza parsia will not be advantageous over 2 mg MT.

The study reveals that estrone could be used to initiate ovarian maturation in Liza parsia even during off-season. But further comprehensive studies are needed to exploit the usage of estrone in inducing maturation in gold-spot mullet.

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LIST OF TABLES, FIGURES AND PLATES

T A B L E S

<u>No.</u>	<u>Description</u>	<u>Between Pages</u>
I	Hormones and their dosages used for the present study	41-42
II	Composition of the diets used for the experiments	41-42
III	Composition of the mineral mixture and vitamin mixture	41-42
IV(a)	Effect of 17α -methyltestosterone on growth, food conversion and body composition of <u>Liza parsia</u> after 40 days (0 - 60 mg/kg)	55-56
(b)	Effect of 17α -methyltestosterone on growth of <u>Liza parsia</u> after 60 days (0 - 30 mg/kg)	55-56
V	Effect of 17α -methyltestosterone on growth, food conversion and body composition of <u>Liza parsia</u> after 45 days (0 - 15 mg/kg)	58-59
VI	Effect of diethylstilbestrol on growth, food conversion and body composition of <u>Liza parsia</u> (0 - 1.8 mg/kg)	62-63
VII	Effect of 17β -estradiol on growth, food conversion and body composition of <u>Liza parsia</u> (0 - 10 mg/kg)	
VIII	Effect of 17α -methyltestosterone and 3,5,3'-triiodo-L-thyronine on growth, food conversion, and biochemical composition of <u>Liza parsia</u>	69-70
IX	Effect of selected dosages of 17α -methyltestosterone and dietary levels of protein on the growth, food conversion and biochemical composition of <u>Liza parsia</u>	79-80

F I G U R E S

<u>No.</u>	<u>Description</u>	<u>Between Pages</u>
1	Effect of selected levels of 17 α -methyltestosterone (MT) on the growth of <u>Liza parsia</u> (0 - 60 mg)	55-56
2	Effect of 17 α -methyltestosterone (MT) on survival, weight gain, specific growth, condition factor, digestibility coefficient and food conversion ratio, after 40 days (0 - 60 mg)	55-56
3	Effect of 17 α -methyltestosterone on protein efficiency ratio, gross conversion efficiency, protein, lipid, moisture and ash, after 40 days (0 - 60 mg)	55-56

4	Effect of selected levels of 17 α -methyltestosterone (MT) on the growth of <u>Liza parsia</u> (0 - 15 mg)	58-59
5	Effect of 17 α -methyltestosterone (MT) on survival, weight gain, specific growth, condition factor, digestibility coefficient and food conversion ratio (0 - 15 mg)	59-60
6	Effect of 17 α -methyltestosterone (MT) on protein efficiency ratio, gross conversion, efficiency, protein, lipid, moisture and ash (0 - 15 mg)	59-60
7	Effect of selected levels of diethylstilbestrol (DES) on the growth of <u>Liza parsia</u>	62-63
8	Effect of diethylstilbestrol (DES) on survival, weight gain, specific growth, condition factor, digestibility, coefficient and food conversion ratio.	62-63

- 9 Effect on diethylstilbestrol (DES) on protein efficiency ratio, gross conversion efficiency, protein, lipid, moisture and ash
- 10 Effect of selected levels of 17 β -estradiol on the growth of Liza parsia
- 11 Effect of 17 β -estradiol on survival, weight gain, specific growth, condition factor, digestibility coefficient and food conversion ratio
- 12 Effect of 17 β -estradiol on protein efficiency ratio, gross conversion efficiency, protein, lipid, moisture and ash 66-67
- 13 Effect of selected levels of thyroid hormone (T3) on the growth of Liza parsia 69 - 70
- * 14
- 15 Effect of T3 on survival, weight gain, specific growth, condition factor, digestibility coefficient and food conversion ratio 70 - 71
- 16 Effect of T3 on protein efficiency ratio, gross conversion efficiency, protein, lipid, moisture and ash 70 - 71
- 17 Effect of combinations of T3 and 17 α -methyltestosterone (MT) on survival, weight gain and specific growth 71 - 72
- 18 Effect of combinations of T3 and 17 α -methyltestosterone (MT) on condition factor, digestibility coefficient and food conversion ratio 72 - 73
- 19 Effect of combinations of T3 and 17 α -methyltestosterone (MT) on protein efficiency ratio, gross conversion efficiency and moisture 72 - 73

20	Effect of combinations of T3 and 17 α -methyltestosterone (MT) on protein, lipid and ash	71 - 72
21	Effects of selected levels of dietary protein and 17 α -methyltestosterone (MT) levels on growth	79-80
22	Effect of selected levels of dietary protein and 17 α -methyltestosterone (MT) on survival, weight gain and specific growth	78 - 79
23	Effect of selected levels of dietary protein and 17 α -methyltestosterone (MT) on condition factor, digestibility coefficient and food conversion ratio	82 - 83
24	Effect of selected levels of dietary protein and 17 α -methyltestosterone (MT) on protein efficiency ratio, gross conversion efficiency and moisture	82 - 83
25	Effect of selected levels of dietary protein and 17 α -methyltestosterone on protein, lipid and ash	84 - 85

P L A T E S

No	Description	Between pages
I & II	Aquaria setup used for the rearing of mullet fry.	38 - 39
III	Tanks used for the rearing of adult immature fish.	50 - 51
IV	Ovary of control gold-spot mullet. Sample taken at the termination of the study. Ovary in oogonia stage.	87 - 88
V	Ovary of the gold-spot mullet administered 10 injections of 1 mg/kg 17 -Methyltestosterone. Ova in central nucleolus stage of development. 10 * 10 mm.	88 - 89
VI	Ovary of the gold-spot mullet administered 10 injections of 2 mg/kg Methyltestosterone. Ova in central nucleolus stage of development.	88 - 89
VII	Ovary of the gold-spot mullet administered 10 injections of 3 mg/kg Methyltestosterone. Ova in central nucleolus stage of development.	88 - 89
VIII	Ovary of the gold-spot mullet administered 10 injections of 0.5 mg/kg estrone. Ova in central nucleolus stage of development. 10 * 10 mm.	88 - 89
IX	Ovary of the gold-spot mullet administered 10 injections of 1 mg/kg estrone. Ova in central nucleolus stage of development.	89 - 90
X	Ovary of the gold-spot mullet administered 10 injections of 1.5 mg/kg estrone. Ova in perinucleolar stage of development.	89 - 90

INTRODUCTION AND REVIEW OF LITERATURE

The production of nutritionally balanced diets for any fish requires research, quality control and biological evaluation. A well balanced diet not only results in higher production, but also provides the nutrients necessary to fasten recovery from diseases or aid the fish in overcoming the effect of environmental stress. In some cases a good quality diet may slow the progress of idiopathic diseases. Hence, nutritionally balanced and quality controlled diets for fish production are of critical importance.

The chief function of the food is to supply the nutrients necessary to satisfy the physiological needs of the organisms such as to supply energy, to build and maintain the cell and tissues and to regulate the metabolic processes.

NUTRITIONAL REQUIREMENTS OF FINFISH

Nutrients in feeds that supply energy are proteins, lipids and carbohydrates. The non-energy nutrients, vitamins and minerals, serve numerous functions in animals. Among the energy nutrients, carbohydrates and lipids form the chief sources of energy; but protein primarily is utilised for growth. These nutrients are required by the animals in appropriate quantities and balanced proportions, and hence,

the diets must be formulated to meet the basic requirements of the animals.

Proteins are indispensable nutrients of animals mainly in three ways: (i) maintenance, the making good of tissue wear and tear, (ii) the repletion of depleted tissues and (iii) growth or formation of new additional proteins. The utilization of dietary protein by fish depends upon (a) the level of other nutrients in the diet, (b) the calorie content of the diet (c) physiological state of the animal and (d) the rearing conditions. The animal's need for nitrogen and essential amino acids is met by dietary protein.

Finfish characteristically require much higher protein levels in the diet than necessary for birds and mammals (Cowey and Sargent, 1972). The optimum dietary protein level required for maximum growth in farmed fishes is 50 to 300% higher than that of terrestrial animals (Cowey, 1975). These quantitative differences have been mainly attributed to the predominant carnivorous and omnivorous habit of fishes and their apparent preferential use of protein over carbohydrate as dietary energy source. Protein requirements are usually expressed in terms of a fixed dietary percentage or ratio of protein to dietary energy. More than thirty species have been

examined in this manner and the results show that the dietary protein requirement of most species range from 35 to 55%. In the mullets dietary protein requirement varies from 40% to 70% (Vallet et al., 1970; Kandasami et al., 1987; Kiron, 1989). The variation in the dietary protein requirements of fish species has been attributed to the species type, physiological conditions, feeding habits, age and size of animals etc. Besides, the amino acids profile of the protein sources used in the diets has significant influence on the protein requirements.

Fishes rely to a large extent on lipids, besides protein, as a source of energy. Lipids in the diet have also been shown to spare protein for growth. Lipids fulfill several functions in fishes: in maintaining the structural integrity of a wide variety of bio-membranes between and within the cells, as a source of energy, as a vehicle for the absorption and transport of fat-soluble vitamins provided in the diet, and contribute to the flavour and textural properties of feed consumed by fishes. Lipids are involved in many other aspects of metabolism, viz., precursor of steroid hormones and prostaglandins and also in the activation of certain enzymes.

A number of reviews on fish nutrition have been

published which contain information on the lipid requirements of fish (Cowey and Sargent, 1972, 1979; Lee and Sinnhuber, 1972; Hashimoto, 1975; Castell, 1979; Watanabe, 1982). Most studies indicate that carnivorous species like the salmonids and sea bass efficiently utilize lipids from their diets as compared to herbivorous and omnivorous fish, provided adequate amounts of essential fatty acids, choline, methionine and tocopherol are also present in the diet. Fish generally require n-3 fatty acids rather than n-6 as essential fatty acids, in contrast to terrestrial animals (Kanazawa, 1985); and the quantitative requirements have been worked out for many fishes (Watanabe, 1982). Marine fish appear to have a greater requirement for highly unsaturated fatty acids such as eicosapentaenoic and docosahexaenoic acids than fresh water species (New, 1987).

Survey of the literature on dietary lipid requirements of finfish shows that diets containing lipid levels from 6 to 15% gives best growth in most of the species (Phillips et al., 1964; Austreng, 1979; Dupree et al., 1979; Paulraj and Arasu, 1987; Kiron, 1989). Several types of oils, either single or in combinations have been commonly used as a source of lipids in fish diets. Although many vegetable lipids contain high levels of n-6 polyunsaturated fatty acids the

best source of n-3 highly unsaturated fatty acids are marine lipids. The oils currently employed in dietary combinations include codliver oil, pollack-liver oil, herring oil, menhaden oil and capelin oil. A variety of plant oils are also being used in fish diets. These include soyabean oil, cotton seed oil, sunflower oil and corn oil etc. Since fish oils and most plant oils are rich in polyunsaturated fatty acids, antioxidants are to be added to them during processing to delay the onset of rancidity.

Besides serving as an energy source, carbohydrates also form the components of certain vital structural and metabolic compounds. There are also a few reports on the protein sparing action of dietary carbohydrates (Cowey ^{and Sargent,} 1979). Studies on the energy requirement of fish have indicated that the carnivorous and omnivorous fish have limited ability to utilise carbohydrates of high molecular weights as an energy source, while herbivorous fish satisfy most of their energy requirements from these energy-yielding macromolecules. Carbohydrate requirement varies from species to species; however, it ranges between 25 to 50% (Cowey and Sargent 1972, 1979; Halver 1972; Millikin, 1982). Various feed ingredients such as cereal grains, plant tubers like potato, tapioca etc. have been used in compounding diets to meet the carbohydrate

needs of finfish.

Vitamins are complex organic substances usually of comparatively small molecular size, but are important for the maintenance of normal metabolic and physiologic functions. They are distributed in feed-stuffs in small quantities and form a distinct entity from other major and minor food components (Cho et al., 1985). Many of the vitamins act as essential cofactors in the enzyme systems functioning in various aspects of carbohydrate, fat and protein metabolism (Cowey and Sargent, 1972). Deficiency of vitamins in diets had been shown to induce severe syndromes in fish (Cho et al., 1985). Therefore, many investigators have directed their attention to find out the requirements of specific vitamins to different fish species. As a dividend of research, considerable knowledge has been accumulated on specific quantitative and qualitative vitamin requirements of only a few species of fish and much of the information has been summarised in several publications (Halver, 1972, 1980; NRC, 1981; New, 1987).

A variety of minerals are known to have important roles in metabolism. They serve as cofactors of enzymes in the metabolic processes; their requirements varying from species

to species. Therefore, many investigators have given attention to find out the dietary requirements of these elements in finfishes. Various compositions of mineral mixtures have been suggested for finfish (New, 1987).

Brief reviews of qualitative and quantitative nutrient requirements for specific groups of fishes are also available for trout, salmon and catfish (NRC, 1973; Rumsey, 1978; Halver, 1982), carp (Jauncey, 1982) seabass, grouper and rabbit fish (Kanazawa, 1984). Cowey and Sargent (1979) reviewed the advances in protein, lipid, amino acid, fatty acid, vitamin and mineral requirement of fishes since their early work (Cowey and Sargent, 1972). In an excellent review Millikin (1982) has delved into interactions of various macro and micronutrients as related to artificial diet formulation for various life stages of several species currently reared in large quantities in fish hatcheries. Rumsey(1977), Ketola (1982) and Watanabe (1982) have contributed outstanding, comprehensive reports on individual nutrients.

Several types of feed ingredients of plant or animal origin or their mixtures have been used to fulfil the requirements of energy and non-energy nutrients (Pfeffer, 1982; Watanabe, 1982).

The majority of the fish species currently farmed in intensive culture systems are either carnivorous or omnivorous and consequently require high protein diets. Hence, protein meals of animal origin, particularly those of marine species, are of great value in aquaculture feeds. Animal proteins are generally rich in essential amino acids, especially lysine and methionine, which are often limiting in plants. Therefore, most of the workers used fish meal alone as a protein source or in combination with other protein sources like soyabean meal cake, feather meal etc. (Pfeffer, 1982). The fish meal content of diet usually vary between 25 and 65% by weight, with higher levels being used in starter and fingerlings ration (Tacon and Jackson, 1985). In view of the high cost of good quality fish meal of relatively constant chemical composition, the feed costs amount to 40 - 60% of the operating costs in intensive culture systems (FAO, 1983). Besides, quality fish meal is in short supply in several countries including India, while the demand is steadily increasing due to the accelerated development in animal husbandry and aquaculture. Therefore, other protein sources like meat meal, bone meal, soyabean meal, groundnut meal, corn gluten meals etc. are being increasingly used in practical fish feeds.

A practical diet that is nutritionally well balanced, easy to handle and store and capable of promoting growth and survival for a number of generations, is the basic need of a long-term fish culture system.

RESPONSE OF FINFISH TO ANABOLIC AGENTS

The constant efforts to develop low-cost feeds of higher efficiency has stimulated continued search for more suitable combinations of known nutrients and for new additives which will increase the feed efficiency, rate of growth and reduce the period of rearing. In this context use of some chemical and biological substances have come to light, which when added to the diets promote faster growth and increase the food conversion efficiency. These selected additives are referred to as growth promoting agents. A variety of substances fall into this category which are primarily administered to the animal as pellet implants, as injections, through water media or through the diets. However, oral administration of growth promoting agents through the diets, offers the most convenient and practical approach (Donaldson et al., 1979; Higgs et al., 1982).

Growth promoting agents are not considered as dietary

essential and their absence in the diet will not have any adverse effect on the animal. The materials generally used as growth promoting agents are antibiotics, hormones, tissue preparations, plant extracts and some chemicals depending upon the recipient animals.

Studies on the use of antibiotics in the diets of finfish were initiated by Wagner (1954) and Milikova and Kotova (1961) with rainbow trout (Salmo gairdneri) and brown trout (S. trutta) fingerlings, respectively; but the results were not encouraging. Later, Leonov (1963), Korneeva (1963 and 1965) and Sukhoverkhov (1967) successfully obtained growth stimulation in carps (Cyprinus carpio) using terramycin in their diets. Sen and Chatterjee (1976) also tested enterocyclin and hcestacyclin in the diets of rohu (Labeo rohita). Another group of substances known as biological stimulants were also used as growth promoting substances in animal diets. Filatov (1948) proposed that any live tissue, separated from the organism and kept under unfavourable conditions, is subject to a biochemical reconstruction. The reconstructed material when introduced into another organism intensifies its biochemical development, and accelerates growth. Generally biological stimulants are prepared from the organs like spleen, liver,

lungs, lymphatic knots, bowels, tracheae with the thyroid gland, ovaries, pregnant womb with embryo in first half of pregnancy, eyeballs etc. The organs are preserved at 3 to 5 degrees centigrade for 5 to 6 days, after which they are dried at 150 to 200 degrees centigrade, powdered and used in the diets. Such preparations were found to promote faster growth in carps (Sukhoverkhov et al., 1963 and Sukhoverkov, 1967).

Apart from these, the use of substances like drugs, enzymes, certain chemicals, chitin and alfalfa extract has also been studied for fishes (Zheltoev et al., 1982 and Sower et al., 1983).

The use of both natural and synthetic hormones as growth promoters has been extensively studied for land and aquatic animals. The hormones generally used are androgens, estrogens, progesterones, thyroid hormones and growth hormones. These were successfully used for growth enhancement in cattle, poultry, lambs, swine etc. Studies on the use of hormonal preparations in fish diets dates as far back as 1936. Tuckmann (1936), Regnier (1938) and Cantilo and Regalado (1942) had achieved faster growth in guppy (Girardinus guppi), swordtail (Xiphophorus helleri)

and brook trout (Salvelinus fontinalis) respectively, using an exogenous anabolic growth hormone such as the mammalian growth hormone in their diet.

A fully acceptable theory explaining the mechanism by which anabolic steroids improve food utilization in aquatic and terrestrial animals has not yet been advanced. Preston (1975) has summarised such attempts as they relate to the effects of estrogen in ruminants and list them as follows: (1) increased ACTH production, causing an increased output of androgens by the adrenals, (2) increased production and/or release of growth hormone, (3) increased secretion of insulin, stimulating amino acid incorporation into protein, (4) increased production of thyroid hormone and (5) direct effect on the tissue in enhancing utilisation of non-protein nitrogen. These theories fall short of explaining all the observed phenomena and contradiction may be found (Donaldson et al., 1979).

The effect of anabolic steroids on food conversion efficiency has to date received limited attention in fish. 17 α -methyltestosterone and testosterone are reported to increase food consumption and food conversion efficiency in many fishes (Higgs et al., 1982). In another study Nirmala

and Pandian (1983) have also reported the increased feeding and increased food conversion when Channa striatus was injected with 17 α -methyltestosterone, testosterone, estroid, docabolin and diethylstilbestrol.

Further, increase in protein and proteases content of the gut was also recorded in response to the steroid hormones, testosterone, 11-ketotestosterone or adrenosterone when administered in doses of 1-10 ppm for 60 days (Lone and Matty, 1981b). Yamazaki (1976) also found histological evidence for increase in proteolytic activity of pancreas and intestine of Oncorhynchus masou treated with MT (10 ppm) for 2 weeks.

Lone and Matty (1980a, 1981c) also noted a general trend of decreasing DNA content and increasing RNA content and ratios of RNA/DNA, protein/RNA and protein/DNA in the muscle, kidney and liver of the carp, Cyprinus carpio as dosage of the MT or 11-Ketotestosterone in the diet was increased. From all the foregoing reports, it appears that anabolic steroid may increase the levels of RNA, and increase the secretion of pancreatic and intestinal proteases, and thereby enhance the nitrogen retention. The increased levels of proteases may also enhance the food conversion efficiency of the animal.

In their review, Higgs et al. (1982) have listed 20 finfish species which have exhibited an anabolic response to steroid treatment. Since then, atleast nine more species have been added to this list : air breathing fishes, Channa striatus (Nirmala and Pandian, 1982; Arul, 1986) and Heteropneustes fossilis (Sindhu and Pandian, 1984); a rose bitterlings, Rhodeus ocellatus ocellatus (Asahina et al., 1983); mahseer Tor khudree (Shyama and Keshavanath, 1988; Gogoi and Keshavanath, 1988); the major carps Catla catla, Cirrhinus mrigala (Deb and Varghese, 1988) and Labeo rohita (Jayaram and Shetty, 1980; Reddy et al., 1987; Deb and Varghese 1988 and Nanjundappa and Varghese 1988); silver carp, Hypophthalmichthys molitrix (Shyama and Keshavanath, 1988) and estuary grouper, Epinephelus salmoides (Chua and Teng, 1980).

Many studies have focused attention exclusively on salmonids, namely, the Atlantic salmon, Salmo salar (Saunders et al., 1978) rainbow trout, Salmo gairdneri (Matty and Cheema, 1978), steel-head trout (Sower et al., 1983), coho salmon, Oncorhynchus kisutch (Yu et al., 1979; Fagerlund et al., 1980), chinook salmon Oncorhynchus tshawytscha (Schreck and Fowler, 1982) and Oncorhynchus rhodurus (Ueda et al., 1984).

Literature survey shows that so far only one mullet species, Mugil auratus has been tested with a steroid hormone, testosterone, which resulted in a negative growth (Bonnet, 1970).

EFFECT OF ANDROGENS

Past experiments established that growth of fish may be stimulated by atleast 14 of the androgen anabolic steroids, effective in mammals (Donaldson et al., 1979; Higgs et al., 1982). Contrary to the situation in ruminants, well documented anabolic effects of estrogen on fish have been reported by only few workers (Cowey et al., 1973; Nirmala and Pandian, 1983; Nagraj and Rao, 1988; Nanjundappa and Varghese, 1988 and Shyama and Keshavanath, 1988).

No compound has been found to date, which promoted weight increments in Salmo species comparable to 17 α -methyltestosterone^(MT). However, scanning through the literature, it is seen that MT administration has resulted in either negative growth in Salmo salar and Salmo gairdneri or has induced moderate positive gain at lower doses in both the species (Fagerlund and McBride, 1977; Saunders et al., 1977; 1978 cited by Higgs et al., 1982). Similarly, moderate growth

was also observed in S.gairdneri irideus and Oncorhynchus nerka when treated with MT at lower doses (1 mg/kg), while negative growth in these species were observed at higher doses (30 mg/kg) by Yamazaki(1976).

Several investigations made on coho salmon, Oncorhynchus kisutch, using various levels of MT in feeds gave better growth at lower and moderate dosages, while negative growth was encountered in doses above 100 mg/kg (McBride and Fagerlund, 1973; Fagerlund and McBride, 1975, 1977; Donaldson et al., 1982). Yu et al., (1979) have also reported significant percent weight gain over control in coho salmon treated with MT at the dose of 2.5 mg/kg dry diet.

Chinook salmon appear to respond weakly to MT. Fagerlund et al. (1980) noted maximum increases in weight over controls of 32 and 25% in this species after feeding MT supplemented diets of 6 ppm and 2 ppm respectively. Responses of similar magnitude were obtained in an earlier study (McBride and Fagerlund, 1973). These results contrast with the findings of Fagerlund et al. (1980) that MT produced a more stronger effect at the dose of 1 ppm on coho salmon, 92% weight gain, than the untreated fish (cited by Higgs et al., 1982). Significant weight increase was recorded in another salmonoid

species, Oncorhynchus keta by Fagerlund and McBride (1977) when fed on diet supplemented with lower dosages of MT.

Most of the non-salmonid species have responded positively to MT. However, guppy (Lebistes reticulatus) recorded length retardation when treated with MT (20-30 mg/kg) in the diet (Clemens et al., 1966). Moderate increase was observed when gold fish, Carassius auratus received moderate dosages of MT, while 30 mg/kg of this steroid resulted in negative growth in this fish (Hirose and Hibiya, 1968a). Another economically important species which has responded positively to MT treatment is the estuary grouper, Epinephelus salmoides. Chua and Teng (1980) found that the most effective dose 9 ppm produced the highest weight gain over the controls in this species.

Moderate dosages of MT seems to be more effective in gaining weight increments over controls in Tilapia species. Guerrero (1975) administered MT through diet to young Tilapia aurea for 21 days and obtained the highest increase in weight over that of the control with 30 ppm of MT. The same dose of MT has promoted growth in another tilapia species, Tilapia mossambica (Guerrero, 1976). A gain in biomass of upto 28 times from their initial weight was recorded in fry of

Oreochromis mossambicus, when fed on a ration containing this hormone at 30 mg/kg diet for 60 days (Macintosh et al., 1985).

Besides rainbow trout and coho salmon, the common carp Cyprinus carpio has been extensively used to study the effect of MT on growth. Lone and Matty (1980a) found that the carp fry Cyprinus carpio given 1 ppm MT through the diet gained 32% more weight than the control. The gain increased to 99.8% when weight was determined 60 days after hormone withdrawal, while the dose of 2.5 ppm caused a slightly smaller gain at 90 days, but 60 days after termination of hormone treatment, more gain was obtained.

Rao and Rao (1983) also noted significant weight gain in MT treated carp, Cyprinus carpio over control. However, retardation of growth with MT at a dose of 10 ppm in Cyprinus carpio was also observed by Lone and Matty (1980). More recently, Deb and Varghese (1988) recorded faster growth of Cyprinus carpio, Catla catla, Labeo rohita at 1 ppm MT treatment, while Cirrhinus mrigala responded better to 3 ppm treatment. In the silver carp, Hypophthalmichthys molitrix and in the mahseer, Tor khudree 4 ppm MT induced a growth depression (Shyama and Keshavanath, 1988). The best growth

promoting potential of MT incorporated diet was 2.5 ppm in the fingerlings of mahseer (Gogoi and Keshavanath, 1988), while the dose of 7.5 ppm resulted in inferior growth in this species.

Relatively higher MT levels seems to be required to elicit positive growth response in fish through injections. The most effective dose of this hormone to induce maximum growth in the fish Heteropneustes fossilis was proved to be 40 mg/kg while the dosages higher than this depressed the growth (Sindhu and Pandian, 1984). Similarly, Channa striatus responded most effectively to injections of MT at the dose of 30 mg/kg (Nirmala and Pandian, 1983).

Testosterone (T) compared to MT proved to be less effective. Ashby (1957) treated fish by adding different concentrations of an alcoholic solution of testosterone (50-60 mg/litre/50 fish) to the water and found retarded growth in brown trout. In contrast, McBride and Fagerlund (1976) have shown that the naturally occurring androgen, testosterone (T) promotes growth in coho salmon, O.kisutch, when fed through diet at the dosage of 1 and 10 ppm. Response of similar magnitude was recorded by Yu et al. (1979) in coho salmon fed a diet administered 2.5 mg/kg of T. Nirmala and

Pandian (1983) observed the highest growth increment when 20 mg/kg of testosterone was injected to Channa striatus.

The compound 11-ketotestosterone has been shown to be a naturally occurring androgen in teleosts (Eckstein, 1970; Katz and Eckstein, 1974) and is more potent than testosterone in its androgenic properties (Arai, 1967; Hishida and Kawamoto, 1970). This natural androgen increases the growth rate of carp Cyprinus carpio (Matty and Lone, 1979) and juvenile coho salmon (McBride and Fagerlund, 1976).

Testosterone propionate (TP) seems to be less potent in anabolic activity. The TP injections could not induce any significant growth increment over the control in the gold fish Carassius auratus (Hirose and Hibiya 1968a) and induced growth retardation in Lebistes reticulatus (Eversole, 1939; Svardson, 1943). In contrast, slight weight increment was observed in murrel fish (Channa striatus) when testosterone propionate was injected at the dose of 10mg/kg, but above this level, retardation of growth was observed (Nirmala and Pandian, 1983).

Experiments conducted by Hirose and Hibiya (1968b) indicated that the injection of the androgen 4-chlorotestosterone acetate (0.5 mg/4 day) promoted the growth

of gold fish and rainbow trout. In contrast, this hormone when fed through the diet at the dosages of 1 mg and 10 mg/kg showed no effect on the growth of coho salmon (McBride and Fagerlund, 1976). Testosterone acetate treated carp (Cyprinus carpio) also recorded better growth (Nagaraj and Rao, 1988). Guerero (1975) administered 1- dehydrotestosterone acetate to young Tilapia aurea for 21 days and obtained significant increase in weight over that of control.

Slight growth increment was observed by Fagerlund and McBride (1975) in O.kisutch when stanozolol was supplemented in the diets of immature channel catfish and gold fish (Bulkley and Swihart, 1973). Two synthetic androgens, dimethazine and norethandrolone were reported to increase weight gain significantly (Matty and Cheema, 1978) in rainbow trout. The androgen, oxymetholone was found to be a growth promoter in coho salmon (McBride and Fagerlund, 1976). While injections of methylandrostenediol could not produce significant weight increment in gold fish (Hirose and Hibiya, 1968a), methelone acetate could not promote growth in Salmo gairdneri (Matty, 1975).

Adrenosterone is a metabolic product of natural androgens in fish (Ozon, 1972a) and has exhibited growth

promoting ability in Cyprinus carpio when given with the food (Matty and Lone, 1979).

Ethylestrenol, a synthetic anabolic steroid has shown growth promoting effect in rainbow trout and Atlantic salmon when incorporated in the diet (Simpson, 1976; Ince et al., 1982). Arul (1986), observed that, when ethylestrenol was injected (5 mg/kg) to Channa striatus growth efficiency was increased by 1.5 times. In contrast to these results oral administration of this hormone resulted in negligible gains in Tilapia melanopleura (Hutchinson and Campbell, 1964).

Sower (1983) observed a growth increment when juvenile steel-head trout was fed on diet supplemented with an antiandrogen, flutamide (20mg/kg food).

EFFECT OF ESTROGENS:

Among estrogens, diethylstilbestrol (DES) has been found to be a very effective growth promoter in cattle.

It is therefore surprising that a growth response in fish has been a matter of dispute, the experimental evidence being quite conflicting. Several workers found no effect from oral administration of DES in fish (Ghittino, 1970; Bulkley, 1972;

Matty and Cheema, 1978; and Shyama and Keshavanath, 1988). However, there are a few reports in support of the anabolic effect of this steroid in fish. Moderate weight gain was recorded for Pleuronectes platessa fed at lower levels (0.2 to 1.2 ppm) of this estrogen (Cowey and Sargent, 1972; Cowey et al., 1973). In the Indian major carp, Labeo rohita, diet containing DES (3ppm) induced significant improvement in weight gain over the control (Nanjundappa and Varghese, 1988). A positive response was also obtained for Channa striatus injected with DES (10mg/kg) by Nirmala and Pandian (1983).

Estradiol (ES) did not provide satisfactory results in most of the fish species. Ashby (1957) treated brown-trout adding an alcoholic solution of estradiol to the water at different concentrations (50 to 300 mg/l), but growth of the fish was much retarded by estradiol and casualties were heavy. Similar response was observed by few other workers (Scott, 1944; Funk, 1972; Funk et al., 1973; and Yamazaki, 1976). In contrast to these results, estradiol accelerated the growth of common carp, Cyprinus carpio by 15% over that of control (Rao and Rao, 1988). Similarly, Nagraj and Rao (1988) found growth promotion in estradiol benzoate treated carp, Cyprinus carpio.

Estrone could not produce any anabolic effect when fed through diet (50-150 mg/kg) in rainbow trout, Salmo gairdneri (Scidmore, 1966). In Tilapia aurea fry estroid, estrone and 17 β -estradiol did not improve the growth significantly (Jensen and Shelton, 1979), whereas the injections of the estrogens estroid, diethylstilbestrol and do^sabolin (an antiandrogen) have promoted growth in Channa striatus (Nirmala and Pandian, 1983).

EFFECT OF THYROID HORMONES

The maintenance of adequate thyroidal status in fish is a prerequisite for normal growth. Thyroid hormones are thought to play a permissive role in growth process, potentiating the effects of other anabolic hormones, most notably growth hormone (Donaldson et al., 1979; Eales, 1979a). However, the benefits of administering thyroid hormones to fish with normally functioning pituitary and thyroid glands have, until recently, been less clear.

The data gathered on salmonid and non-salmonid fishes, demonstrate that fish growth and condition factor can be manipulated to positive or negative direction according to T4 or T3 dosages (Donaldson et al., 1979; Higgs et al., 1982).

Pharmacological doses of either T4 or T3, well in excess of their respective degradation rates (Eales, 1977a; Eales 1979b) and consequently resulting in plasma hormone titres many fold higher than the upper limits of their respective physiological ranges, have often been employed by investigators (Higgs et al., 1982). Such doses generally depress food intake, growth, and condition factor (Massey and Smith, 1968; Higgs et al., 1976, 1979) and enhance protein catabolism (Ray and Medda, 1976). Under a given set of experimental conditions, T3 induces these effects at lower doses than T4 (Higgs et al., 1982). This can be explained at least in part by the greater biological potency of T3 and its slower plasma degradation rate.

The efficiency of thyroid hormone as a growth promoter may be affected by the influence of several factors (i) fish size and/or water temperature (Thornburn and Matty 1963; Ray and Medda, 1976), (ii) diet composition (Fagerlund et al., 1983; Higgs et al., 1982) and level of dietary intake (Narayan Singh and Eales 1975b; Fagerlund et al., 1980) and (iii) season, treatment duration, route of hormone administration and dietary goitrogen content (Higgs et al., 1982). Thus in order to promote growth without morphological aberrations the dose of thyroid hormone employed should be

evaluated carefully in the context of above variables.

INTERACTIONS OF HORMONES

Very little attention has been paid to the study of various types of interactions between hormones on fish growth. Higgs et al. (1977a) have tried the combination of bovine growth hormone (bGH), MT and thyroid hormone (T4) on coho salmon, Oncorhynchus kisutch. These authors have demonstrated that hormone combinations significantly enhance fish growth.

Recently, the combination of MT and DES in the diet has been found to promote growth in Tor khudree and Hypophthalmichthys molitrix (Shyama and Keshavanath, 1988).

While a combination of DES + chlomiphene citrate -an antiandrogen (5:15 mg/g) produced gain in weight 15% more than the controls, combinations of MT + flutamide, MT and DES and estradiol + progesterone did not have any significant effect in weight compared to the control in the juvenile steel-head trout, Salmo gairdneri (Sower et al., 1983).

Effects of Hormones on Food utilisation

The application of steroid hormones to enhance the rate of efficiency of conversion of the ingested food in animal husbandry resulted in substantial savings in the production cost of meat. But it has received little attention in the field of aquaculture. However, there are few studies dealing with the food utilization of the hormone treated fish, to find out whether, the steroid hormones are appetite stimulants or anabolic growth promoters.

Moderate increase in food conversion efficiency (FCE) was observed in coho salmon and rainbow trout when fed on a diet containing MT (Simpson, 1976; Fagerlund et al., 1979). When coho salmon fry were daily fed to satiation on diets containing protein and/or lipid at two levels with or without MT, relatively high food conversion and protein efficiency ratio were attained for all the treated groups as compared to the control (Fagerlund et al., 1983).

While Lone and Matty (1980a) showed that MT supplemented diets improved the food conversion efficiency (FCE) in common

carp, Deb and Varghese (1988) did not observe any variation in food conversion efficiency between MT treated and untreated common carp groups. However, higher FCE were observed in fingerlings of Indian major carps, Catla catla, Labeo rohita, Cirrhinus mrigala when they received the dose of 1 mg MT or 3 ppm DES after 98 days as compared to the control (Deb and Varghese, 1988). Similarly, 40 mg/kg MT enhanced 39% FCE in catfish, Heteropneustes fossilis (Sindhu and Pandian, 1984). In the murrel, Channa striatus exposed to various androgens, Nirmala and Pandian (1983) reported higher FCE in fish groups treated with MT, testosterone and testosterone propionate. Similarly, increased FCE in testosterone treated chinook salmon and 11-ketotestosterone treated common carp was reported by Schreck and Fowler (1982) and Lone and Matty (1980a) respectively.

Effect of Hormones on Body Composition

The effect of anabolic steroids on the body composition had been worked out on several species of fish.

Studies made earlier shows that anabolic steroids exert only minor effects on moisture and protein contents of juvenile salmonids (Hirose and Hibiya, 1968b; Fagerlund et

al., 1979). Hirose and Hibiya (1968a) reported a slight decrease in protein and a decrease in non-protein nitrogen content when rainbow trout received 4-cholorotestosterone acetate orally, while Fagerlund et al. (1979) noted that, MT increased the protein content in coho salmon. A decrease in moisture content was noticed in MT treated coho salmon (Fagerlund and McBride, 1975), Atlantic salmon (Saunders et al., 1977) and chinook salmon (Fagerlund and McBride, 1979).

Fat content of coho was affected only marginally by administration of MT in doses of 0.2 and 1 ppm (Fagerlund and McBride, 1975), but 10 ppm increased the lipid content significantly. MT also significantly increased the lipid level in steel-head trout and pink salmon (Fagerlund and McBride, 1977). However, significant reduction in lipid content resulted from administration of MT and oxymethalone to coho salmon (McBride and Fagerlund 1976; Fagerlund and McBride, 1977; Fagerlund et al., 1979; 1980). The effect of MT on lipid content of muscle seems to be dependant on diet composition. When coho ^{were} fed diets containing high or low levels of lipid and or protein for four months (Fagerlund et al., 1983) the fish receiving low lipid diets had the lowest body lipid content, but adding MT to high lipid diets reduced whole lipid content regardless of dietary protein level.

Effect of Hormones on the fish ovary:

At present seeds for mullet culture are collected from the wild. However, due to the limitation in adequate availability of quality seeds and because of the season related maturation it has become essential to develop techniques for controlled maturation and spawning of the mullets. Maturation refers to the cyclic morphological changes that the female and male gonads undergo to attain full maturity. Attainment of full maturity, almost always marks a change in the growth pattern resulting from the 'reproductive drain' due to the diversion of the material meant for somatic growth to the gonads.

Oogenesis is the process of changes in the ovary from immature stage to ripening stage. Changes in the various cellular organelles of the oocyte during oogenesis have been described in a number of teleost species (Wallace and Selman, 1981; de Vlaming, 1982). In the first stage, the oogonia undergo proliferation by mitotic divisions and become primary oocytes when the chromosomes become arrested at the diplotene stage of the first meiotic prophase. Oocytes, unlike male gametes, enter a period of growth which varies from species to species. Enlargement of oocyte is caused mainly by the

accumulation of yolk.

Oogenesis and vitellogenesis stages have been described for a few Mugil species (Abraham, 1963; Abraham et al., 1966; Livni, 1971). Description of ovarian lamellae based on microscopical examination has been given for Mugil auratus, M.chelo and M.capito (Thang, 1969a).

Several ovarian stages have been described in Mugil species i.e. stages I to VII in Mugil chelo and O to V in Mugil cephalus (Brusle, 1981) and in Mugil capito by El Maghraby et al. (1974b) etc.

The hormones play an important role in the maturation of the ovary. Various teleost gonadotropin preparations induced oocyte maturation and ovulation in teleosts, i.e. carp gonadotropin (Burzawa-Gerard, 1974), pike and eel gonadotropins (Huang et al., 1981); maturational hormones from salmon (Idler and Ng, 1979). Pituitary hormones also seem to accelerate the process of oocyte maturation and ovulation (Ng and Idler, 1978, 1979; Idler and Ng, 1979). .op

Sex steroids function at various levels. They play a role in the genesis of the gonad both in differentiation and maintenance of somatic tissues, mainly the gonial ducts, and in gametogenesis. Steroidogenesis develops in immature fish when the endocrinological regulation of the future adult is developing and then participate in this regulation in adult. When gametes are ready for fertilisation, steroids act to bring the sexes together, stimulating the morphological characteristics and modulating sexual behaviour (Yamazaki and Watanabe, 1979). Both these actions may be retained after fecundation.

In immature but already sexually differentiated teleost, the effects of sexual steroids on gametogenesis vary according to species and experimental conditions (i.e. doses, mode of administration and treatment time and length).

Review of literature indicates that the exogenous androgenic steroids have either a degenerating or a suppressing effect on the development of the teleost ovaries. However, the ovaries of the coho salmon O.kisutch was found to be unaffected, when fed on a diet containing 10 mg or 50 mg MT/kg (McBride and Fagerlund, 1973), but when the concentration of this steroid was increased from 100 mg to 500 mg/kg, marked degeneration was noted in the ovary

(Fagerlund and McBride, 1975). Methyltestosterone was found to induce degeneration of the ovary of steel-head trout when incorporated in the diets at the level of 35 mg/kg diets (Sower et al., 1983).

Control as well as testosterone treated (200 ug) adult rainbow trout were found to be immature and their ovaries were in the state of previtellogenesis (Magri et al., 1984). When testosterone propionate was given to the female L.reticulatus in smaller dosages (0.3 to 0.5 mg/day) ova genesis was suppressed (Eversole, 1941), while the injections of the same steroid at levels of 0.025 - 0.25 mg/week, inhibited the ovary development (Eversole, 1944). Suppression of ovary ripening and adult female masculinization were observed in testosterone propionate treated L.reticulatus at the dose of 25 mg (Svardson, 1943).

The injections of estrogens seemed to stimulate growth of oocytes in the fresh water silver eel (Olivereau and Olivereau, 1979), and gonial divisions and vitellogenesis in the carp (Pantie and Lovern, 1978) but had no effect in rainbow trout (Upadhyay, 1977; Billard et al., 1982; Sower et

al., 1983) and led to cessation of vitellogenesis in pink salmon (Funk et al., 1973).

Further, when estradiol was added to aquarium water (6.5 to 15 mg/week), the external feminization was induced in L.reticulatus (Scott, 1944). In this fish, when fed on a diet containing estrone (45 rat units 3 times weekly) the ovaries were not affected (Berkowitz, 1938), but at the dose of 300,000 IB unit of this hormone, the degeneration of the adult female ovaries were inhibited (Svardson, 1943).

The ovarian development was also found to be retarded, when Nagraj and Rao (1988) fed carp Cyprinus carpio on diet containing 17 β - estradiol benzoate (800 mg/kg).

In the rainbow trout the process of exogenous vitellogenesis is regulated by ovarian estrogens (Takashima et al., 1971, 1972; Hara and Hirai, 1978; Elliott et al., 1979; van Bohemen and Lambert, 1980; van Bohemen et al., 1981a). Estradiol is the primary regulatory steroid (van Bohemen et al., 1981b), but estrone also participates significantly, although in an indirect manner (van Bohemen et al., 1981b).

However not much attention has been given to estrone by

earlier workers to study its effects on the sexually differentiated ovary of the fish.

Scope of the present work

Considerable efforts have been made to develop suitable supplementary feeds for the mullet Liza parsia (Ghosh et al., 1972, 1975; Das et al., 1979; Rangaswami and Raman, 1979). Besides, the role of salinity in feed intake and feed utilization (Paulraj and Kiron, 1988), the feeding rate (Kiron and Paulraj, 1988) and feeding frequency (Kiron and Paulraj, 1990) on this mullet have also been studied. Further, a comprehensive study was made using purified and compounded diets to find out the dietary requirements of protein, lipid, and selected vitamins of this fish (Kiron, 1989). Despite all these studies there is no information on the efficiency of any so called "growth promoting agents" to maximize the production of gold-spot mullet. Further, among the mullet species, with the exception of Mugil auratus (Bonnet 1970), there is no information on growth promoters. Therefore, the present study was made on Liza parsia fry.

Similarly, there is no information on the efficiency of steroids in advancing maturation in Liza parsia. Therefore,

experiments were undertaken to elucidate the effect of intramuscular injection of two steroids, MT and estrone, on the ovary of Liza parsia.

CHAPTER I I

MATERIALS

AND

METHODS

MATERIALS AND METHODS

Six feeding and two injection experiments were conducted in the laboratory. Feeding experiments were conducted to study the nutritional response of selected levels of steroid hormones and to determine optimum dosages in the diets of the mullet, Liza parsia, fry. Out of the six sets of feeding experiments, four sets were conducted using individual steroid hormones, viz., 17α - methyltestosterone (MT), diethylstilbestrol (DES), and 17β - estradiol (ES) with a view to finding out the most suitable and best growth promoting steroid hormone for this species. Based on the findings of these experiments one set of experiments was designed and conducted to determine the effect of individual as well as combined action of the steroid hormone, 17α - methyltestosterone and a thyroid hormone (T3). One experiment was also conducted to study the protein sparing action of 17α - methyltestosterone using diets containing selected dietary protein levels.

Both the injection experiments were conducted to study the effects of different dosages of the androgen 17α - methyltestosterone and the estrogen, estrone (En) on the immature ovary of the adult mullet, Liza parsia. Except for the experimental variables other biotic and abiotic parameters were maintained quite homogeneous in all the

treatments and replicates (3 for each treatment). The experimental design permitted an unbiased out-flow of data, aiding in successful statistical interpretations.

A) FEEDING EXPERIMENTS:-

i) Experimental Facilities:

Plastic tubs measuring 54 cm diameter and 30 cm height were used to rear the fish fry, for the feeding experiments. The tubs were arranged in vertical wooden steel-racks.

For feeding and injection experiments, compressed oil-free air was supplied to each of the tubs or tanks through rectangular air-diffuser stones of 30 x 15mm size, connected to the main air delivery system by plastic tubing, through plastic regulators. The air supply was maintained quite uniformly throughout the experimental period through the regulators. The aeration was suspended for at least one hour in the morning, while cleaning the tanks.

Sea water, salinity 32-35‰, collected from the open sea, off Cochin (depth 25-30m), was transported to the laboratory in plastic jerry cans (capacity 20l), filtered

thrice using 60 μ mesh bolting silk cloth and pooled into 600L capacity plastic pools. Salinity was adjusted to 15‰ (Paulraj and Kiron, 1988) by diluting the sea water with dechlorinated fresh water, and maintained in the sea water holding facility which consisted of a series of fibre glass tanks of 1000L capacity, each equipped with a biological filter for further clarification. For reducing the bacterial load the water stock was irradiated for 2 hrs. every day using a 125 W uv lamp. The used sea water was recycled for two more runs following the procedure mentioned above.

ii) **Experimental Animals and their Acclimatization:**

Liza parsia fry, mean total length 2.0 to 2.5cm and mean live-weight 200 to 300 mg were obtained either from Narakkal Fish Culture Facility of the Central Marine Fisheries Research Institute, Cochin or from the Fish Farm of the Kerala Agriculture University, at Puduvaipu, both located in the Vypeen Island, off Cochin. The fish were transported in plastic seed transportation bags of 20L capacity each holding over 100 fry in the ambient sea water and partly filled with oxygen to the nutrition experimental facility of the Central Marine Fisheries Research Institute, Cochin.

In the laboratory the fry were introduced into

large fibreglass pools, gradually acclimatizing them to the experimental salinity. During this transit phase, which lasted for two to three days, the fish were maintained on a diet of phytoplankton. Subsequently, they were hand-graded to ensure minimal size/weight variation and introduced into perspex glass tanks of 90cm length x 60cm width x 30cm depth and fed a starter semi-moist compounded diet to get them used to artificial compound diets and to the laboratory conditions. The entire acclimatization period was fixed as two weeks. Then the fish were transferred to the experimental plastic tubs and 25 fish were maintained in each tub.

At the start of the experiments and thereafter at regular intervals, depending upon the experiment, all the fish were weighed. The total length in cm and weight in mg were noted for each fish at the start and after termination of an experiment. The fish were weighed on a Mettler electronics balance. The entire procedure of recording the length and weight data of each fish was completed within seconds, giving least stress to the fish.

iii) Formulation and Preparation of Diets:

Semi-moist diets (moisture content 30-40%) were

TABLE 1: HORMONES AND THEIR DOSAGES USED FOR THE PRESENT STUDY

Name of hormone	Dosages (mg / kg diet)	Feeding period in days
17 α -methyltestosterone (MT)	0, 10, 20, 30, 40, 50 and 60	40 and 60
17 α -methyltestosterone (MT)	0, 2, 4, 6, 8, 10 and 15	45
Diethylstilbestrol (DES)	0, 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8	60
17 β -estradiol	0, 1, 2, 4, 6, 8 and 10	60
Combination of Thyroid hormone (T3) and MT	0, 3(T3), 6(T3), 9(T3), 2(MT), 4(MT), 3(T3) + 2(MT), 6(T3) + 2(MT), 9(T3) + 2(MT), 3(T3) + 4(MT), 6(T3) + 4(MT) and 9(T3) + 4(MT)	45
17 α -methyltestosterone (MT) at 3 protein levels (P = protein)	35% P + 1MT, 35% P + 2MT, 35% P + 3MT, 30% P + 1MT, 30% P + 2MT, 30% P + 3MT, 25% P + 1MT, 25% P + 2MT and 25% P + 3MT	60

TABLE II : COMPOSITON OF DIETS USED FOR THE EXPERIMENTS

FEED INGREDIENTS (in g/100g diet)	35% protein diet	30% protein diet	25% protein diet
Fish meal (Lipid free)	29	22.5	20.0
Ground nut oil cake (lipid free)	30	25.5	23.0
Corn oil	4	4	5
Cod liver oil	2	3	3
Tapioca	18	28	32
Rice bran	12	12	12
Gelatin	3	3	3
Mineral mixture *	1.5	1.5	1.5
Vitamin Mixture *	0.5	0.5	0.5
Proximate Composition of the feeds:			
Crude protein	34.8 ± 1.0	30.2 ± 0.58	25.0 ± 0.51
Total lipids	7.5 ± 0.35	9.1 ± 0.40	10.2 ± 0.41
Crude fibre	12.03 ± 0.56	13.14 ± 0.50	17.0 ± 0.52
Ash	14.52 ± 0.72	12.93 ± 0.45	17.0 ± 1.0
Nitrogen free extract	30.13 ± 0.53	34.91 ± 1.0	17.0 ± 1.0
Energy KJ/g	16.13 ± 0.35	15.94 ± 0.18	15.73 ± 0.31

② These diets were used only in one experiment conducted to test the protein sparing action of 17 methyltestosterone.

* See Table III

TABLE III : COMPOSITION OF THE MINERAL MIXTURE AND
VITAMIN MIXTURE USED IN THE DIETS

MINERAL MIXTURE	g/100g
Calcium biphosphate	13.58
Calcium lactate	32.7
Ferric citrate	2.97
Magnesium sulphate	13.2
Dibasic Potassium phosphate	23.98
Sodium biphosphate	8.72
Sodium chloride	4.35
Aluminium chloride	0.015
Zinc sulphate	0.3
Cuprous chloride	0.01
Manganese sulphate	0.08
Potassium iodide	0.015
Cobaltous chloride	0.1
VITAMIN MIXTURE	mg/g
Choline chloride	500
Inositol	200
L - Ascorbic acid	100
Nicotinic acid	75
Calcium pantothenate	50
Riboflavin	20
Thiamine hydrochloride	5
Pyridoxine hydrochloride	5
Folic acid	1.5
Cyanocobalamin	1.1
Biotin	0.5
Cholicalciferol	0.2
Menadione	4
L - Tocopherol acetate	40

used for the experimental studies as the initial feeding trials had indicated a preference for the same.

Locally available ingredients, viz., groundnut oil cake, tapioca, fish meal, rice bran and gelatin were the main sources used for compounding diets. Lipid sources included were corn oil and codliver oil, which provided the essential n-6 and n-3 fatty acids such as linoleic, linolenic, eicosapentaenoic and docosahexaenoic acids (Watanabe, 1982). The composition of mineral and vitamin mixtures used in the diets is shown in Table III. Gelatin was used as a binder in addition to getalinised tapioca.

Proximate composition of each of the ingredients was determined before feeds were formulated. Diets were formulated to contain about 35% protein as shown in Table II for all the experiments. However, to study the protein sparing action, besides the common experimental diet containing 35% protein, two other diets with protein levels of 30% and 25% were prepared (Table II). The energy content of the diets for protein - sparing action experiment was adjusted by varying the levels of tapioca, the main source of carbohydrate and lipids (Table II). The proximate composition of the ingredients and finished feeds was

determined by standard methods (AOAC, 1984) and are shown in Table II.

The individual feed ingredients were grounded in a domestic grinder, sieved through 200 μ mesh sieve and stored in cleanly washed, oven-dried bottles. All the powdered ingredients were weighed for the respective lots of the experimental diets. Each time feed was prepared to supply a fortnight ration. The fat - soluble vitamins were added to the oil mixture and mixed thoroughly.

Experimental diets were prepared as follows:

Gelatin was dissolved in cold double distilled water (30ml/100gm diet) taken in a container. Then it was boiled over a water bath; tapioca powder was then added on to gelatin and the contents were boiled and mixed. To this was added premixed mixture of groundnut oil - cake powder, fish - meal and mineral mixture and the diet was mixed thoroughly. After reducing the heat, oil mixture was added and churned. After cooling to room temperature water - soluble vitamin mixture was added to the feed-dough and blended thoroughly. The pH of the diet was maintained near neutral with 0.5 N NaOH. The prepared feed was cooled and stored in a freezer.

The overall moisture content of the diet ranged between 30 and 40% during the different experiments.

iv) **Hormone Administration:**

The steroid hormones, 17 α -methyltestosterone, diethylstilbestrol, 17 β -estradiol, estrone and a thyroid hormone, 3,5,3-triiodo-L-thyronine (T3) were procured from Sigma Chemicals Company St. Louis, MO 63178, USA.

The specified quantity of the selected hormone was dissolved in minimum solution of 95% ethanol, and sprayed on to the feed dough kept in a tray (length 45cm x 30cm width x 15cm depth) with continuous churning. After the addition of required quantity of hormone in the diet, the feed dough was continuously mixed, smeared and resmeared by hand under the continuous circulation of fan-air, for about half-an-hour to evaporate maximum amount of ethanol from the diet. The diet so prepared was kept in air-tight plastic dishes and maintained in a freezer. Everyday a block was taken, thawed, weighed for the respective treatments on a dry matter basis and offered to the fish. To observe the digestibility pattern of the animals 1% chromium oxide was incorporated in the diets, whenever it was required.

v) **Monitoring of Water Quality:**

Salinity of the water was determined using a A.O. hand refractometer (American Optical Corporation, U.S.A) and it was maintained 15‰ throughout all the experiments. Dissolved oxygen was monitored using a Elico oxygen meter. The recorded values of salinity and oxygen contents were cross checked with the respective titrimetric methods (Strickland and Parsons, 1972) frequently. Ammonia content in water was determined in samples drawn before and after water change in the experimental system. On collection, the water samples were fixed with 4% ethanol solution and stored in refrigerator before it was taken up for analysis, within 2 hrs. The ammonia concentration was determined using the phenol-sodium hypochlorite method (Solarzano, 1969). pH of the water samples from the experimental tanks was determined thrice a week using an Elico digital pH meter with an accuracy of 0.001. All pH determinations were done at room temperature. The experimental facility was located in well lighted rooms and hence experiments were conducted providing natural photoperiod.

vi) **Data Collection:**

Experimental duration depended upon the objectives

of each experiment and ranged from 30 to 60 days. The length and weight of the animals were measured at regular intervals and at the end of the experiments, adopting similar procedures.

During the experimental period, daily observations were made on their feeding behaviour, swimming movements, acceptability of food and patterns of faecal output. On termination of an experiment, the animals were quick-frozen to avoid post-mortem changes. Later a sample was drawn for determining the moisture content. The rest of the animals in each replicate were freeze-dried and stored in desiccator for biochemical analysis.

Survival Rate:

Mortality of the fishes were recorded daily from each of the experimental treatments. At the end of the experiment final percentage survival was determined as follows:

$$\text{Percentage survival} = \frac{\text{No} - \text{N}}{\text{No}} \times 100$$

Where No is the initial number and N the number of dead fishes.

Growth Rate:

$$\text{Mean weight gain(\%)} = \frac{W_t - W_o}{W_o} \times 100$$

Where: W_o = mean initial weight
 W_t = mean final weight

Since growth pattern over a longer period was a monotonically decreasing function of time (Kruger, 1965) specific growth rate (SGR) was calculated as percent of daily growth rate.

$$\text{SGR} = \frac{W_t - W_o}{t/2(W_t + W_o)} \times 100$$

Where t is the duration of the experiment in days and W_o and W_t same as above.

Condition Factor (CF):

(CF) was obtained using the following formula

$$\text{CF} = \frac{W_t}{L_t^3} \times 100$$

Where W_t is the mean final weight and L_t is the mean final length.

Food Utilisation Indices:

The data obtained on food consumption during the experimental duration had been used for computing several related aspects.

$$\begin{aligned} \text{Feed Conversion Rate(FCR)} &= \frac{\text{Feed intake}}{\text{Weight gain}} \\ \text{Protein Efficiency Ratio(PER)} &= \frac{\text{Weight gain}}{\text{Protein intake}} \\ \text{Gross Conversion Efficiency(GE)} &= \frac{\text{Weight gain}}{\text{Feed intake}} \times 100 \\ \text{Digestibility coefficient (Da)} &= \frac{I - F}{I} \times 100 \end{aligned}$$

where, I = Feed intake and F = Faecal Output

vii) Proximate Composition Analysis of Body and Feed:-

Proximate analysis (CMFRI, 1982; AOAC, 1984) was performed on triplicate samples of each diet and on a composite sample of fishes from each replicate. The moisture content was determined by oven-drying at 70°C for 48 hours. The freeze - dried fish and feed samples for chemical analysis were ground in a microgrinder. Crude protein was determined by the micro-kjeldahl method using the conversion

factor $N \times 6.25$. Samples were extracted with petroleum ether in a Tecator system for crude fat (soxhlet method) determinations. Moisture - free ether extracted samples were digested with weak acid and then weak base in a Tecator fibretec system, washed with acetone and finally the organic residue ignited to determine the crude fibre. Ash was determined in samples after incineration in a muffle furnace at 550°C . Nitrogen - free extract was calculated by difference from the above values. In the digestibility studies, the content of the chromic oxide indicator was measured spectrophotometrically (Furukawa and Tusukahara, 1966). Analytical measurements on pooled faecal samples were carried out wherever necessary using the same technique, described above. The productive energy value of feeds was determined using a Gallenkamp ballistic bomb calorimeter.

viii) Statistical Analysis:

The data for diet response had been subjected to statistical analysis to arrive at valid conclusions. One way analysis of variance was performed to the treatment effects and the "t" test was employed to locate the significant difference between means. Data from the experiment where

combination of T3 + MT as well as their individual doses was tested the 3 x 2 factorial design analysis was performed to test the significant differences among the treatments. Similarly 3 x 3 factorial design analysis was also carried out on the data derived from protein sparing action experiment.

B) INJECTION EXPERIMENTS:

Two experiments were conducted to observe the histological changes in the ovary of the adult fish associated with the injection of 17 α -methyltestosterone and estrone.

During August 1986, a stock of the gold-spot mullet, measuring 11-12cm length and 45-50 g weight, were collected from the fish ponds at Narakkal using a cast-net. Immediately the fish were transferred at the rate of 10 fish per tank (1000L) which were arranged adjacent to these fish ponds, in the experimental shed of Narakkal Fish Culture Facility of Central Marine Fisheries Research Institute.

Few fishes were collected randomly from the rearing plastic pools using handnet to observe the state of the gonads (ovaries) of which females were found to be

immature having only oogonia.

Initially for two days all the fish were reared in the pools containing filtered water collected from the fish ponds. Same pond water was used for the rearing of the animals throughout the experiments, the salinity of which ranged between 15 to 20‰.

Acclimatization of fish to the laboratory conditions and a compounded diet was done. After two weeks, healthy animals were segregated and transferred them to fibreglass tanks (1000L capacity). All the fish in one tank were considered as one treatment group and all of them were fed a compounded diet (Table II) throughout the experimental duration.

i) **Hormone Administration:**

The selected dosages of steroid hormones 17 α -methyltestosterone and estrone were 0.0, 0.5, 1.0 and 1.5 as well as 0, 1, 2 and 3 mg/kg body live - weight respectively.

Stock solutions of these steroid hormones were prepared in 95% ethanol. From this appropriate quantity of stock solutions were diluted to 1% by adding saline (0.6

NaCl) (Hiroshi et al., 1985). The volume of the hormone solution injected into the fish was maintained constant at 0.2 ml for all the tested dosages. Similar doses of ethanol devoid of any steroid hormones were given to the control fish. All injections were given intramuscularly (i.m.) at the caudal region mid-way between the lateral line and ventral fin. The injections were given within a handling duration of one minute with minimum stress to the individuals.

ii) Histological Investigation:

Histological studies were done on ovaries of fish injected with different levels of two steroid hormones, 17 α -methyltestosterone and estrone. At the end of the experiments, fishes were dissected and their ovaries collected one by one, cut into small pieces of manageable size and fixed in Bouin's fluid for 24 hours in labelled bottles. Then thoroughly washed with running water for 5-6 hours to remove all the excess fixative and then it was dehydrated in alcohol grades (30 minutes in each) as follows:

30% > 50% > 70% (two changes)

90%, rectified spirit (95%) and absolute alcohol (two changes of 10 minutes each). From absolute alcohol tissues were cleared in chloroform by immersing for 10

minutes and then filtered in wax, two changes of one and half hours each (wax bath). Finally, the tissues were embedded using molten wax (56 to 58^o C) by pouring it into small aluminium boats. Extreme care was taken to prevent formation of air-bubbles inside the wax blocks. The tissue embedded in wax was then trimmed to small blocks and mounted on a wax holder of the microtome for sectioning.

Thin sections (5-7 μ) of the ovaries were cut with a manually operated rotary microtome. The wax ribbons were then mounted on albumin applied glass slides. Few drops of water were carefully added onto the slide, so that the ribbons floated over it. The slides were then warmed (40-45^o C) over a slide-warmer to spread the wax ribbons uniformly. Excess water was drained off and the slides were kept for overnight incubation at 37^o C and then stained in Ehrlich Haematoxylin-Eosin.

After dewaxing with xylene solution the sections were hydrated with alcohol grades in the order 100%, 90%, 70%, 50% and 30%, keeping for five minutes in each grade of alcohol and finally with distilled water (two washes of 15 seconds each). After hydration, the sections were stained by keeping in haematoxylin for 30 minutes. The stained slides

were kept under running tap water for 10 minutes and placed in lukewarm water till the appearance of blue colour in the sections and again rinsed in distilled water. The stained slides were then dehydrated by keeping two minutes first in 50% and then in 70% alcohol and then stained the sections with 0.5% Eosin(made in 70% alcohol) by giving 4-5 dips. The tissues were then transferred to 95% alcohol for 1-2 dips to remove excess Eosin and finally gave two changes of 1-2 dips each in absolute alcohol for complete dehydration.

Two changes of ten minutes each were given in the xylene to clear the slide and permanent slides were made using DPX mountant.

iii) Microphotography:-

Sections were observed under the light microscope and selected slides were subjected to microphotograph using a vanox-S Microscope with automatic microphotographic system at the magnification of 10 x 10 and 10 x 40 mm.

CHAPTER III

RESULTS

RESULTS

1. 17 α -METHYLTESTOSTERONE (MT)

The results of the two sets of experiments conducted to study the effects of selected levels of the androgen 17 α -methyltestosterone on the survival, growth, feed efficiency and proximate composition of Liza parsia fry are shown in Figs. 1 to 7 and Tables IVa, b and V.

Experiment 1:-

In the first set of experiments compounded diets, containing selected levels of MT ranging from 0 mg to 60 mg/kg, with an interval of 10mg/kg, were fed to the fish fry for sixty days. As heavy mortality was encountered in the fish groups receiving diets containing 40mg to 60 mg MT they were discontinued after forty days, and data on growth, feed efficiency and proximate composition were collected. The remaining fish groups were reared for sixty days and growth and survival data were collected.

The rearing conditions during the experimental period were as follows: salinity 15 ± 1 ppt; temperature $27.4 \pm 2.1^{\circ}$ C; pH 7.956 ± 0.114 ; ammonia 0.252 ± 0.113 mg/l; oxygen 4.82 ± 0.33 ppm.

The survival rates (Table - 5a, b) were significantly

Response Parameters	control	MT levels in the diet (mg / kg)					
		10	20	30	40	50	60
Mean initial weight (g)	0.794	0.793	0.786	0.791	0.79	0.794	0.798
Mean final weight (g)	1.47	1.493	1.41	1.288	1.166	1.043	0.922
Specific growth rate (SGR)	2.128	2.129	1.939	1.55	1.171	0.784	0.403
Condition factor (CF)	1.295	1.296	1.35	1.38	1.422	1.452	1.494
Survival %	97	90	87	80	73	67	53
Gross conversion efficiency (GCE)	25.4	25.6	23.2	23	18.2	11.8	6.3
Digestibility coefficient (Da)	78.13	78.28	76.32	75.27	72.88	70.23	64.58
Food conversion ratio (FCR)	3.936	3.896	3.201	4.347	5.483	8.427	15.862
Protein efficiency ratio (PER)	0.725	0.733	0.689	0.657	0.521	0.339	0.18
Proximate composition							
Moisture %	71.27	71.12	72.87	73.64	74.13	74.81	75.95
Protein %	60.2	60.15	57.87	57.35	56.7	55.54	53.1
Lipid %	22.3	22.72	21.08	20.86	20.4	19.5	19.1
Ash %	14.18	14.05	14.8	15.2	15.7	16.1	16.6

TABLE IV (b): EFFECT OF 17 α -METHYLTESTOSTERONE (MT) ON
GROWTH OF LIZA PARSIA AFTER 60 DAYS
(0 - 30 mg/kg)

Response Parameters	MT levels in the diet (mg/kg)			
	control	10	20	30
Mean final weight (g)	1.802	1.682	1.414	1.193
Specific growth rate (SGR)	2.231	2.151	2.021	1.834
Condition factor (CF)	1.280	1.292	1.113	1.025
Survival %	95	88	77	65

Fig. 1 Effects of selected levels of 17 α -methyltestosterone (MT) on the growth of *Liza parsia*.

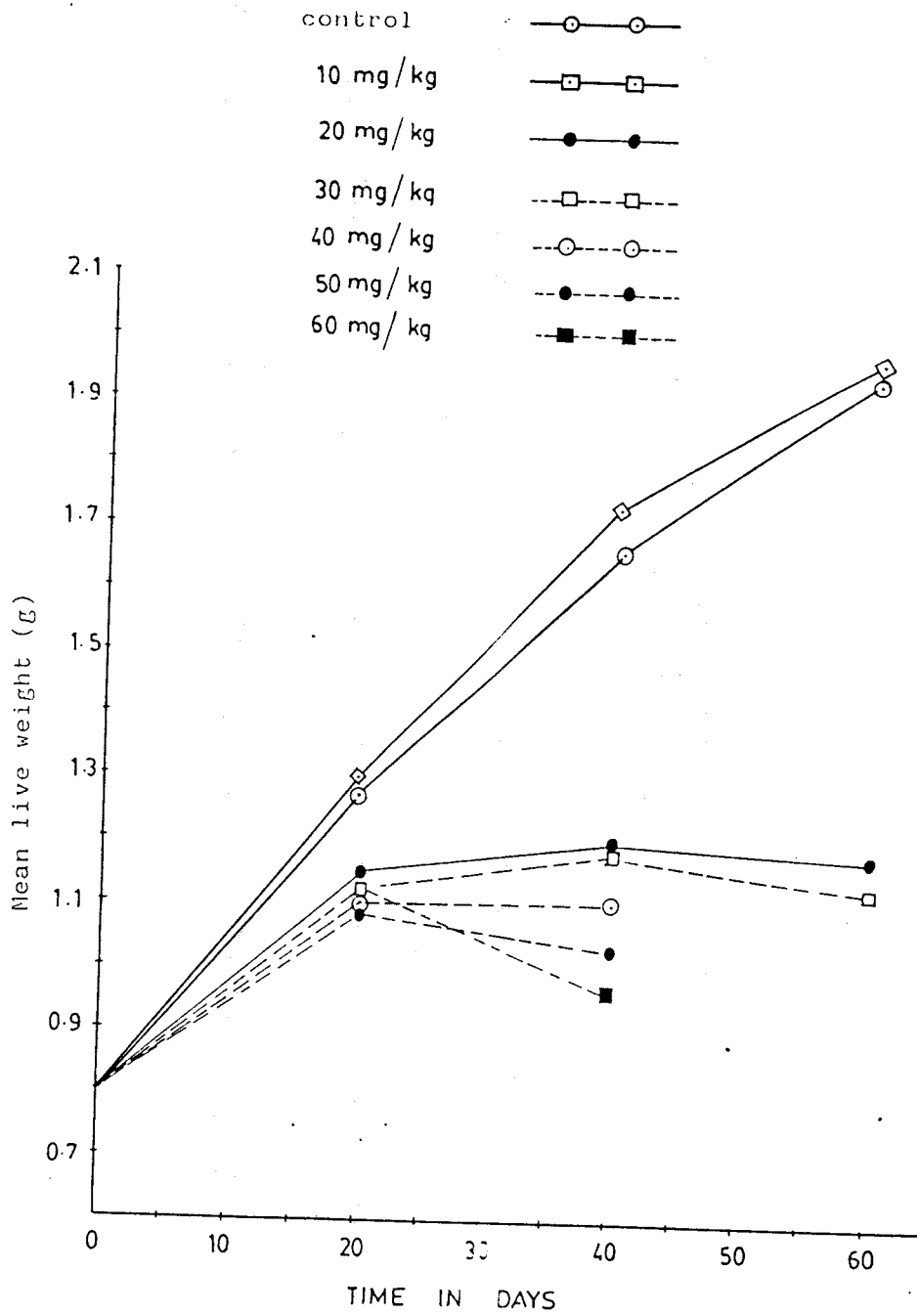
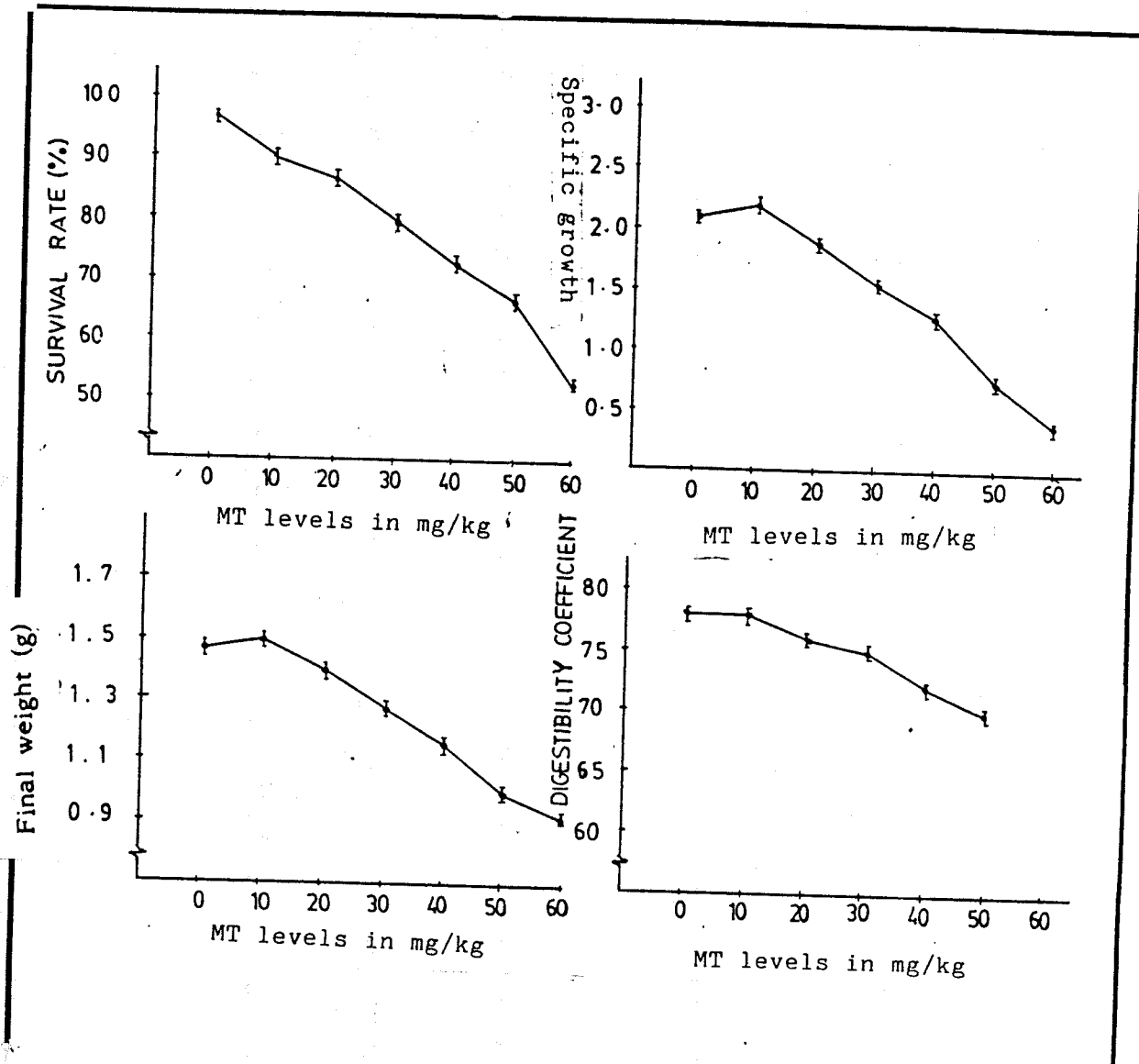


Fig. 2. Effects of selected levels of 17α -methyltestosterone (MT)

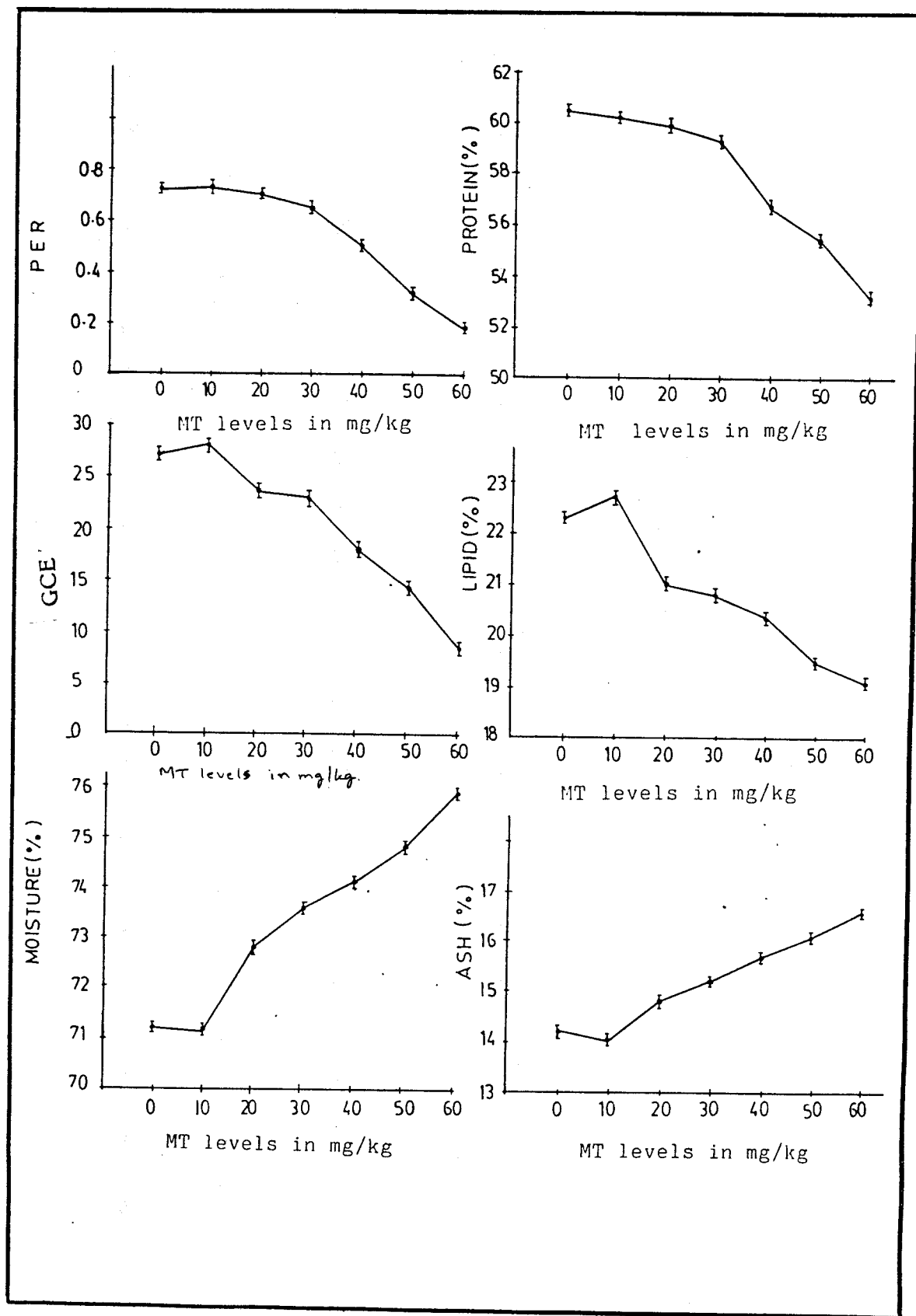


influenced by the MT levels. The survival rate ($P < 0.01$) exhibited a declining trend (Fig.2) with increasing MT dosages. After 40 days of rearing, the highest (97%) survival rate was recorded for the steroid-free control diet and the survival rate was only 53% for the fish groups receiving 60 mg MT in the diet. Almost a similar pattern (Table-IVb) was evident upto the 60th day in the fish groups fed on 10 mg to 30 mg MT diets. The steroid-free control diet provided the best survival rate of 95% followed by the 10mg MT diet (88% survival) and the lowest rate of survival was obtained for the 30mg MT diet after 60 days.

After 40 days of rearing there was no significant difference in the weight gains of the fish between the 10mg MT diet and the control (Table IV a & Fig.1&2). All the diets with MT concentrations exceeding 10mg produced significantly less weight gains than the control. After sixty days the lowest (1.193g) and the highest (1.802g) weight gains were observed in fish receiving the 30mg MT diet and the control diet respectively.

The fish groups fed on diets containing 20mg MT onwards gave significantly ($P < 0.01$) lower specific growth (Fig-2) than the control fish after 40 days. The specific growth for

Fig. 3. Effects of selected levels of 17 α -methyltestosterone (MT)



the 10mg/MT fish did not differ significantly from the control fish. The lowest specific growth (0.403) was recorded for the fish fed on the 60 mg MT diet after 40 days of feeding. However, after 60 days the specific growth of fish fed on 10mg to 30 mg MT diets were significantly lower than that of control fish (Table - IVb).

Statistical analysis revealed that the condition factor (CF) of fish from various treatments were significantly influenced ($P < 0.01$) by the MT dosage. However, after 40 days, no significant difference in CF was evident between the fish groups fed on the 10 mg MT diet and the control diet (Table - IVa). The fish in the remaining treatments showed lower values than the control animals, with a steady decline in the CF as the concentration of MT in the diets increased.

The protein efficiency ratio (PER), food conversion ratio (FCR) and the apparent digestibility coefficient (D_a) recorded in the fish treated with 10mg MT dose were similar to that of fish fed on the steroid-free control diet (Table IVa & Fig-2&3). All other fish groups showed lower values for PER, and D_a than the control. However, the best FCR (3.201) was recorded for groups fed on the 20mg MT diet.

The proximate analysis of the fish revealed that the

body composition (Table IVa & Fig-3) is significantly influenced ($P < 0.01$) by the MT levels in the diets. However there was no significant difference in the protein content of the fish between the steroid-free diet and those receiving 10mg MT in the diet. Trends obtained for protein and lipid were similar (Fig -3). A small peak for protein and lipid was observed for the 10mg MT diet and thereafter a decline was noted. The minimum values of protein (53.01%) and lipid (19.12%) were observed for the diet containing 60 mg MT.

Moisture content was also affected significantly by the various steroid dosages in the diet (Table - IVa). The fish fed on the 10mg MT diet had the lowest moisture content (71.12%) while the highest moisture content (75.95%) was found in fish fed the 60 mg MT diet. Moisture content in the remaining fish groups ranged between 71.27% and 74.81% (Table 5a). A similar trend was observed for ash with the lowest (14.05%) and the highest (16.6%) values for fish receiving 10 mg MT and 60 mg MT respectively. The fish from remaining treatments had ash content ranging between 14.18% and 16.1%.

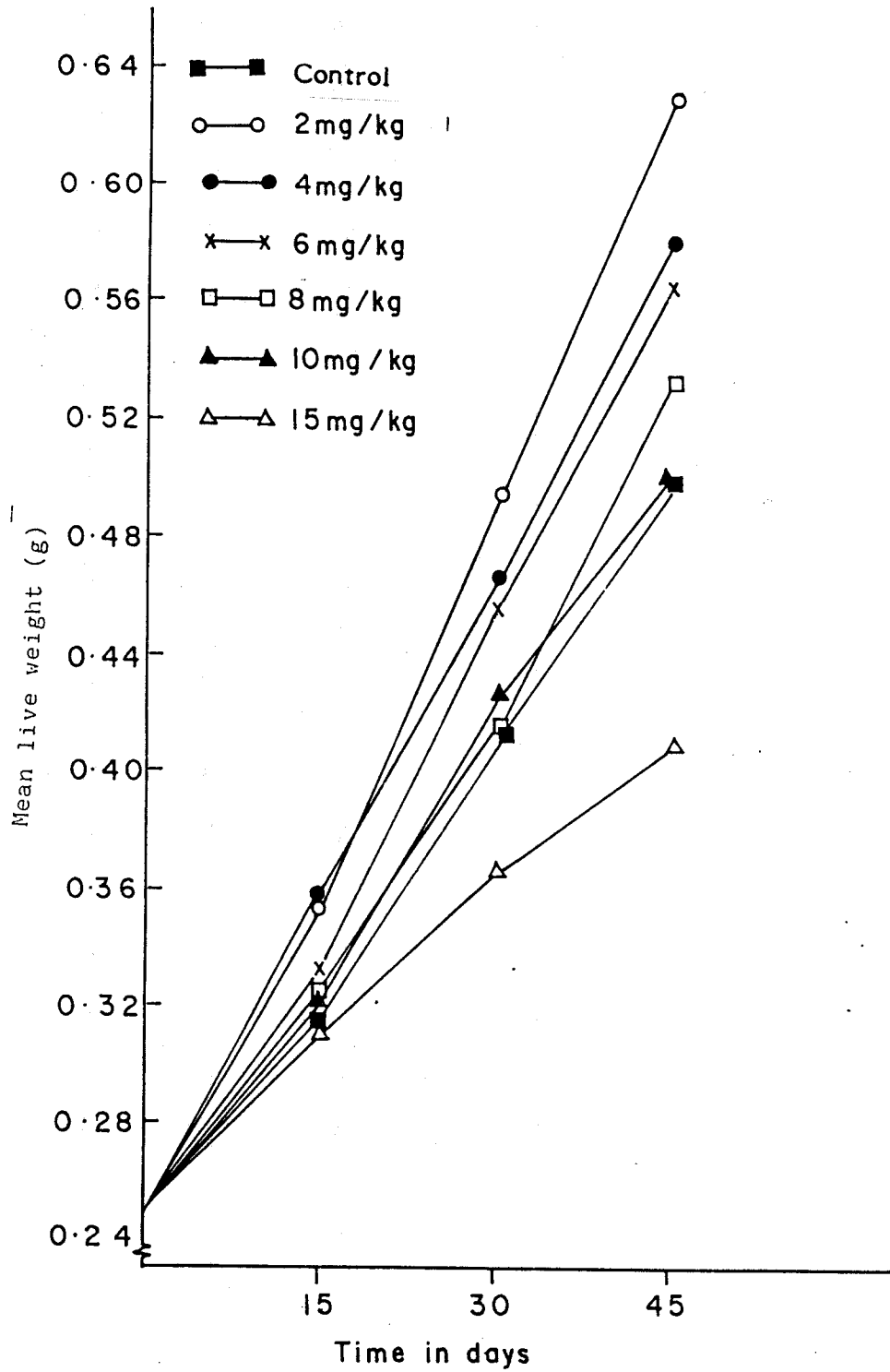
Experiment - 2

Based on the response attained in the first experiment,

TABLE V : EFFECT OF 17 α - METHYLTESTOSTERONE (MT) ON GROWTH, FOOD CONVERSION AND BODY COMPOSITION OF LIZA PARSIA AFTER 45 DAYS (0 - 15 mg/kg)

Response Parameters	I	MT levels in the diet (mg/kg)						I
		control	2	4	6	8	10	
Mean initial weight (g)	I	0.266	0.265	0.266	0.265	0.267	0.265	0.268
Mean final weight (g)	I	0.502	0.628	0.58	0.566	0.533	0.506	0.464
Specific growth rate (SGR)	I	1.969	3.022	2.625	2.509	2.232	2.009	1.65
Condition factor (CF)	I	1.105	1.298	1.235	1.201	1.167	1.12	1.075
Survival (%)	I	96	93	93	92	88	88	84
Gross conversion efficiency (GCE)	I	27.7	38.1	34.6	33.4	30.5	27.8	24
Digestibility coefficient (Da)	I	78.2	79.33	78.89	78.91	78.53	78.24	77.65
Food conversion ratio (FCR)	I	3.511	2.623	2.888	2.988	3.274	3.591	4.157
Protein efficiency ratio (PER)	I	0.792	1.09	0.989	0.955	0.872	0.795	0.546
Proximate composition	I							
Moisture %	I	70.26	70.13	70.31	70.76	70.87	71.14	72.22
Protein %	I	60.46	62.8	61.33	60.43	60.33	60.13	57.86
Lipid %	I	22.54	24.23	23.66	23.1	22.73	22.65	21.23
Ash %	I	14.2	12.7	13.4	13.6	13.8	14.9	15.1

Fig. 4 Effects of selected levels of 17α -methyltestosterone (MT) on the growth of Liza parsia.



with wide range of dosages, another experiment was carried out to define the optimum level of 17 -methyltestosterone (MT) required for promoting maximum growth, food conversion and nutrient deposition in the tissues and the results are shown in Fig 5-7 and Table V.

The rearing conditions during the experiment, which lasted for forty five days, were as follows: salinity 15 ± 1 ppt; temperature $28.6 \pm 2.2^{\circ}$ C; pH 7.935 ± 0.118 ; ammonia 0.254 ± 0.112 mg/l; oxygen 4.80 ± 0.25 ppm.

The best survival rate (96%) was recorded for the control diet; and the diets supplemented with 2mg and 4mg MT gave 93% survival. The percentage survival rate ranged from 92 to 84 for the remaining treatments with the lowest (84%) in the treatment given 15 mg MT.

Trend obtained for the growth (Fig-5) showed that all the fish groups, with the exception of the fish receiving the 15mg MT diet, produced better growth than the control, throughout the experimental period. The growth attained upto the 15th day was quite similar for all the fish groups and significant difference was evident between groups only during the subsequent rearing period. However, the 10 mg MT diet produced growth comparable to that of the control fish after

Fig. 5 Effects of selected levels of 17 α -methyltestosterone (MT)

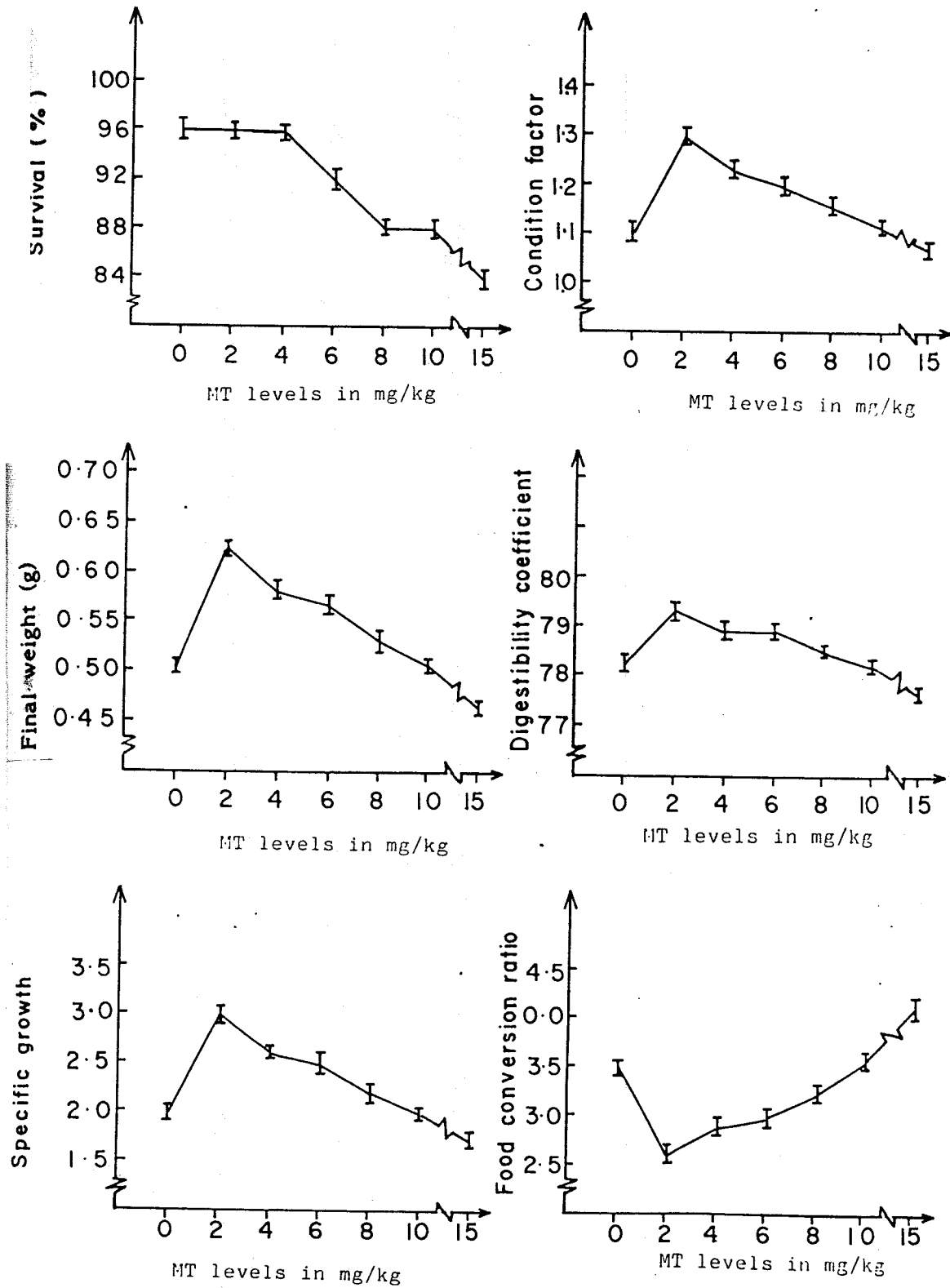
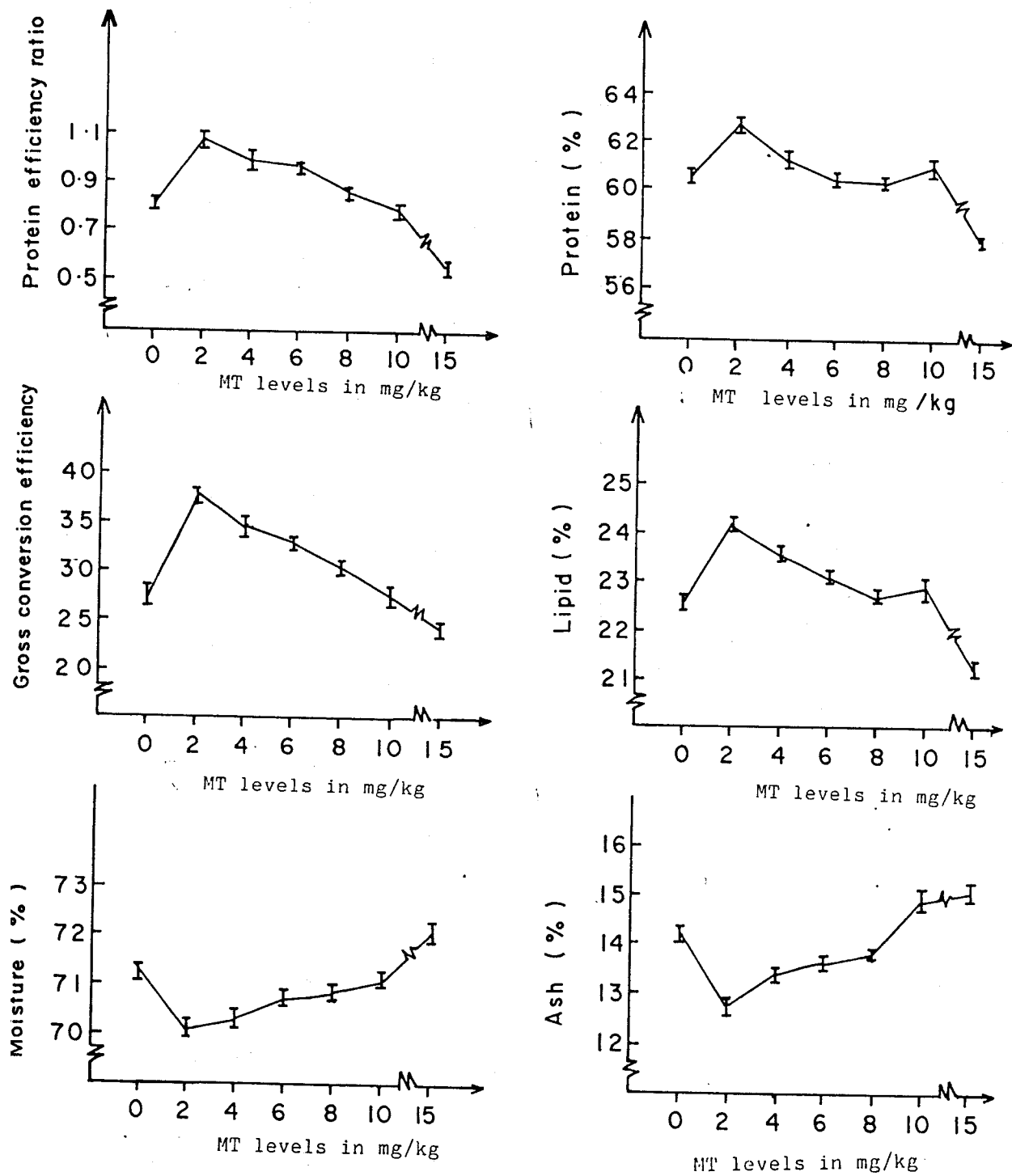


Fig. 6. Effects of selected levels of 17α -methyltestosterone (MT)



45 days. While the 2 mg MT diet produced the best growth, 4mg MT diet was found to be the second best growth promoter after 45 days (Table V). The fish receiving 15 mg MT diet showed a consistently slower growth than the fish from control and other treatments.

The specific growth rate (Fig - 6) followed a similar trend to that of the percent weight gain with the highest (3.022) and the lowest (1.650) for the diets with 2 mg and 15 mg MT diets respectively (Table V).

There was no significant difference ($P < 0.01$) in the condition factor of the fish between the control and the dosages of 10mg and 15mg MT (Table - 6). MT dosages from 2 mg to 10 mg produced better condition factor than the control (Fig. 6). After 45 days, the highest CF value (1.298) was recorded for the 2 mg MT diet fed fish. While the control diet fed fish had CF of 1.105, the 15 mg MT diet gave the lowest CF of 1.075.

The digestibility coefficient (Da) and protein efficiency ratio (PER) were higher for most of the treatments than the control (Table V). However, no significant difference was observed between the control diet and the diet containing 10mg MT. The highest PER (1.09), Da (79.33) and the

best FCR (2.623) were recorded for the 2mg MT diet. A steady decline in Da, PER and gross conversion efficiency (GCE) occurred when MT levels in diets exceeded 2mg/kg. The fish groups receiving the highest MT dosage (15 mg/kg MT) in this experiment gave lower values for PER, GCE and Da than the groups fed on the steroid-deficient diet. FCR showed an inverse trend with that of PER and Da (Fig. 6&7). Diets containing 2 mg, 4mg and 6mg MT provided better FCR than the control and other dietary MT concentrations.

Protein, lipid and moisture contents of the fish were significantly ($P < 0.01$) affected by the various levels of 17 α -methyltestosterone. The protein and lipid levels in the fish were relatively high for the 2mg MT diet (Fig 7). Inclusion of 15 mg MT in the diet resulted in markedly low protein and lipid levels (Table V). The highest (62.8%) and the lowest (57.86%) values for protein content were obtained for the fish on the 2 mg and 15 mg MT diets respectively. The results obtained for moisture and ash are presented in Table V. The moisture and ash levels were lowest for the fish group fed on the 2mg MT diet. The differences in moisture levels observed among the fish fed 2mg to 8mg MT diets were not significant (Table V & Fig.7).

2. DIETHYLSTILBESTROL (DES)

One experiment was conducted for a period of 60 days to study the effects of the estrogen diethylstilbestrol (DES) on growth, food conversion and proximate composition of the mullet and the results are presented in Fig. 8, 9 & 10 and Table VI. The selected dosages of the steroid ranged from 0mg to 1.8mg/kg diet, with an interval of 0.3 mg.

The rearing conditions during the experimental period were as follows: salinity 15 ± 1 ppt; temperature $27.4 \pm 2.1^{\circ}$ C pH 7.902 ± 0.114 , ammonia 0.245 ± 0.061 mg/l, oxygen 4.82 ± 0.33 ppm.

Table VI shows the percentage survival of the various fish groups fed the experimental diets. Only, the 0.3 mg/kg DES diet gave a survival rate comparable to that of the control fish groups (94.5%). In all other treatments, survival rate was found to be lower than the control, which ranged from 78.2% to 87.4% (Fig.9).

Growth trend (Fig.8) indicates that except for the fish groups receiving 0.3 mg DES in the diet all the diets produced lower weight gains than the control throughout the experimental period. After 60 days the fish fed the diet containing 0.3 mg DES had marginal difference in percent

TABLE VII EFFECT OF DITHYLSTILBESTROL (DES) ON GROWTH, FOOD CONVERSION AND BODY COMPOSITION OF LIZA PARSIA AFTER 60 DAYS (0 - 1.8 mg/kg)

Response Parameters	I	DES levels in the diet (mg/kg)						I
		Icontrol	0.3	0.6	0.9	1.2	1.5	
Mean initial weight (g)	I	0.354	0.352	0.352	0.357	0.356	0.354	0.354
Mean final weight (g)	I	0.823	0.836	0.75	0.719	0.707	0.658	0.609
Specific growth rate (SGR)	I	2.203	2.265	1.859	1.715	1.656	1.427	1.199
Condition factor (CF)	I	1.201	1.237	1.17	1.129	1.105	1.073	1.025
Survival %	I	94.8	94.5	86.6	87.4	84.5	80.8	78.2
Gross conversion efficiency (GCE)	I	26	26.1	22.6	21.2	20.5	18	15.7
Digestibility coefficient (Da)	I	78.32	78.45	78.03	77.63	77.63	76.84	75.38
Food conversion ratio (FCR)	I	3.841	3.822	4.409	4.715	4.86	5.535	6.357
Protein efficiency ratio (PER)	I	0.743	0.747	0.648	0.605	0.588	0.516	0.449
Proximate composition	I							
	I							
	I							
	I							
	I							
Moisture %	I	72.7	72.86	73.29	73.14	73.28	73.42	73.87
Protein %	I	59.36	59.8	59.23	59.1	58.8	58.63	58.63
Lipid %	I	22.16	22.43	22.13	21.7	21.3	21.16	20.76
Ash %	I	14.27	13.9	14.4	14.8	15.1	15.2	15.4

Fig. 7 - Effects of selected levels of diethylstilbestrol the growth of Liza parsia.

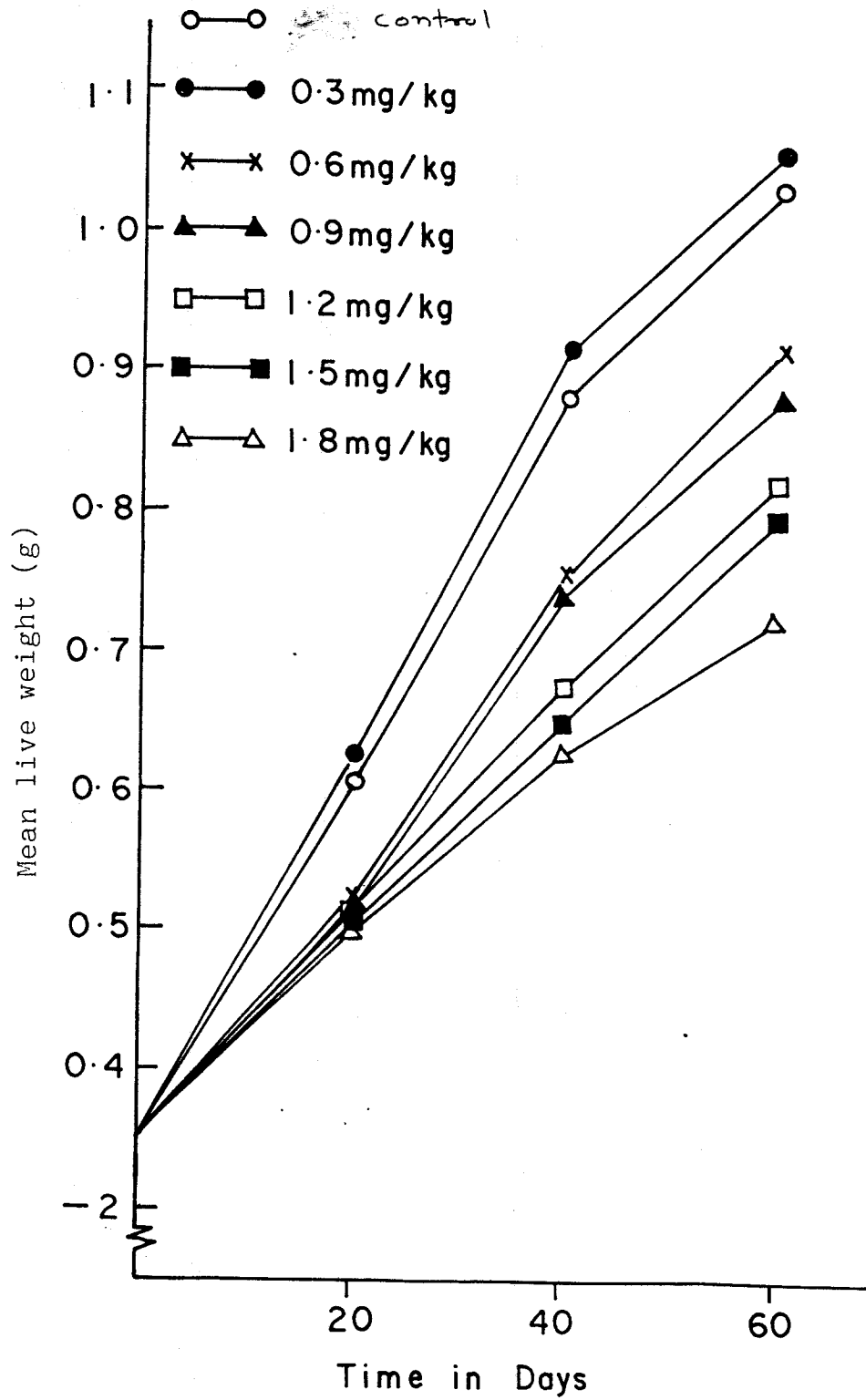


Fig. 8. Effects of selected levels of diethylstilbestrol (DES).

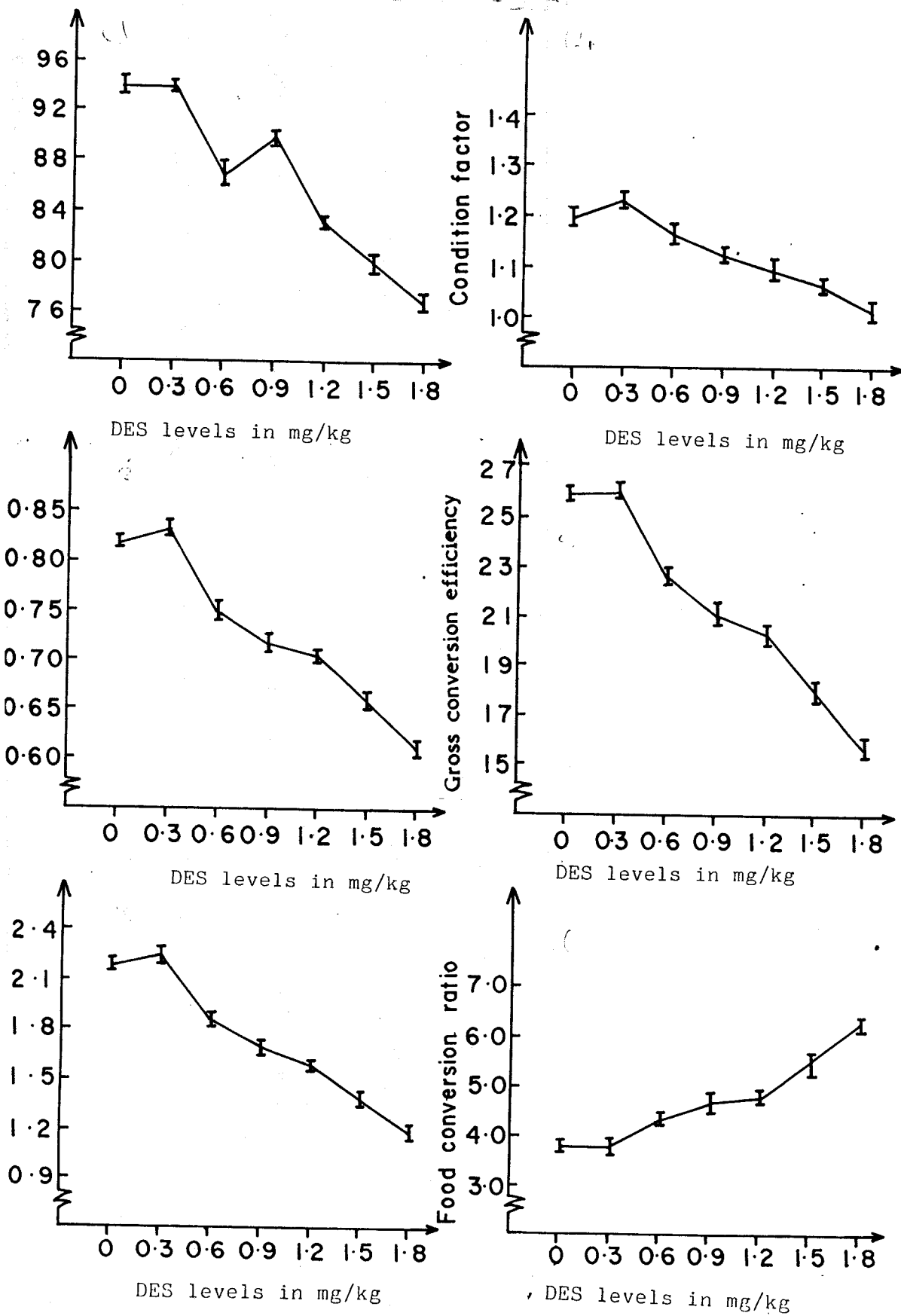
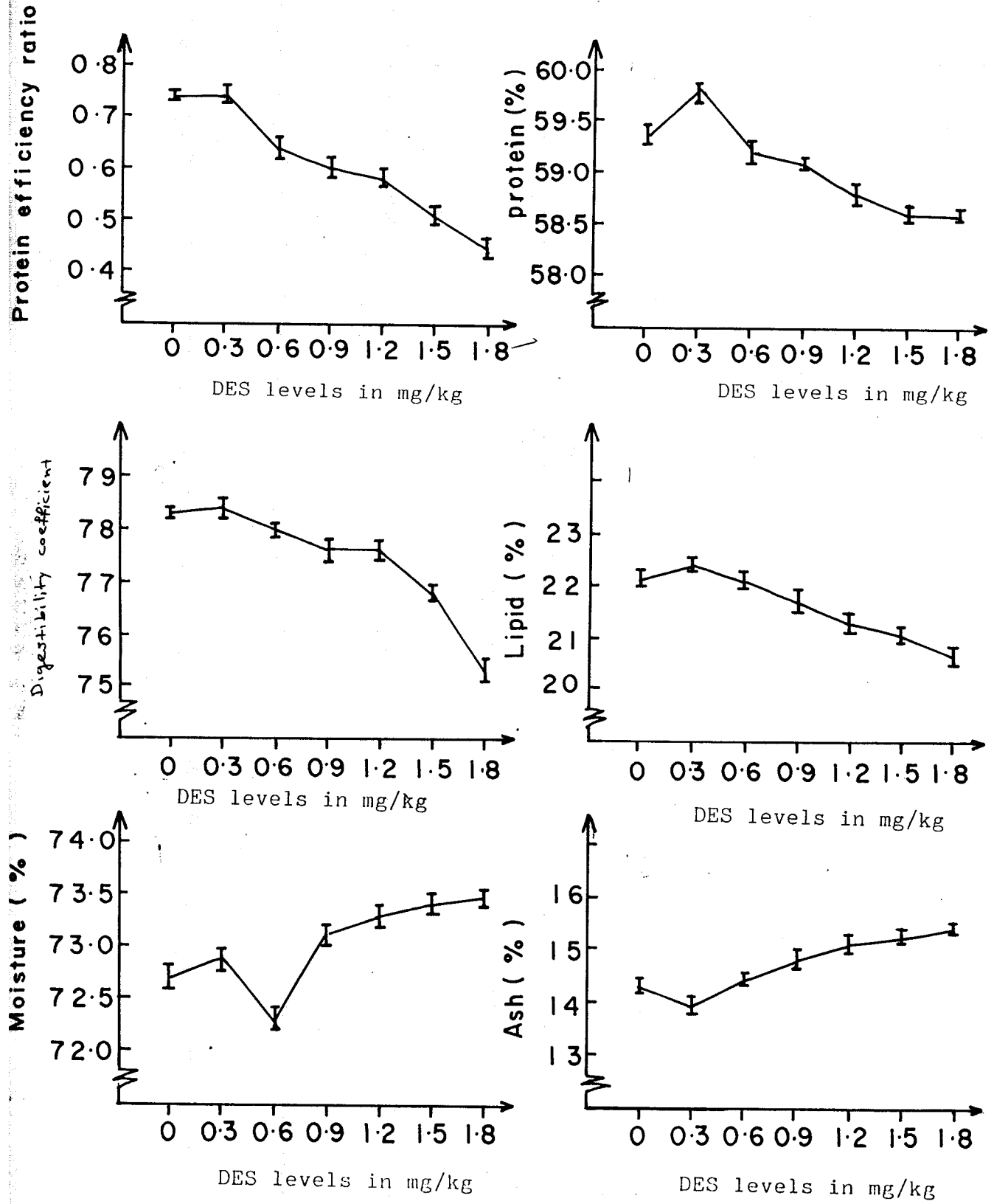


Fig. 9. Effects of selected levels of diethylstilbestrol (DES).



weight gain (136.3%) with that of the control (132.1%).

The SGR (Fig.9) of the fish fed 0.3 mg DES diet was comparable (2.265% per day) to that of the control (2.203% per day). Dietary DES levels exceeding 0.3mg resulted in lowered SGR (Table VI).

The DES did not affect the condition factor (Fig.9) significantly ($P < 0.05$). The maximum value of 1.237 was noted in the fish groups fed on the diet supplemented with 0.3 mg DES which was comparable to the CF value observed in the control (1.201), while the minimum (1.025) was recorded for the fish groups treated with the 1.8mg DES diet.

The values obtained for FCR, PER, Da and GCE were significantly lower ($P < 0.05$) among the DES treated fish groups. However, though slightly better values for these parameters were noted in the diet containing 0.3 mg DES, they were not significantly different from the values recorded for control fish (Table VI). The trend for Da, PER and GCE exhibited a decline when the DES concentration in the diets exceeded 0.6 mg. In contrast, FCR increased as the dietary level of DES increased (Fig.9 & 10). The highest Da (78.45) PER (0.747) and GCE (26.1) were recorded for the diet containing 0.3 mg DES and the lowest Da (75.38), PER (0.449)

and GCE (15.7) were recorded for the 1.8 mg diethylstilbestrol diet. Similarly, the best FCR (3.822) was observed in the treatment with 0.3 mg DES, which however, was not significantly different from that of the control fish (3.841). The diet containing 1.8 mg DES gave poor FCR (6.357) in this experiment.

The moisture, protein, lipid and ash contents of fish did not vary significantly among the treatments. Among the fish groups, only those fed on the diet containing 0.3 mg/kg DES had marginally higher protein and lipid levels than the control (Fig.10 & Table VI). The fish given 1.8 mg DES had the maximum moisture (73.87%) and ash (15.4%) contents (Table VI).

3. 17β - ESTRADIOL (ES)

One experiment was conducted to examine the effects of 17β - estradiol (ES) hormone on growth, food conversion and body composition of the mullet. In this experiment compounded diets containing different dosages of estradiol, viz., 0mg, 1mg, 2mg, 3mg, 4mg, 6mg, 8mg and 10mg/kg were fed to the fry of mullet Liza parsia for sixty days. The rearing conditions during the sixty days were as follows: salinity 15 ± 1 ppt; temperature $31.2 \pm 2.5^{\circ}$ C; pH 7.823 ± 0.101 ; ammonia

0.265±0.070 mg/l; oxygen 4.9±0.35 ppm.

Survival rates recorded from the different treatment groups are shown in Fig.12. The fish groups on the estradiol-free diet and those fed on the 4mg estradiol diet gave the highest survival of 93%. The fish groups fed on diets containing 1mg and 2mg ES gave survival rates of 90% and 87% respectively. In the remaining treatments the survival ranged between 77 and 83%.

ANOVA revealed that there were significant differences ($P < 0.01$) in growth between the 17β -estradiol treated fish groups and the control fish groups after 20, 40 and 60 days. All the fish groups recorded higher weight gains over the control fish after 20 days (Fig.11). The highest weight gain (107.52mg) over the control was found in the fish fed the diet containing 2mg 17β -estradiol. The order of weight gain recorded after 20 days was as follows: 2mg > 4mg > 6mg > 1mg > 8mg > 10mg > 0mg (Fig.11).

After 40 days the picture was slightly different (Fig. 11). Though fish groups fed the 2mg ES diet maintained higher growth rates as compared to all other fish groups, growth of the fish groups receiving 4mg and 6mg ES declined. No significant difference ($P < 0.01$) was noted between the 4mg ES

diet and the control fish, while fish on the 6mg ES treatment recorded lower weight gain than the control. The highest weight gain was recorded in the fish fed the diet containing 2mg ES (98.2mg) after 40 days.

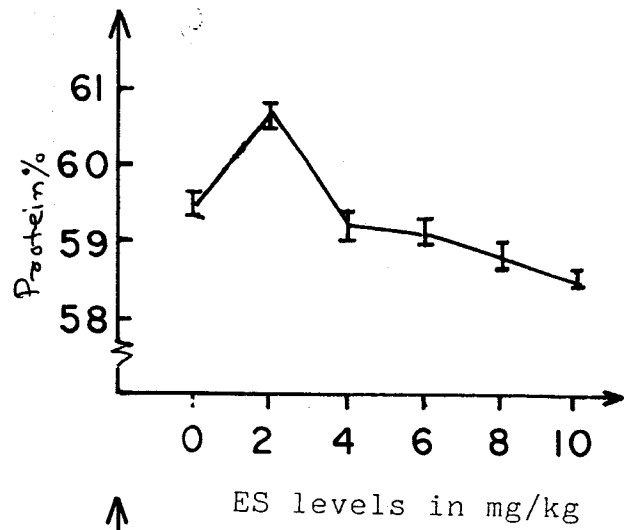
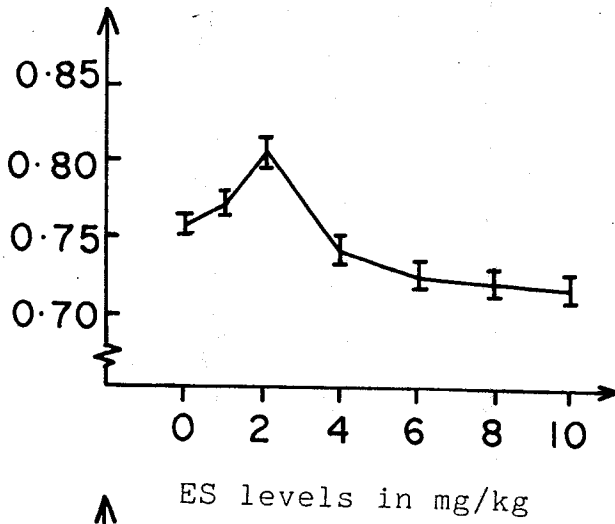
After 60 days also, fish groups receiving 2mg ES recorded the highest weight gain (90.48mg) as compared to all other treatments. The next best weight gain (10.09mg) was observed in the fish administered 1mg ES through diet. All other fish groups produced less growth than the control (Table VII). However, no significant difference was observed between the 4mg ES diet and the control diet. The lowest negative gain (-74.75mg) over the control was recorded for the diet containing 10mg ES. The final order of growth increment recorded after 60 days was as follows:

2mg > 1mg > 4mg > 0mg > 6mg > 8mg > 10mg/kg ES (Table VII).

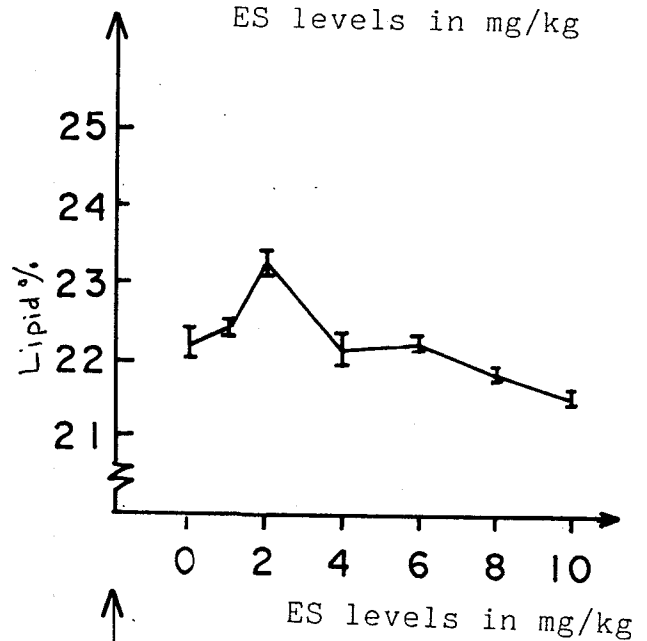
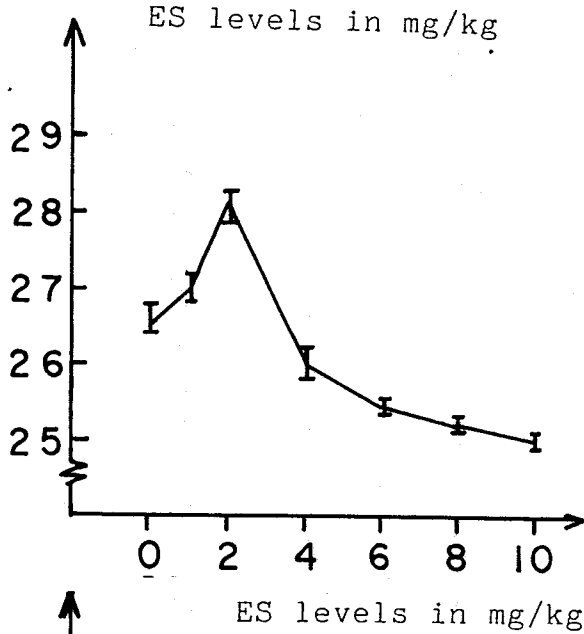
Inclusion of 2mg 17 β -estradiol in the diet resulted in significantly greater SGR than the control (Fig.12). Diet containing 4mg ES (2.661) gave a smaller increment in SGR than the control (2.229). The remaining fish groups had less SGR than the control. SGR decreased with the increase in dietary ES level in excess of 4mg (Fig.12). However, no significant difference in SGR was noted between

Fig. 12 Effects of selected levels of estradiol (ES)

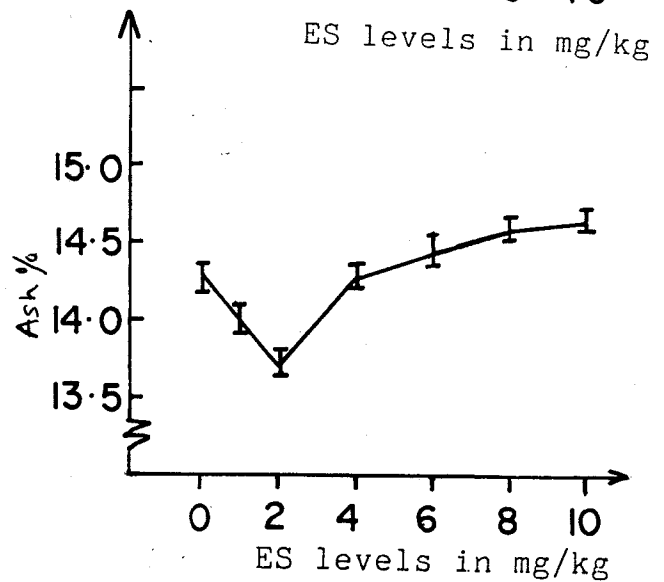
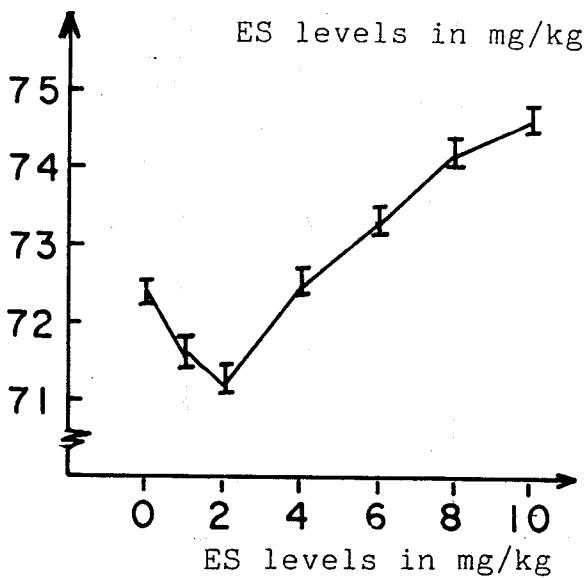
Protein efficiency ratio



Gross conversion efficiency



Moisture (%)



the 1 mg ES diet and the control diet.

Although the condition factor of fish were significantly ($P < 0.05$) influenced by the ES levels in the diet, the observed differences among the fish groups fed on 1mg, 2mg and 4mg ES were not significantly different from each other. Similarly, not much difference in CF was observed among 6mg, 8mg, 10mg and control fish groups. The maximum (1.18) and the minimum (1.018) CF were observed in the fish fed on diets containing 2mg and 10mg 17β -estradiol respectively. Remaining treatments recorded CF ranging between 1.131 to 1.071.

The dietary ES levels had significant ($P < 0.01$) influence on PER and Da. However, only slight differences in these parameters were observed for the diets containing ES levels of 6mg and above. PER and Da (Fig. 12 & 13) were highest for the 2mg ES diet. The best PER (0.804) and Da (77.98) were obtained for the fish groups fed on the 2mg diet and relatively poor values of PER (0.714) and Da (77.49) were recorded for 10mg diet (Table VII). PER and Da showed an increasing trend upto 4mg ES in the diet and thereafter a declining trend was observed (Fig 11 & 12). The best FCR (3.552) was observed for the 2mg ES diet. The FCR showed a

decrease upto 2mg ES in the diet and thereafter it showed an increase (Fig 12). The 17β -estradiol-free control diet provided a FCR value of 3.75.

Significant differences ($P < 0.05$) were observed in the protein and lipid contents when the fish were treated with various concentrations of 17β -estradiol. However, the body levels of protein and lipid were not significantly different ($P < 0.05$) among the fish groups administered the 4mg and 6mg ES as well as the control diets. Similarly, there was no significant difference in protein and lipid between the 8mg and 10mg ES diets. Fish fed the 1mg and 2mg ES diets had better levels of protein and lipid than the control. All other fish had relatively less body percentage of protein and lipid than the control groups. The highest protein (60.72%) and lipid (23.46%) were observed in the treatments with 2mg/kg and 1mg/kg ES respectively. The lowest levels of the nutrients, protein (58.5%) and lipid (21.5%), were recorded in the fish fed on the diet containing 10mg ES.

While there was no significant variation ($P < 0.05$) in the ash content, the moisture content of the fish were affected by the ES dosages ($P < 0.05$). Except for the fish groups fed on the 2mg ES diet (13.72%) all other treatments have recorded

similar values for ash content which ranged between 14.01% and 14.65% (Table VII). The lowest moisture content (71.19%) was observed at the 2mg ES concentration and the highest (74.59%) in the fish group receiving the 10mg ES diet. In the fish from remaining treatments moisture content ranged between 71.62% and 74.24%. Moisture and ash showed a decreasing trend upto the dose of 2mg and thereafter steadily increased as the ES dose increased in the diet.

4. EFFECT OF 17 -METHYLTESTOSTERONE (MT) AND A THYROID HORMONE, 3,5,3'-TRIIODO -L - THYRONINE(T3) MIXTURE:-

One set of experiments was conducted to study the individual and combined effect of thyroxine (T3) and MT. Compounded diets containing two selected dosages of MT (2mg and 4mg/kg) and three selected dosages of thyroxine (T3) (3mg, 6mg and 9mg/kg) and their combinations., viz, 2mg MT + 3mg T3, 2mg MT + 6mg T3, 2mg MT + 9mg T3, 4mg MT + 3mg T3, 4mg MT + 6mg T3 and 4mg MT + 9mg T3/kg diet, were fed to the mullet Liza parsia for forty five days.

The rearing conditions, during the experimental period, were as follows: salinity 15 ± 1 ppt; temperature 23.1 ± 3.4 C; pH 7.794 ± 0.210 ; ammonia 0.260 ± 0.116 mg/l and oxygen 4.83 ± 0.39 ppm.

COMPOSITION OF LIZA PARSIA AFTER 45 DAYS

Response Parameters	control	3 T3	6 T3	MT and 9 T3	T3 levels 2 MT 4 MT	in the diet (mg/kg) 2 MT + 3 T3	2 MT + 6 T3	2 MT + 9 T3	4 MT + 3 T3	4 MT + 6 T3	4 MT + 9 T3	
Mean initial weight (g)	0.234	0.231	0.234	0.233	0.236	0.237	0.234	0.231	0.233	0.232	0.232	0.24
Mean final weight (g)	0.451	0.475	0.492	0.36	0.56	0.507	0.448	0.475	0.52	0.408	0.48	0.481
Specific growth rate (SGR)	2.056	2.284	2.449	1.198	3.095	2.595	2.03	2.286	2.716	1.648	2.339	2.347
Condition factor (CF)	1.117	1.156	1.205	1.037	1.301	1.242	1.115	1.156	1.276	1.093	1.198	1.198
Survival %	94	89	95	85	90	94	81	79	86	75	86	80.1
Gross conversion efficiency (GCE)	28.1	30.9	32.8	16.3	37.7	33.3	27.4	28.8	32.5	22	29.5	30.8
Digestibility coefficient (Da)	78.53	78.72	78.94	75.87	79.38	79.07	78.51	78.7	79.15	76.13	78.79	78.83
Food conversion ratio (FCR)	3.557	3.23	3.047	6.12	2.646	3.002	3.637	3.461	3.073	4.536	3.38	3.244
Protein efficiency ratio (FER)	0.803	0.884	0.937	0.466	1.079	0.951	0.785	0.825	0.929	0.629	0.845	0.88
Proximate composition												
Moisture %	71.41	70.7	71.23	72.86	70.43	70.53	71.45	70.28	70.16	73.35	71.81	71.92
Protein %	59.06	61.5	60.4	58.26	62.2	60.5	59.13	61.43	62.43	58.63	61.16	61.16
Lipid %	22.16	23.83	23.2	20.73	24.16	23.76	22.16	23.15	24.2	21.2	23.2	23.03
Ash %	14.1	13.9	13.4	15	12.6	13.3	14.2	13.9	12.9	14.6	13.5	13.6

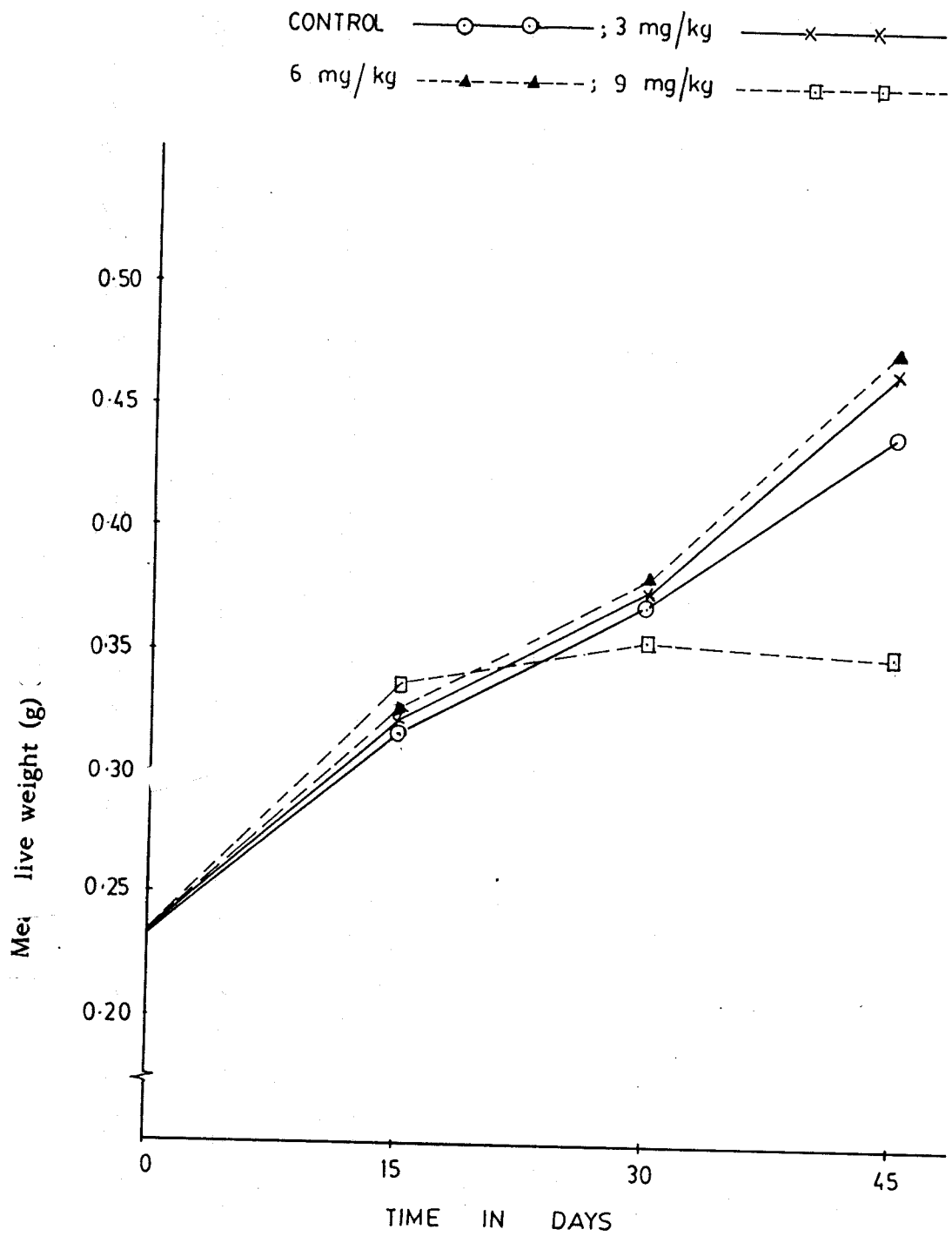


FIG. 13 EFFECT OF THYROID HORMONE T_3 ON GROWTH

(i) Efficacy of thyroid hormone:-

The percentage of survival (Table 9 & Fig 14) ranged from 83% to 97%, with ^{the} highest being recorded for the fish groups fed on the T3 free-diet and those fed on the diet supplemented with 6mg T3; while the lowest survival (83%) was for the 9mg T3 diet. The fish receiving 1mg T3 gave the second best survival rate (90%) among the T3 treated fish groups.

The growth response of the fish to the selected levels of T3 are shown in Fig.13. All the values obtained for growth increment after 15, 30 and 45 days are highly significant ($P < 0.01$). However, the growth of the fish fed on diet containing 3mg T3 and control were almost similar upto 30 days, but after 45 days there was significant difference in growth between the two groups (Fig 13).

The mullet fed on the diet containing 9mg T3 gave the best growth, initially, during the first 15 days. Thereafter, a declining trend (Fig 13) was observed and the lowest growth was recorded for this diet at the end of 45 days. The diet containing 6mg T3 produced better growth after 30 and 45 days than the other diets. After 45 days the highest weight gain (9.18%) over the control was recorded for the fish fed on the

Fig. EFFECT OF COMBINATION OF MT & T₃ ON GRO

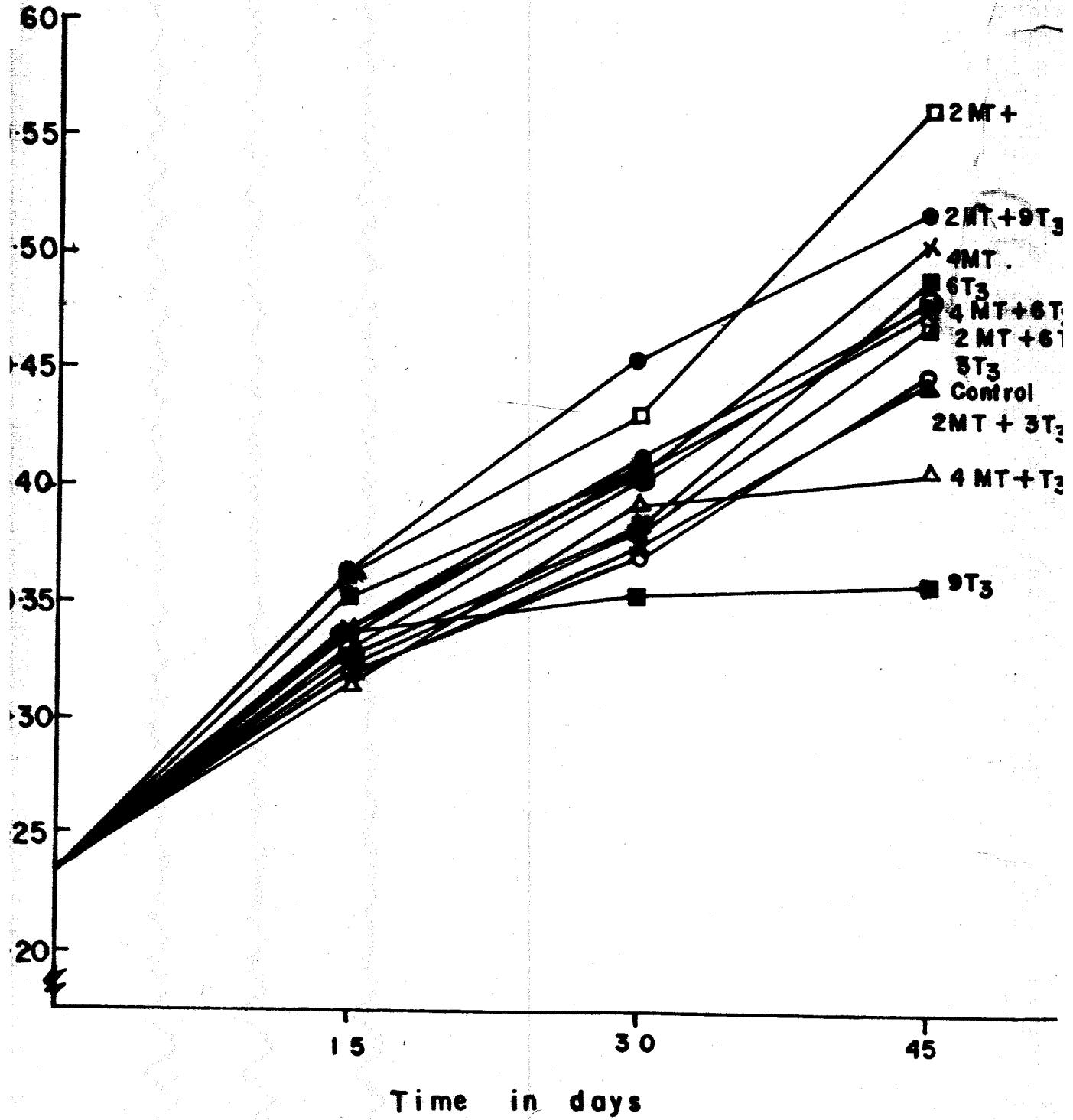


Fig. 15

Effect of selected levels of T3

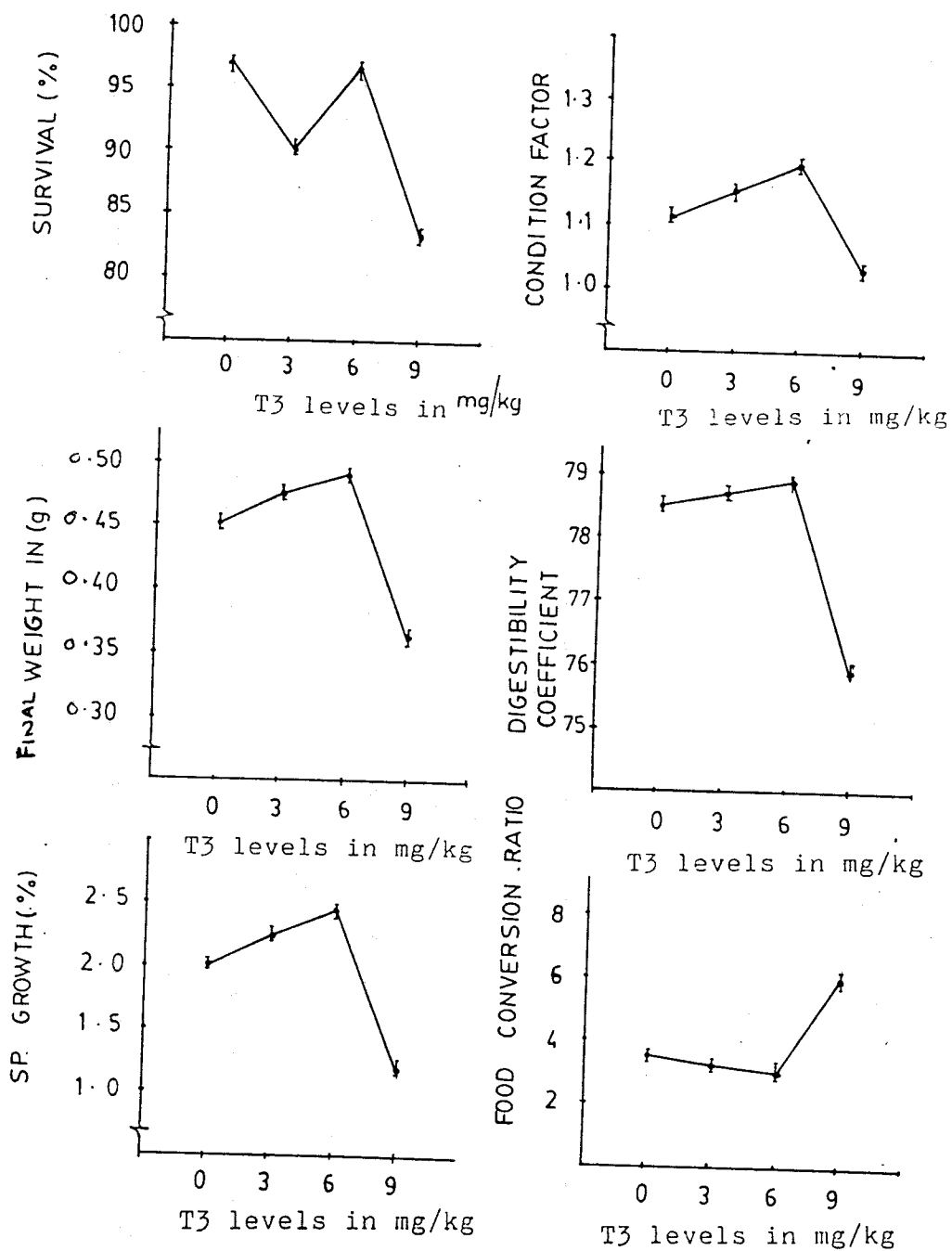
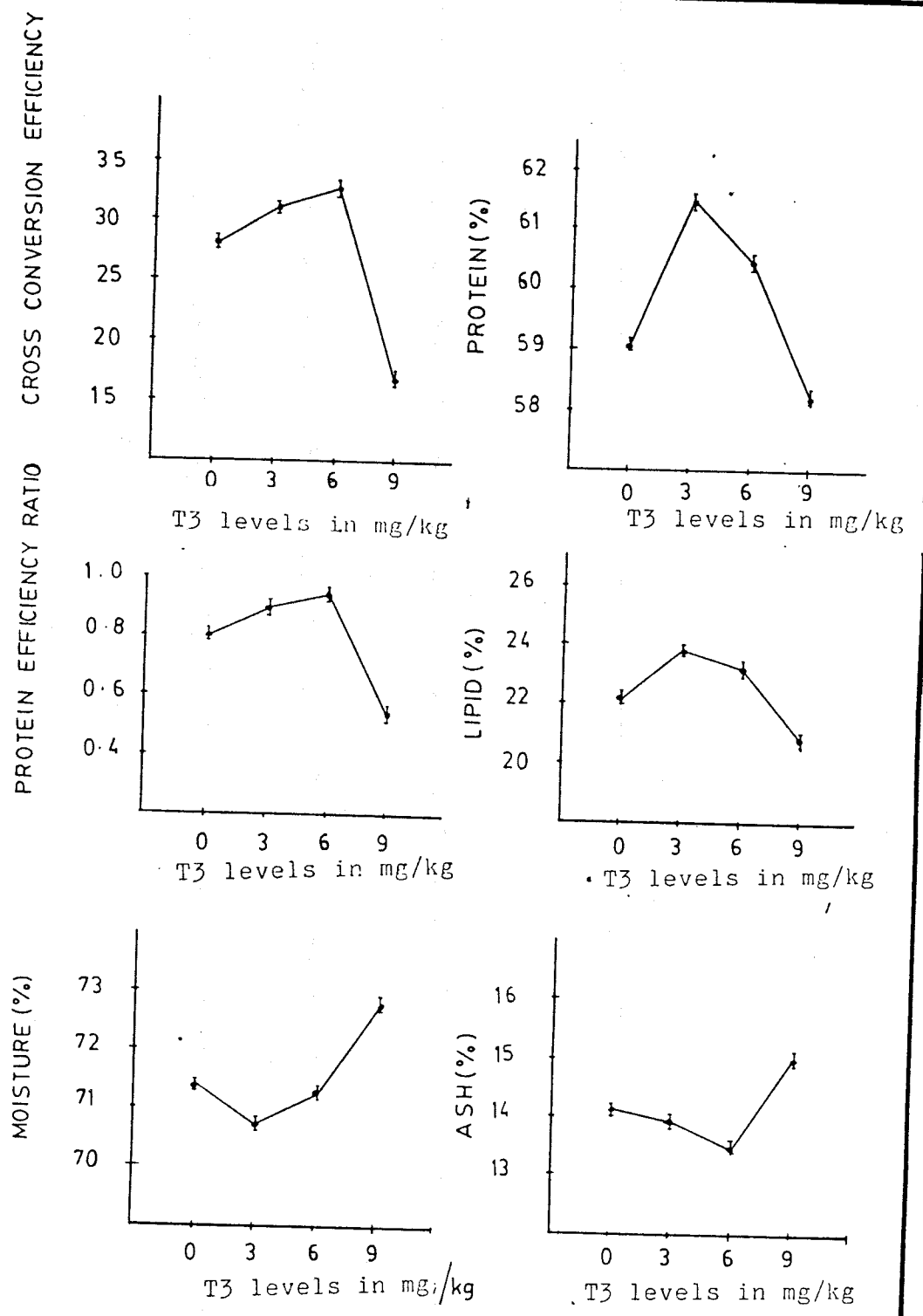


Fig. 16 Effect of selected levels of T3



diet containing 6mg T3. The 9mg T3 diet produced less growth than the control after 45 days.

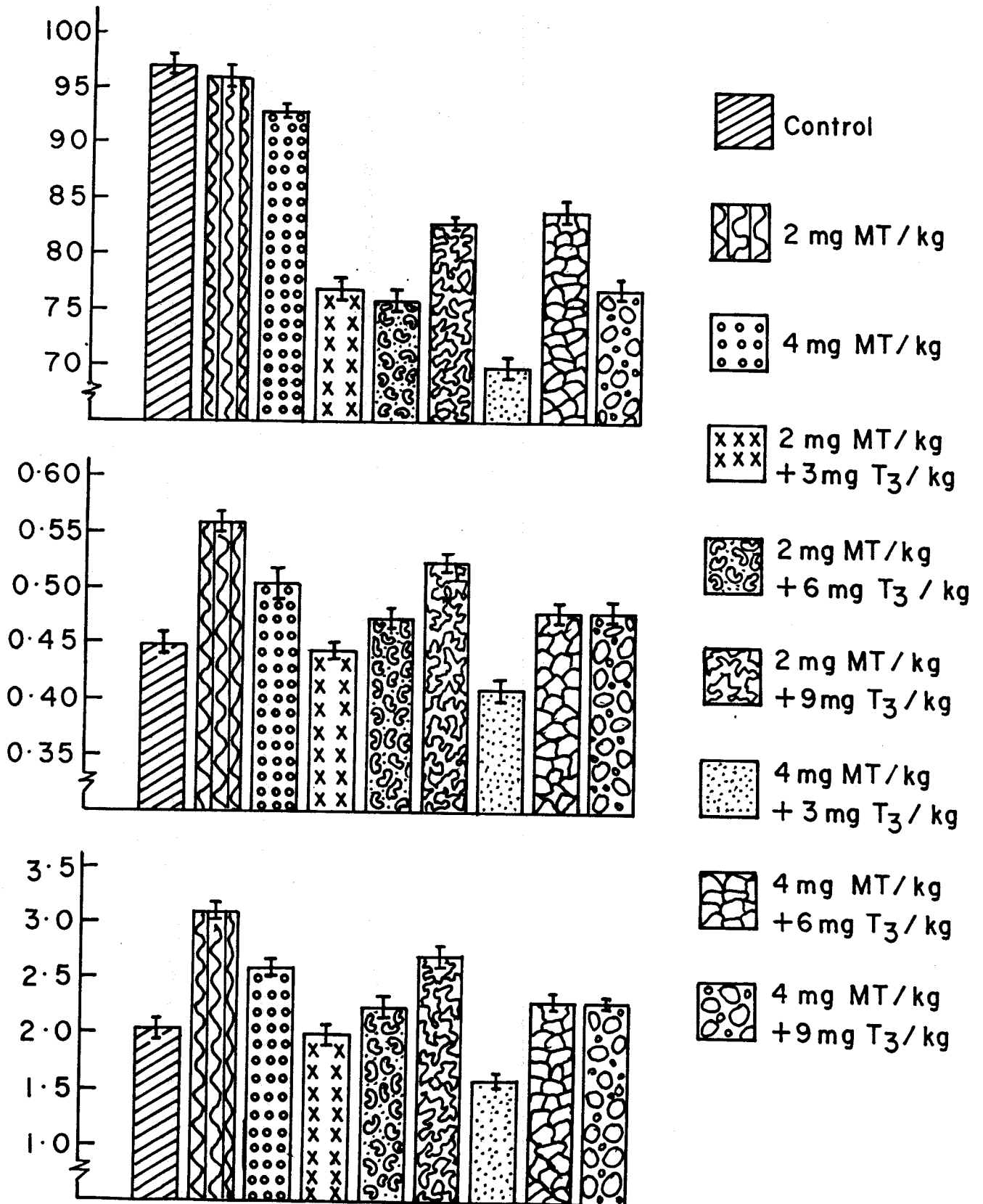
Specific growth rate was better for the fish fed on the 6mg T3 diet (2.449%) than the control (2.056%). The lowest SGR (1.198%) was recorded for the fish group treated with 9mg T3 (Table VIII).

The condition factor (Fig.14) also showed a pattern similar to that of the SGR (Fig.14). The CF values recorded for 6mg T3 (1.205) and 3mg T3 (1.156) were slightly better than the control (1.117), while the dose of 9mg T3 gave lower value (1.037) than the control (Table VIII).

Data obtained for FCR, PER and GCE are given in Table VIII. Fish receiving the diet incorporated with 6mg T3 showed better FCR (3.047), Da (78.94), PER (0.937) and GCE (32.8) than the fish on other dietary T3 dosages (Fig.15 & 16). No significant difference ($P < 0.01$) in digestibility coefficient was observed between the fish groups fed on the diet containing 6mg and 3mg T3. The fish groups receiving 9mg T3 gave relatively poor results for FCR (6.12), Da (75.87), PER (0.466) and GCE (16.3).

The results of body composition analysis are shown in

Fig. 17 Effects of combinations of selected levels of 17 α -methyltestosterone (MT) and T₃.



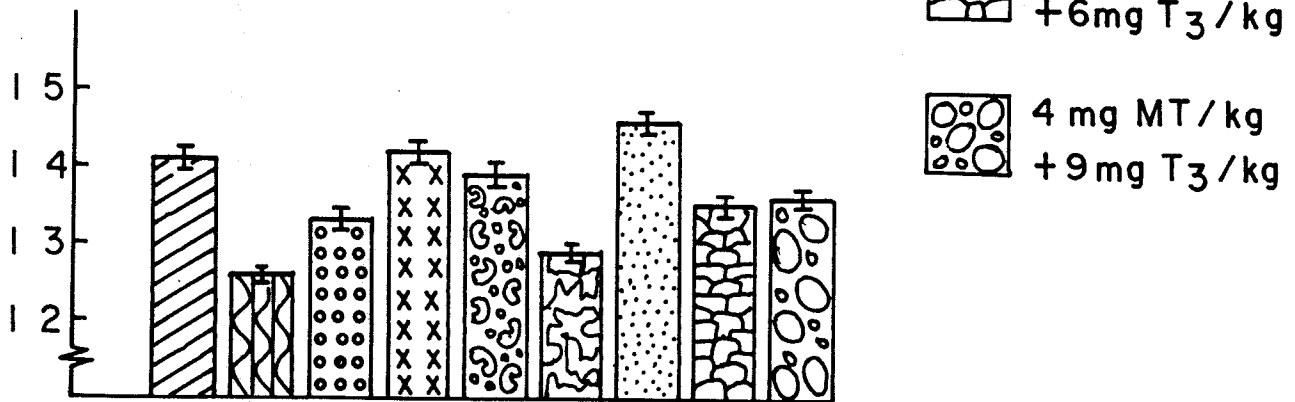
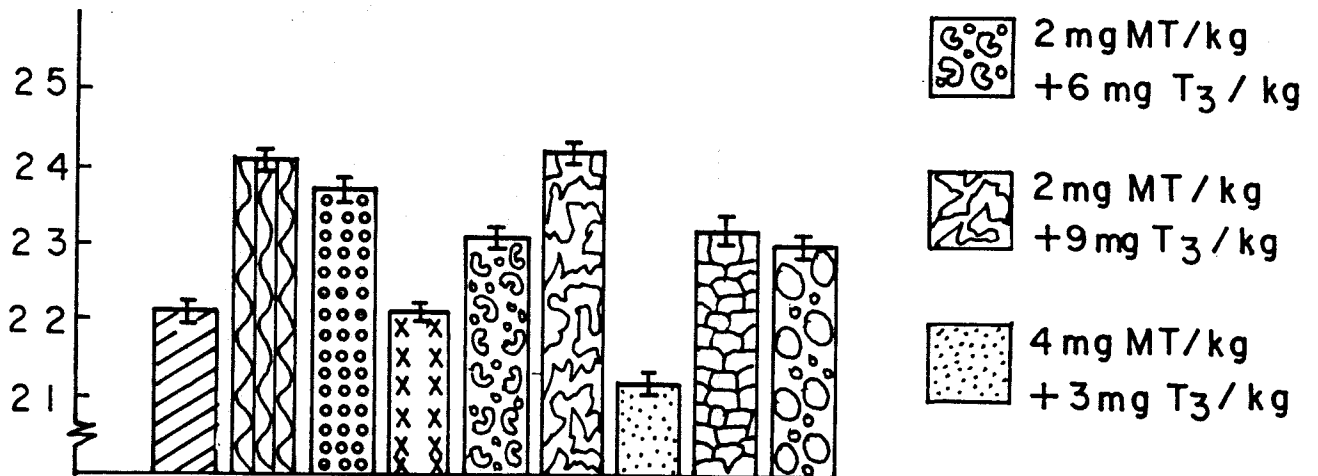
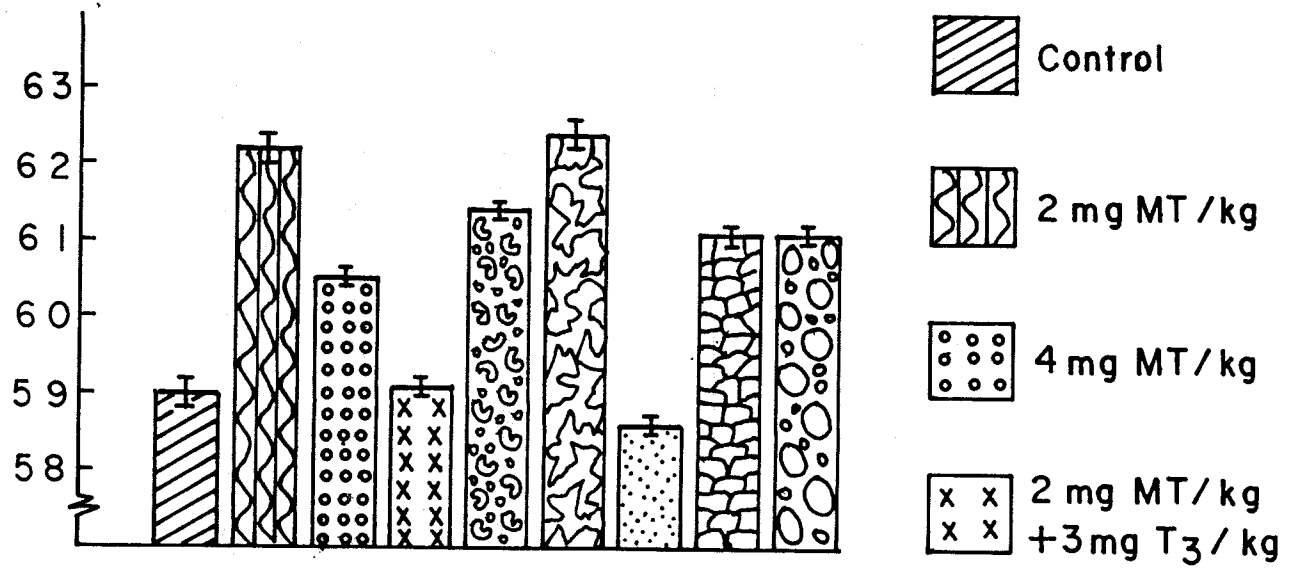


Fig.16 and Table VIII. The fish receiving 3mg T3 diet had relatively higher protein (61.5%) and lipid (22.83%), and lower ash (13.9%) and moisture (70.7%) contents than the fish from other treatments. In contrast, poor values for protein (58.26%), lipid (20.73%), ash (15.0%) and moisture (72.8%) were observed in the 9mgT3 diet.

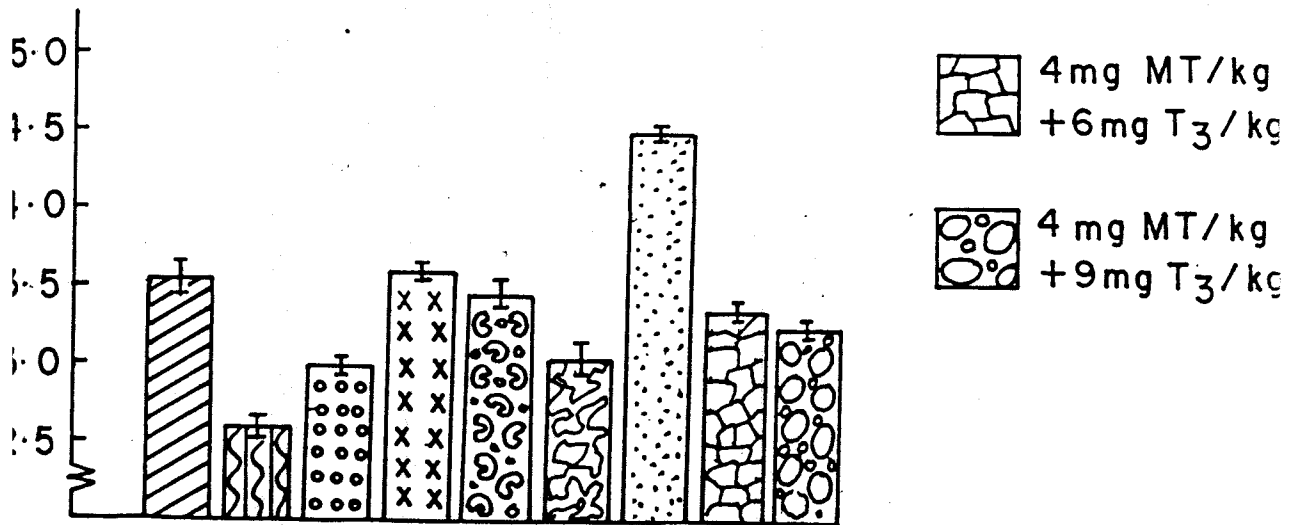
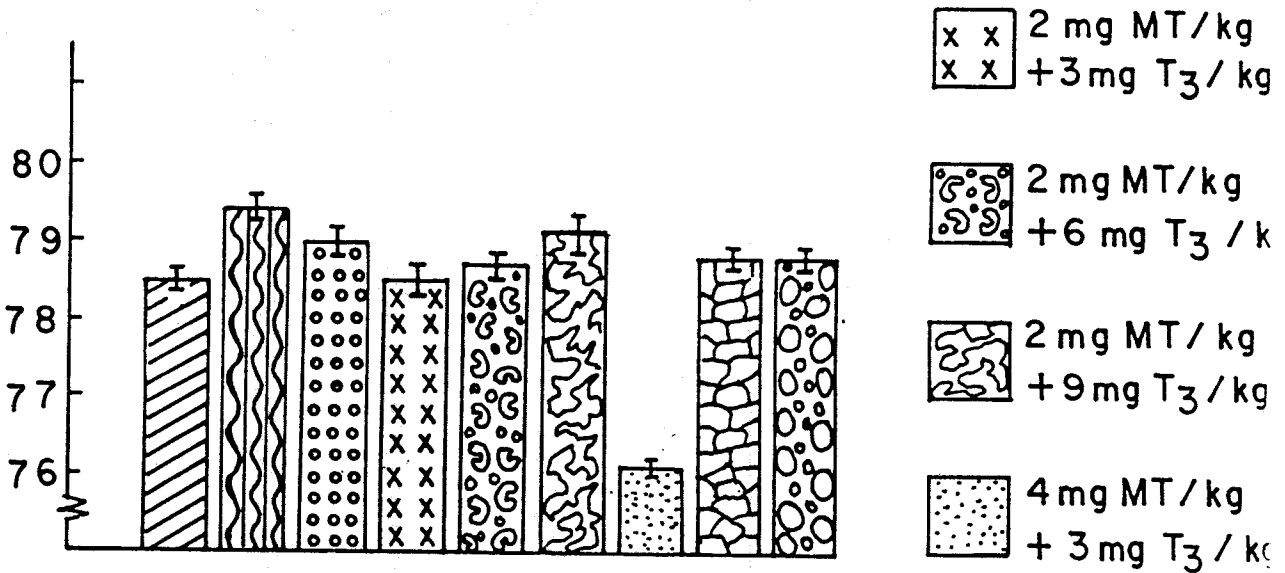
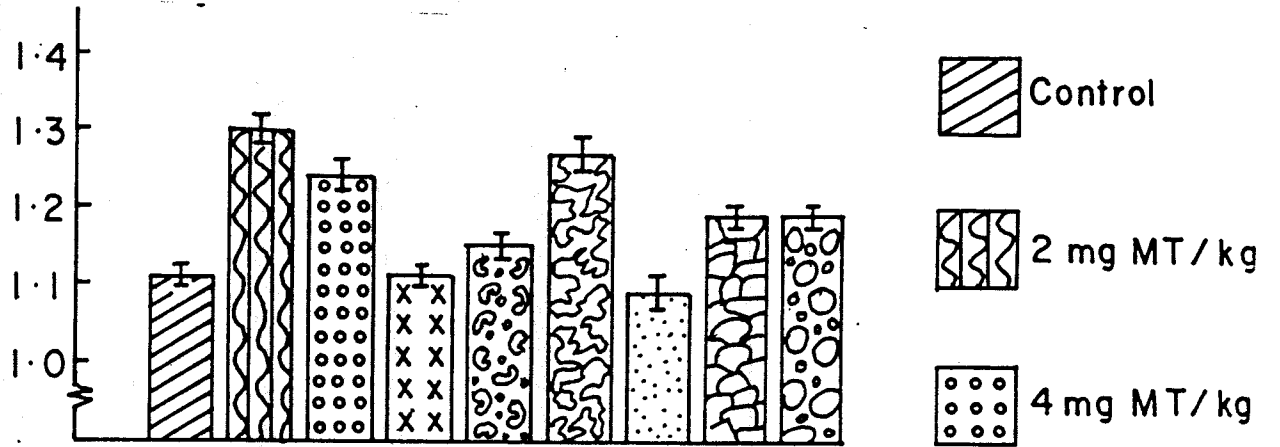
(ii) Efficiency of 17 α -methyltestosterone (MT):-

There was not much difference in the survival rate (Table VIII) recorded for the control groups (97%) and the fish administered 2mg MT through the diet (96%). The diet containing 4mg MT gave a survival rate of 93%. Significant difference ($P < 0.01$) was observed in growth increment between the MT treated fish and the control fish, after 15,30 and 45 days. Growth was higher for the MT treated diets as compared to the control fish. The percentage gain over the control was 24.27 and 12.60 for 2mg and 4mg MT diets respectively.

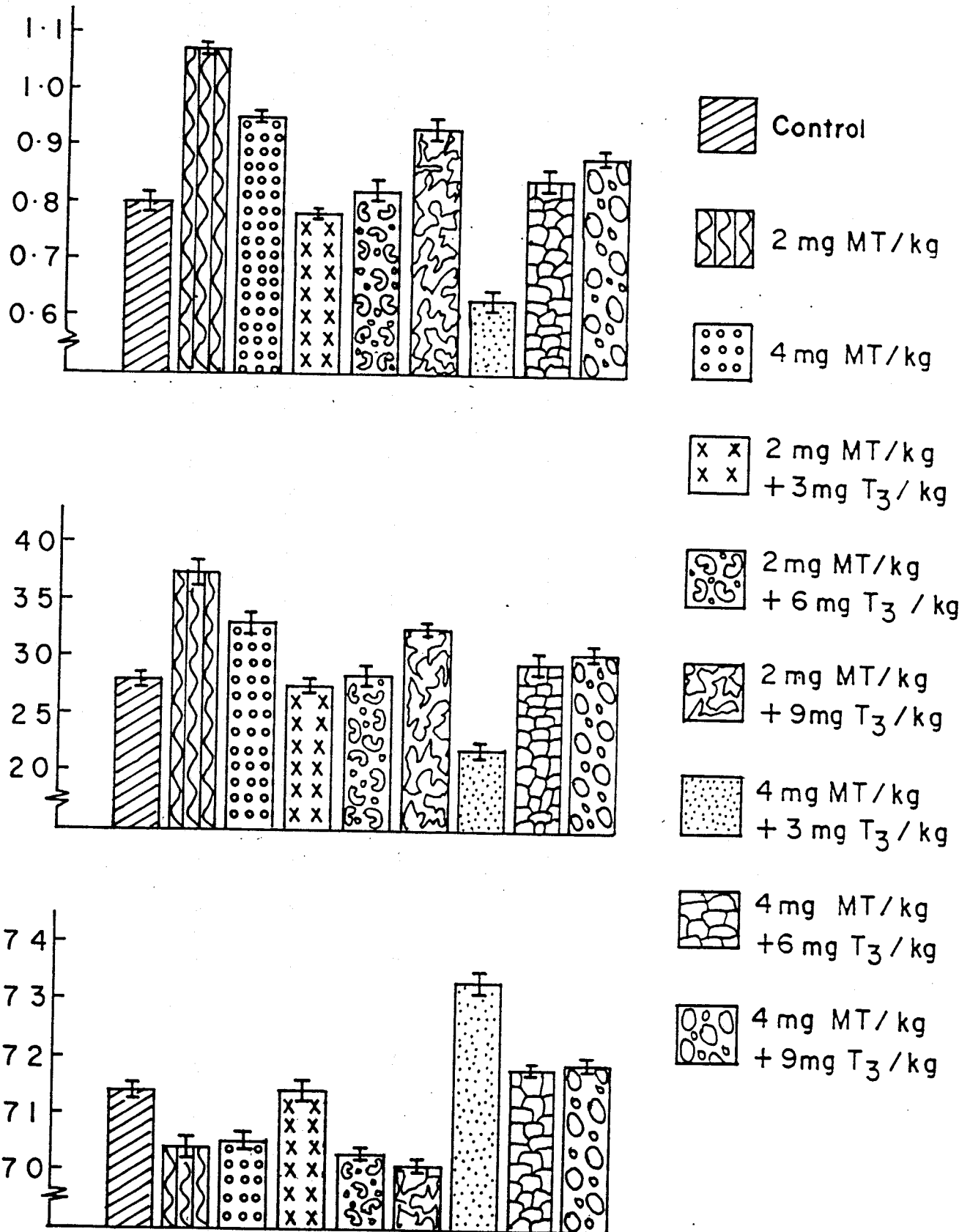
The best specific growth rate (3.095%) and condition factor (1.301) were obtained with the diet containing 2mg MT (Table VIII). The control diet produced significantly less SGR and CF than the 2mg and 4mg MT diets.

Significant difference in digestibility coefficient was

Fig. 18. Effects of combinations of selected levels of 17 α -methyltestosterone (MT) and T₃.



g. 19 Effects of combinations of selected levels of 17 α -methyltestosterone (MT) and T₃.



noted between fish fed on the hormone deficient diet and the 2mg MT treatment. Relatively high PER (1.079) and Da (79.38) and low FCR (2.646) were recorded for the 2mg MT diet, which were significantly better than the control (Fig. 18 & 19 and Table VIII).

The proximate principles in the body of the mullet were also affected significantly ($P < 0.01$) by the MT dosages (Fig 20). However, the moisture contents of the fish groups treated with MT were significantly lower than the control animals. Both the MT dosages produced fish with higher protein and lipid levels and lower levels of ash than the control fish. The maximum protein (62.2%) and lipid (24.16%) contents and minimum ash (12.6%) contents were observed in the 2 mg MT diet.

(iii) Efficacy of T3 and MT combinations (MT)

The results are presented in Table VIII and Fig 17 to 20. The hormone-free, control diet provided the highest survival (97%). Among the groups fed the diets containing various combinations of MT and T3, the highest (84%) and lowest (76%) survival rates were found at the dosages of 4mg MT + 6mg T3 and 2mg MT + 6mg T3 respectively.

ANOVA of growth data recorded after 45 days showed that the hormone treatments have highly significant ($P < 0.01$) effect on growth. However, no significant difference was evident in the growth after 15 days among most of the fish groups. Similarly, after 30 and 45 days the control fish and fish fed on the diets containing 2mg MT + 3mg T3 did not show any significant difference in weight gain. The growth recorded in fish receiving the diets containing 4mg MT + 6mg T3 and 4mg MT + 9mg T3 were similar (Table VIII).

Among the fish groups fed on various dietary hormone concentrations the dosage of 2mg MT + 9mg T3 produced the best growth promotion after 15 (14.44) and 30 (22.92) days; thereafter, a slight decline was noted in this group. At the termination of the experiment this treatment recorded the second best weight gain (15.41%) over the control as compared to other treatments. The fish groups receiving 2mg MT gave the highest weight gain (24.27%) over the control after 45 days. The fish groups fed on diet supplemented with 9mg T3, 2mg MT + 3mg T3 and 4mg MT + 3mg T3 recorded negative weight gains over the control after 45 days which were (-20.16%), (-0.47%) and (-9.55%) respectively.

The highest SGR (2.716) and CF (1.276) and lowest SGR

(1.648) and CF (1.093) were recorded at the dosages of 2mg MT + 9mg T3 and 4mg MT + 3mg T3 respectively (Fig.17 & 18, Table VIII). In the remaining fish groups fed on combination of MT and T3 the SGR values ranged from 2.03 to 2.347 per day, and the CF between 1.115 to 1.198.

Analysis of variance revealed significant differences ($P < 0.01$) between most of the treatments in the results obtained for FCR, Da, PER and GCE. However, among the fish groups treated with the dosages of 2mg MT + 6mg T3, 4mg MT + 6mg T3 and 4mg MT + 9mg T3 there was no significant difference in FCR, Da and PER, but these values were slightly better than the control animals. The best FCR (3.073) Da (79.15) PER (0.929) and GCE (32.5) were recorded for the 2 mg MT + 9 mg T3 diet, while relatively poor FCR (4.536), Da (76.13), PER (0.629) and GCE (22.0) were recorded in the fish groups fed the 4mg MT + 3mg T3 diet (Table VIII).

The body levels of protein, lipid, ash and moisture in the fish were significantly ($P < 0.01$) influenced by the dietary treatments. However, there were no significant differences in the levels of protein, lipid, ash and moisture between the fish receiving diets containing 4mg MT + 6mg T3 and 4mg MT + 9mg T3. The fish receiving diets containing 4mg

MT + 6mg T3 and 4mg MT + 9mg T3 had almost equal levels of lipid and protein in the body (Fig.21, Table VIII). The highest levels of body protein (62.43%) and lipid (24.2%) and lowest levels of ash (12.9%) and moisture (70.16%) were recorded for the fish fed the diet containing 2mg MT + 9mg T3. The lowest levels of protein (58.63%) and lipid (21.2%) and highest ash (14.6%) and moisture (73.35) levels were observed in the fish administered the diet with 4mg MT + 3mg T3.

The fish groups fed diets either containing T3 or MT hormone, gave better survival (over 83%) than their combination treatments, which ranged between 70 and 84%. However, the control and the 6mg T3 treatments recorded the highest percentage of survival (97) followed by the fish fed on the diet containing 2mg MT (96%). The lowest survival (70%) was recorded in the fish fed the diet containing 4mg MT + 3mg T3.

In the fish fed the diets containing either MT or T3 hormones at different dosages the growth was found to increase linearly with time. However, the diet containing 9mg T3 produced growth increment only upto the initial 15 days; thereafter, a decline was noted resulting in poor growth after 45 days. The MT dose of 2mg provided the highest

percentage weight gain. The diet containing a combination of 2mg MT + 9mg T3 produced superior growth upto 30 days; thereafter, a decrease in growth was observed in this group. However, it recorded the second best percent weight gain overall.

The overall highest (24.27%) and lowest (-20.16%) percentage weight gain over the control were recorded in the fish fed the diets containing 2mg MT and 9mg T3 respectively. The fish fed the diet containing 2mg MT showed the best SGR (3.095%) followed by those fed the 2mg MT + 9mg T3 diet (2.716%) per day. The lowest SGR (1.198% per day) was for fish fed on the diet containing 9mg T3.

When all the treatment groups were ranked according to SGR and percentage weight gain over the control after 45 days the following sequence emerged.

2mg MT > 2mg MT + 9mg T3 > 4mg MT >
 6mg T3 > 4mg MT + 9mg T3 > 4mg MT + 6mg T3 >
 2mg MT + 6mg T3 > 3mg T3 > control > +
 2mg MT + 3mg T3 > 4mg MT + 9mg T3 > 9mg T3.

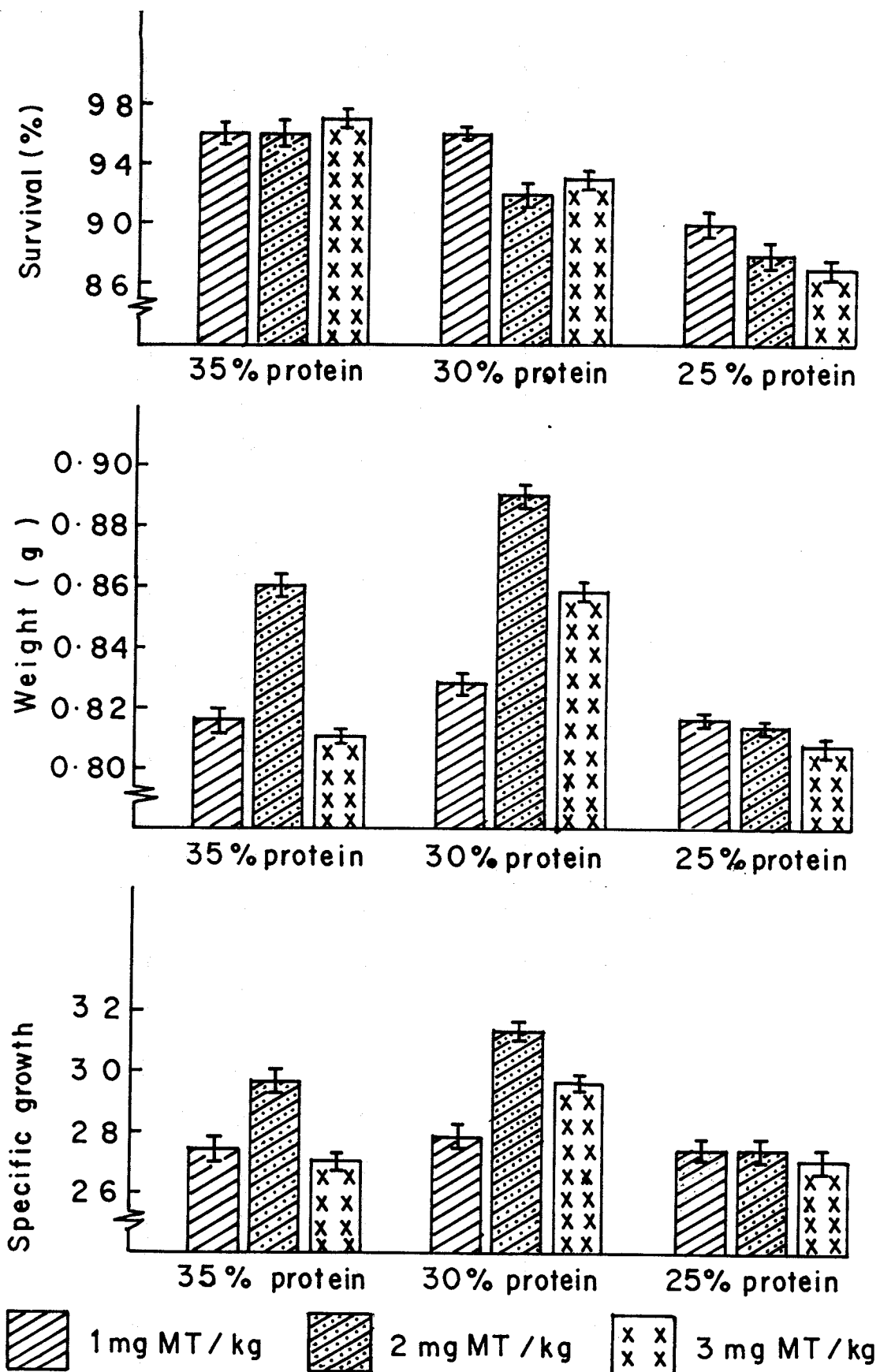
All the values obtained for the condition factor (CF) were highly significant ($P < 0.01$). The best CF (1.301) was recorded for the fish receiving the 2mg MT diet which was

followed by the fish fed the diet containing 2mg MT + 9mg T3, which were significantly ($P < 0.01$) higher than the CF recorded for the fish fed on the control diet (1.117). The lowest values for CF (1.0037) was noted at the dosage of 9mg T3.

Results obtained for the FCR, Da, PER and GCE were highly significant ($P < 0.01$) among the various treatments. The Da, PER and GCE (Fig.18 & 19) were higher for the fish groups receiving the 2mg MT diet which also recorded the best FCR among all other fish groups. The best FCR (2.646), Da (79.38), PER (1.079) and GCE (37.7) were recorded for the diet containing 2mg MT as against the poor FCR (6.120), Da (75.87) PER (0.446) and GCE (16.3) for the 9mg T3 diet.

Protein, lipid, moisture and ash levels (Fig.20, Table VIII) were significantly influenced by the diets. However, no significant difference was noticed for these body contents among the fish groups fed on diet containing 2mg MT and 2mg MT + 9mg T3. The highest protein (62.43%), and lipid (24.2%) and the lowest moisture (70.28%) levels were observed for the 2mg MT + 9mg T3 treated fish groups, which were not significantly different from the fish fed the 2mg MT diet. The ash (12.6%) content was relatively low in the fish fed the 2mg MT + 9mg T3 diet (12.9%). Similarly, protein

Fig. 29 Effects of selected levels of 17 α - methyltestosterone (MT) and dietary protein levels



(58.26%), lipid (20.73%) and ash (15.0) levels were relatively low in the fish groups fed the 9mg T3 diet.

5. PROTEIN SPARING EFFECT OF 17 α -METHYLTESTOSTERONE

In this experiment compounded diets containing three different protein levels, viz., 35%, 30% and 25% and for each of the protein levels three different dosages of MT (1mg, 2mg and 3mg) were prepared and fed to Liza parsia fry for a period of 60 days.

The rearing conditions during the experimental period were as follows: salinity 15 ± 1 ppt; temperature $28.2 \pm 3.4^{\circ}\text{C}$; pH 7.856 ± 0.104 ; ammonia 0.247 ± 0.090 mg/l and oxygen 4.77 ± 0.31 ppm.

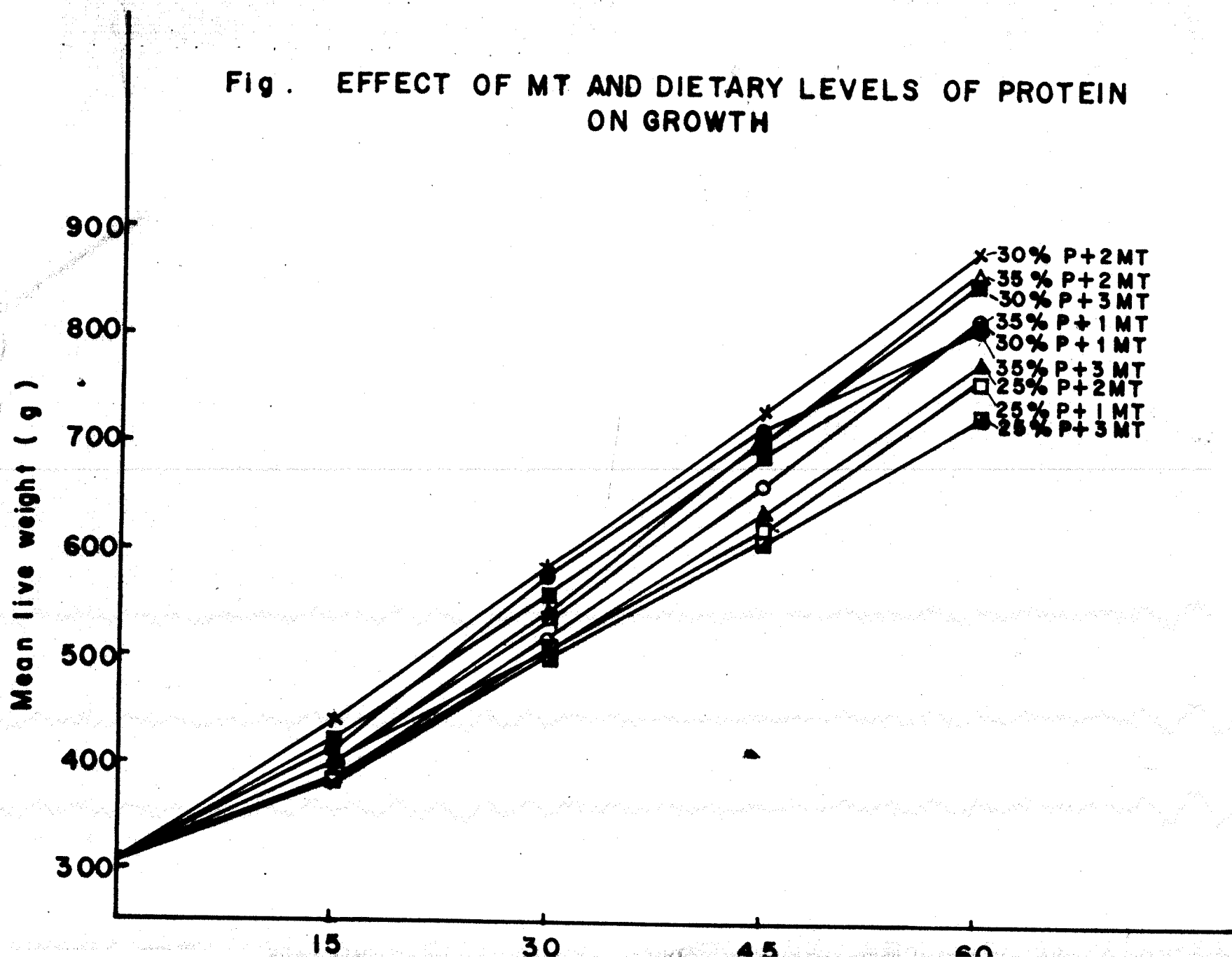
Survival:

Irrespective of the MT concentration in the diet the percentage survival was above 96% in all the fish groups receiving the 35% protein diet, while the fish groups fed the 30% diet had survival ranging from 90 to 93%. At the 25% dietary protein level 1mg MT produced survival rate below 90%, while the lowest was recorded in the diet containing 3mg MT. The best survival rate (97%) amongst all the fish groups was exhibited by the fish fed on the 35% protein + 3mg MT and

TABLE IX: EFFECTS OF SELECTED DOSAGES OF 17 -METHYLTESTOSTERONE AND DIETARY LEVELS OF PROTEIN (P) ON THE GROWTH, FOOD CONVERSION AND BODY COMPOSITION OF LIZA PARSIA AFTER 60 DAYS

Response Parameters	MT levels in mg/kg and protein (P) levels in percentage								
	35 % protein			30 % protein			25 % protein		
	1 MT	2 MT	3 MT	1MT	2MT	3MT	1MT	2MT	3MT
Mean initial weight (g)	0.308	0.306	0.305	0.311	0.307	0.31	0.308	0.308	0.309
Mean final weight (g)	0.818	0.859	0.81	0.828	0.884	0.858	0.787	0.775	0.735
Specific growth rate (SGR)	2.747	2.968	2.705	2.781	3.131	2.965	2.545	2.52	2.431
Condition factor (CF)	1.235	1.29	1.232	1.238	1.301	1.29	1.134	1.112	1.03
Survival %	96	96	97	96	92	93	90	88	87
Gross conversion efficiency (GCE)	30.9	32.2	30.1	31.5	33.5	32.2	29.95	29.91	29.63
Digestibility coefficient (Da)	79.22	79.28	79.12	79.32	79.45	79.28	78.82	78.8	78.2
Food conversion ratio (FCR)	3.23	3.1	3.322	3.21	3.019	3.1	3.412	3.41	3.53
Protein efficiency ratio (PER)	0.879	0.919	0.86	0.89	0.945	0.919	0.845	0.84	0.834
Proximate composition									
Moisture %	71.45	70.1	71.68	70.93	70.16	70.7	73.1	73.2	73.31
Protein %	59.33	62.56	61.83	59.71	63.58	62.54	58.5	58.5	58.1
Lipid %	23.64	24.23	23.46	22.7	23.58	23.01	22.54	22.53	22.2
Ash %	13.17	12.6	13.16	13.2	12.8	13.8	14.1	14.2	14.2

Fig. EFFECT OF MT AND DIETARY LEVELS OF PROTEIN ON GROWTH



the lowest recorded in the treatment of 25% protein + 3mg MT (87%). The response of the fish to the dietary treatments are illustrated in Fig.22 and Table IX.

Factorial analysis (3 x 3) was used to test the significance of differences in the growth among the treatments. It was found that the fish growth was significantly ($P < 0.01$) affected by the combinations of various levels of protein and steroid hormone in the diets after 15, 30, 45 and 60 days.

(i) Response of fish groups receiving 1mg MT:

Among the diets containing 1mg MT, the fish fed the diet containing 30% protein showed a steady increase in weight gain over that recorded for the 35% and 25% protein diets upto the termination of the experiment. This was followed by the fish groups receiving the 35% protein diet. However, after 30 days the fish groups fed on the 25% protein + 1mg MT diet recorded 72.72% weight gain, which was significantly higher than the 35% protein + 1mg MT treatment. Thereafter, a decline was observed in the diet containing 25% protein + 1mg MT. At the termination of the experiment the fish fed on the 30% protein diet gave the highest percentage weight gain

(168.31) followed by 35% (164.87) and 25% (155.07) protein diets.

(ii) Response of fish groups receiving 2mg MT:-

After 15 days trend shows (Fig.21) the diet containing 30% protein level gave the highest weight gain (44.74%), which was followed by the groups fed on the diet containing 25% protein, (30.67%). After 30 days, the picture was different. The 30% protein diet continued to record the best percentage weight gain (70.25) which was closely followed by 35% protein treatment (74.67). The fish groups receiving 25% protein diet produced significantly lower weight gains (62.08%).

The same trend was evident upto the termination of the experiment. After 60 days, the highest weight gain (186.27%) was noted in the fish fed on the diets containing 30% protein + 2mg MT, followed by 35% protein (178.19%) and 25% protein (151.14%).

(iii) Response of fish groups receiving 3mg MT:

Fish groups receiving 25% protein + 3mg MT gave the highest weight gain (37.13%) during the initial 15 days and

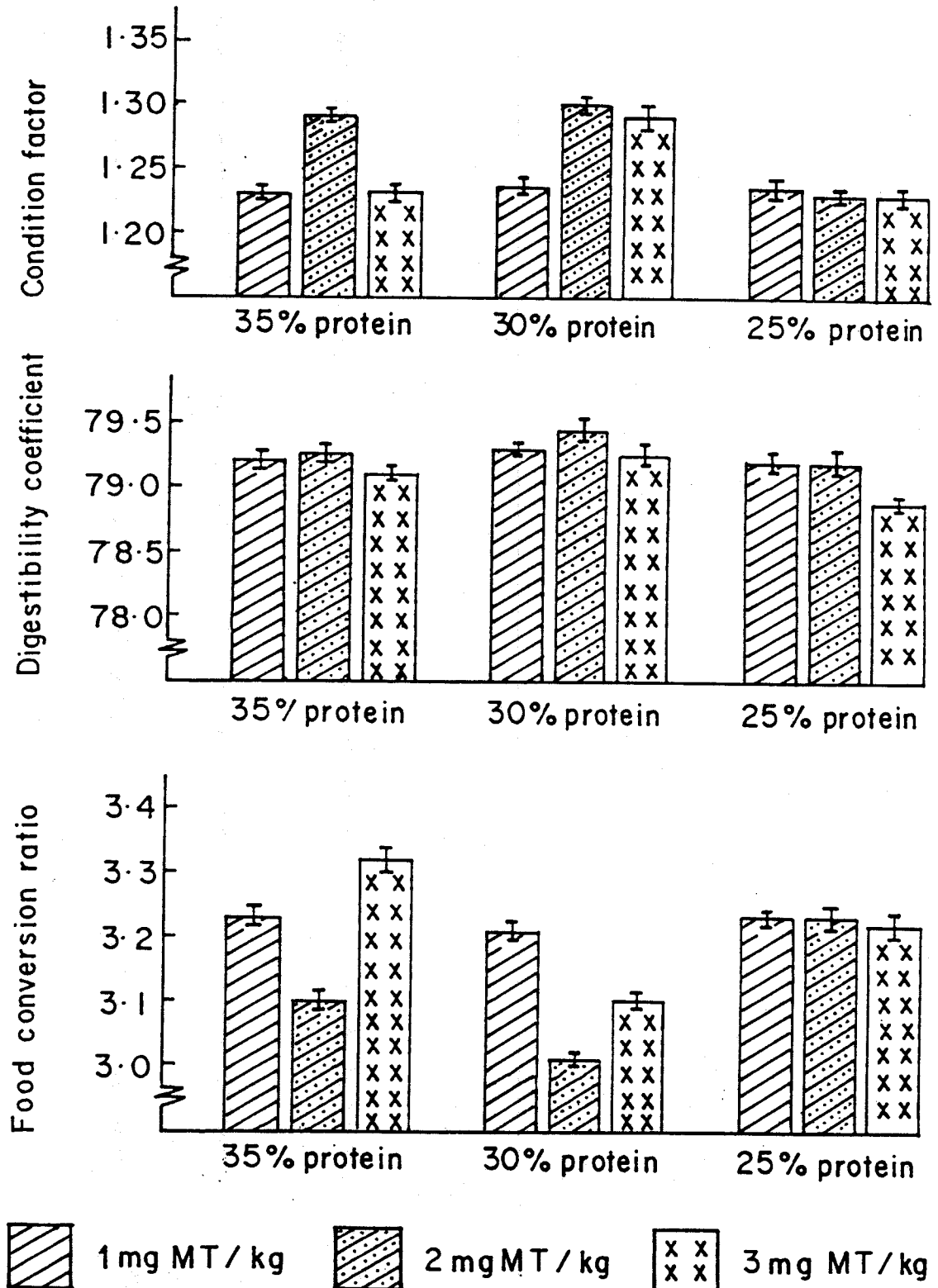
the next best weight gain was obtained for the 35% protein diet. The results obtained after 30 days were quite different from that seen after 15 days. The fish fed on the 35% protein diet had better weight gains over initial (87.01%) as compared to the diets containing 30% (80.07%) and 25% (61.93%) protein levels.

Trend observed after 45 and 60 days, were similar to that obtained after 15 days. The fish groups fed on the 30% protein + 3mg MT diet gave maximum percentage weight gain (177.88) after 60 days, which was followed by 35% protein + 3mg MT treatment (113.94%). The lowest weight gain (138.23%) was provided by the 25% protein diet.

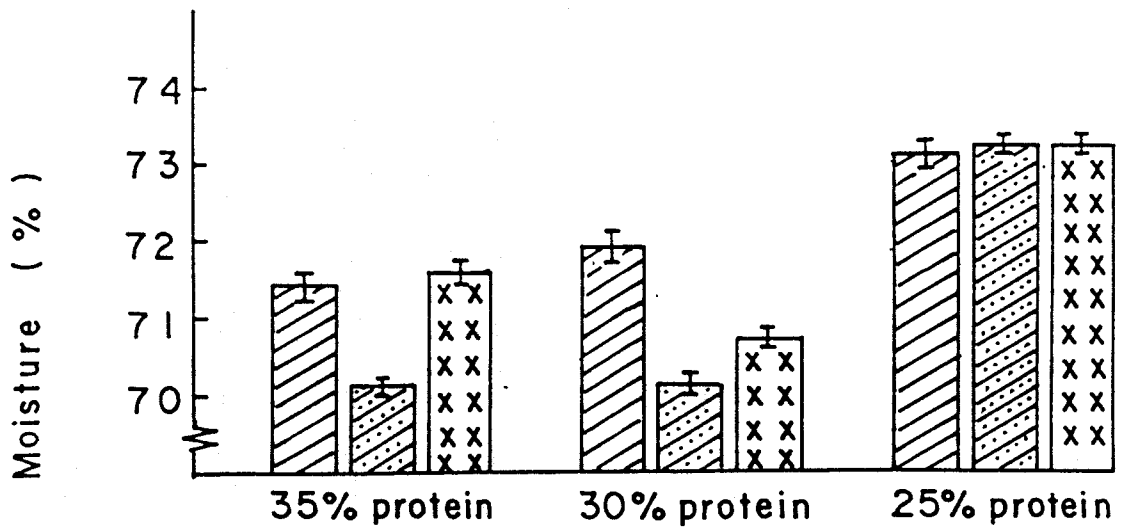
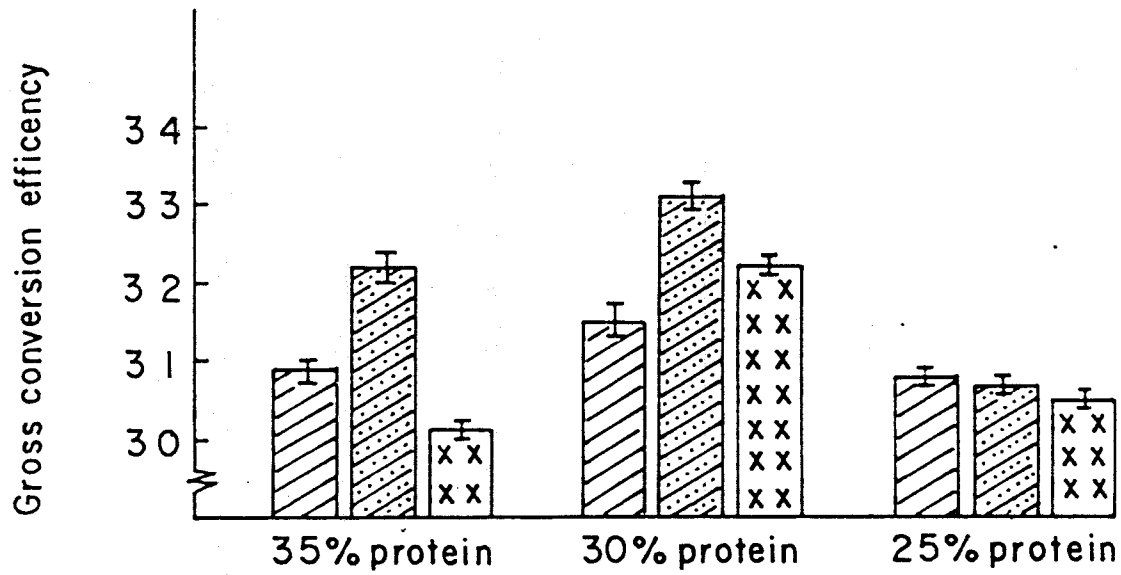
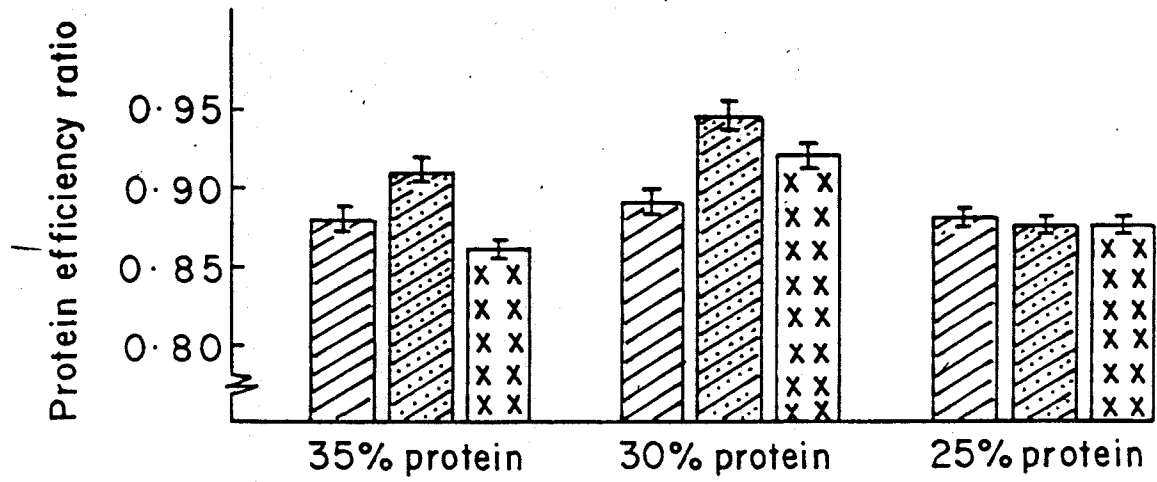
Irrespective of the protein level in the diets, the dosage of 1mg MT produced lower weight gains than the 2mg and 3mg steroid dosages. In contrast, the best percent weight gains were observed in fish groups fed the 2mg hormone diets at all the protein levels. The diet containing 30% protein and 2mg MT produced the best weight gain (186.27%), which was closely followed by the diet containing 35% protein + 2mg MT (178.11%) after 60 days. The lowest weight gain (138.23%) was noted in the fish fed the diet containing 25% protein + 3 mg MT.

Specific growth rate and condition factors (Fig.22 & 23

23 Effects of selected levels of 17 α - methyltestosterone (MT) and dietary protein levels.



4 Effects of selected levels of 17 α -methyltestosterone and dietary protein levels.






 1 mg MT / kg
  2 mg MT / kg
  3 mg MT / kg

Table IX) were significantly influenced by the different dietary treatments.

Among the fish groups fed the diet containing 35% protein and different levels of, MT the 2mg MT diet recorded the highest values for SGR (2.968 per day) and CF (1.290). At 35% protein level there was no significant difference ($P < 0.01$) in SGR and CF values between 1mg and 3mg MT diets. Among the diets containing 30% protein, fish fed the 2mg MT diet recorded the highest SGR (3.131 per day) and CF (1.290). The fish fed 25% protein plus different MT levels did not show much differences in their SGR and CF. However, the fish receiving MT at the concentration of 1mg/kg of feed showed relatively better values for SGR (2.545 per day) and CF (1.112). Overall, the fish fed the 30% protein diet with 2mg MT showed highest values for SGR and CF while the lowest protein level in the diet produced poor results for these parameters.

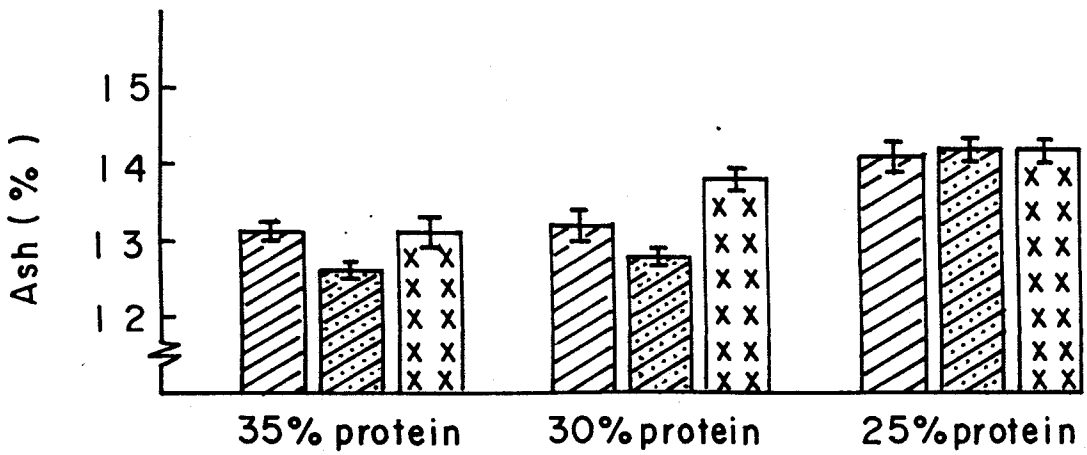
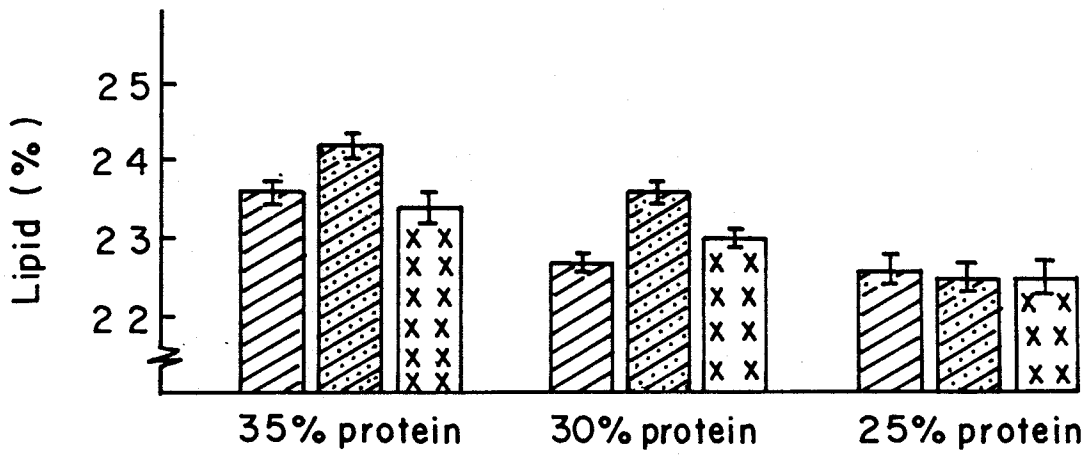
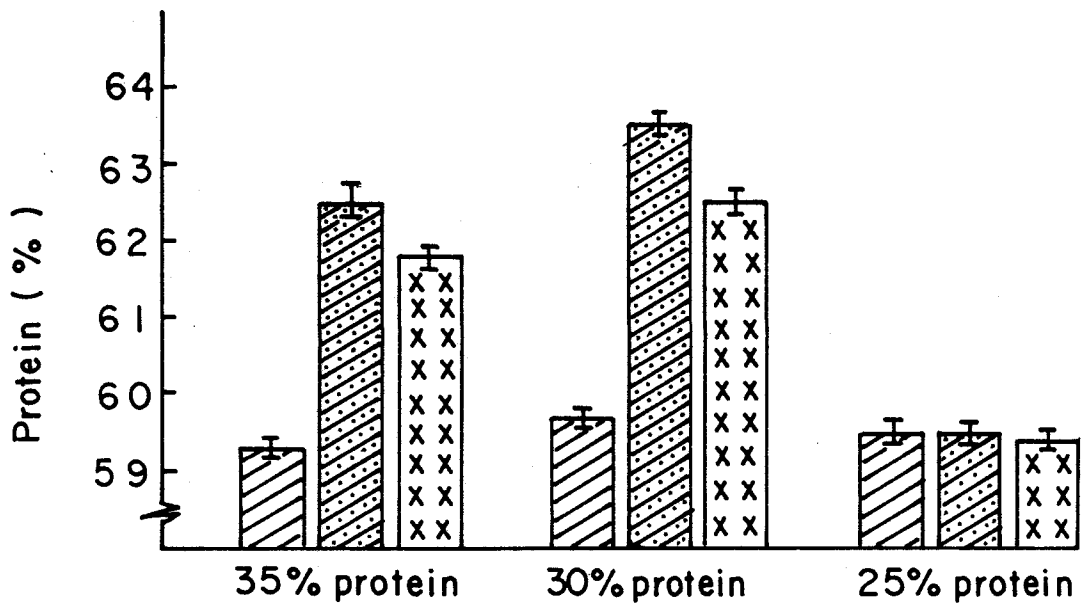
The overall growth response of the fish assessed from the weight gain, specific growth rate, and condition factor was in the following order: 30% P + 2mg MT > 35% P + 2mg MT > 30% P + 3mg MT > 30% P + 1mg MT > 35% P + 1mg MT > 35% P + 3mg MT > 25% P + 1mg MT > 25% P + 2mg MT > 25% P + 3mg MT.




FCR, Da, GCE and PER:

The feed conversion ratio (FCR) digestibility coefficient (Da) Protein efficiency ratio (PER) and the gross conversion efficiency (GCE) were also significantly affected ($P < 0.01$) by the dietary treatments (Fig.23 & 24 Table IX).

The fish groups fed the 35% protein diet + 2mg MT recorded the best FCR (3.10) but it was not significantly different from that of the fish groups fed the 35% protein diet with 1mg MT. A slight peak was observed for PER (79.28) and GCE (32.2) at the dose of 2mg MT, and the same group produced non-significant higher values for Da as compared to the fish groups fed on the 35% protein diet containing 1mg MT. Among the 35% protein diet receiving fish groups, the diet containing 3mg MT gave slightly poor values for FCR (3.322), Da (79.12), PER (0.860) and GCE (30.1) as compared to the dose of 2mg and 1mg MT.

Among the 30% protein with different doses of MT there were not much differences in the FCR, Da, GCE, and PER for the fish groups receiving 1mg and 2mg MT levels, though the diet with 2mg MT produced relatively better FCR (3.019), Da (79.45), PER (0.945) and GCE (33.1%). Relatively poor values for FCR (3.21), PER (0.890) and GCE (31.5%) were observed at



 1 mg MT / kg
  2 mg MT / kg
  3 mg MT / kg

the dose of 1mg MT (Table IX).

Among the 25% diet protein receiving fish groups, significant differences were not observed for the values of FCR, Da, PER and GCE. Slightly better values for Da (78.82), PER (0.845) and GCE (29.45%) were recorded for 1mg MT fed groups.

Overall, the 25% diet in combination with 1mg, 2mg and 3mg MT exhibited poor response as compared to the diets of 30% and 35% protein. Among 30% and 35% protein diet fed fish groups, the fish receiving 30% protein and 2mg MT gave the best values for FCR, Da, PER and GCE.

Proximate composition:

The percentage of protein, lipid, moisture and ash of the fish body were significantly influenced ($P < 0.01$) by the dietary treatments.

The fish groups fed on combination of 35% protein and 2mgMT/kg had relatively greater protein (62.56%) and lipid (24.23%). Significant difference was not observed in the body levels of lipid, moisture and ash between the fish groups fed the diets of 35% protein containing 1mg and 3mg of MT.

However, protein content was significantly lower in fish fed the diet containing 35% protein + 1mg MT than the other groups.

Significantly higher percentages of protein (63.5) and lipid (23.58) and lower levels of moisture (70.16%) and ash (12.8%) were observed in fish fed the 30% protein with 2mg MT, than the other 30% protein diet receiving fishes. In contrast to this, the fish given 30% protein + 1mg MT diet exhibited significantly poor results for protein (59.71%) lipid (22.7%) moisture (71.93%) and ash (13.2%) even as compared to the combination of 30% protein + 3mgMT/kg.

The results obtained for the body composition of the fish fed the 25% protein plus different dosages of MT were not significantly different ($P < 0.01$) from each other. Comparatively, better results for protein (58.5%), lipid (22.54%) moisture (73.1%) and ash (14.1%) were observed in the fish fed the diet of 25% protein + 1mg MT, which did not show significant differences with other fish groups (Fig.25, Table IX).

Among the fish groups fed the 35% and 30% protein diets, better levels of body components were found in fish on diets containing 30% protein + 2mg MT, followed by the fish groups

fed on the combination of 35% protein + 2mg MT (Table 10).

6. EFFECT OF INJECTION OF MT AND ESTRONE ON OVARY DEVELOPMENT:-

The hormone dosages for the injection experiments were selected based on the response obtained by various workers in teleosts. 17 α - Methyltestosterone dosages were 0 to 1.5 mg/kg and estrone 1 mg, 2 mg and 3 mg/kg live - body weight of the fish.

Both the experiments were conducted simultaneously and therefore the same stock of fishes were used. Initial examination of the gonads of a sample of fish (5 nos) were made and all were found to be immature. The rearing conditions during the experiments were as follows: salinity 17 ± 1 ppt, temperature $28.3 \pm 2.2^{\circ}$ C, pH 7.6 ± 0.24 , ammonia 0.25 ± 0.10 mg/l, oxygen 4.7 ± 0.3 ppm.

Results obtained in the present study are shown in Plates IV to XI. After termination of the experiments i.e. after 30 days, the ovaries of hormone treated and control females were collected and fixed and processed to study the histological changes.

2) 2 mg/kg - The ovary of L.parsia treated with estrone at this dose were found to be in perinucleus stage (Plate X). About 20 - 25% oocytes were in perinucleus stage which could be easily distinguished by the peripheral arrangement of 4 - 6 numbers of small nucleoli on the inner side of the nuclear membrane. The size of the nucleoli ranged between 3 - 4 μ m in diameter. Nucleii were larger, measuring 35 - 45 μ m in diameter.

3) 3 mg/kg - The ovary of the fish injected with 3 mg estrone was in a slightly advanced stage than the 2 mg/kg estrone (Plate XI). The ovary had about 30 - 35% perinucleolus oocytes, which could easily be identified by the peripheral arrangement of 6 to 8 numbers of small nucleoli on the inner side of the nuclear membrane. Nucleii were about 45 to 50 μ m in diameter.



CHAPTER IV

DISCUSSION

DISCUSSION

Acceleration or control of growth can be a very useful management tool in aquaculture, especially for the hatchery production and nursery rearing of fish. Previous studies have shown that growth of finfish can be significantly enhanced by oral administration of a variety of steroid hormones (Higgs et al., 1982). Most of these studies dealt with the effects of steroid hormones on fresh water fishes, with very few exceptions (Higgs et al., 1982). The effects of selected levels of 17α -methyltestosterone (MT), diethylstilbestrol (DES), 17β -estradiol(ES) and combination of MT with thyroid hormone(T₃) on the survival, growth, food utilization and body composition of the mullet Liza parsia have been examined through oral administration for the first time in the present study. The results of the present feeding experiments have shown either positive or negative influence of the steroid hormones on the survival, growth, food utilization and body composition of Liza parsia fry. The response of the fish to the different hormones and their dosages varied significantly.

Response of the fish to the dietary MT:-

The present study shows that growth of Liza parsia can be accelerated by treatment with MT at optimum

levels. Further, the result suggests that the growth response of the fish to MT is dose-dependent.

Growth promotion was exhibited only by those fish fed the diets containing less than 10mgMT, with the highest anabolic effect for the fish fed on the diets containing 2mg MT. The dosages from 4 mg to 8 mg MT provided moderate growth, but the growth showed a steadily decreasing trend as dosages increased. These results suggest that the potency of the hormone declines with increasing concentration. Thus MT concentration of 10 mg in the diet produced almost similar growth to that of the control showing that the moderate dose of 10 mg of MT do not adversely influence the fish physiology. However, since there was no weight gain over the control when MT levels exceeded 10 mg/kg diet during any phase of the rearing period, it can be assumed that higher concentrations of the 17 α - methyltestosterone may not have any anabolic effect in the fry of this fish. In fact MT concentrations exceeding 10 mg/kg diet have a growth depressing effect in Liza parsia. These results suggest that 2 mg 17 α - methyltestosterone is the optimum to obtain maximum growth in this species, while the dosage of 10 mg/kg seems to be the critical level at which the normal physiology of the fish is not impaired; therefore no apparent growth

changes are seen at this concentration as compared to the control. But higher MT levels have a definite growth depressing effect on the fish fry. It is most likely that MT at supra-optimal levels either interferes with the anabolic processes of the fish or high dosages may prove toxic to the fish fry. The heavy mortality that occurred in fish groups receiving 40 to 60 mg MT can be ascribed to the above conclusion.

Results of similar magnitude have been observed, with lower concentration of MT in the diets, in the fry of many fish species. Maximum growth response has been achieved with 1 ppm MT in Oncorhynchus gorbuscha, O.kisutch and Salmo gairdneri (Fagerlund and McBride, 1977); but Yu et al (1979) observed highest weight gains in the fry of O.kisutch with a diet containing 2.5 ppm MT, when fed to satiation. Lower dosages (1 to 3 mg/kg in the diet) of 17 α -methyltestosterone are also reported to be very effective in accelerating growth in the fingerlings of many fish species: the mahseer, Tor khudree and carps, Catla catla, Labeo rohita, Cirrhinus mrigala and Cyprinus carpio (Deb and Varghese, 1988). Similarly, one year old gold-fish Carassius auratus responded with highest growth increment when received the 1 ppm MT through diets (Yamazaki, 1976). Thus the lower levels, 1 to 3

mg/kg, of this hormone promotes growth in various size groups of several fish species. However, such low levels induced only marginal weight gains in O.nerka (Fagerlund and McBride, 1975) and O.tschawytcha (Schreck and Fowler, 1982). In contrast, the juveniles of Salmo gairdneri recorded lower growth over the control when fed on 1 to 5 mg MT (Sower *et al.*, 1983).

In one year old gold-fish Carass. s auratus no apparent changes in the growth occurred at the dose of 10 mg MT while 30 mg MT diet retarded the growth (Yamazaki, 1976) as observed in L.parsia fry in the present study. In contrast, moderate weight gains were noted in the fry of O.gorbuscha, when fed on a 10 ppm MT diet (Fagerlund and McBride, 1977), but superior growth occurred in the fry of Cyprinus carpio fed on a 220 mg MT diet (Rao and Rao, 1988).

The condition factor, a measure of the relationship between weight and length is expressed for the mullet as $a = W/L^t$ where $t = 3$ in the present study (Kiron, 1989). Data obtained in the present study reveals that condition factor increases with the fish growth suggesting that growth attained is more due to the weight increment than the length increase. The steroid treatments gave higher values for

condition factor than the control fish upto the dose of 10 mg/kg, beyond this level all the fish groups recorded lower values than the fish fed on the steroid deficient diet (Table IVa, b and V). It shows that fish receiving diets containing more than 10 mg MT concentration do not add weight proportional to the normal length attained initially.

A similar response was observed in the fry of pink salmon O.gorbuscha by Fagerlund and McBride (1977), wherein condition factor showed an increase upto the dose of 10 mg MT after 44 days. The condition factor is a sensitive indicator of changes in the diet and environment and such changes may temporarily influence the trends towards lower condition factor. Since the diet composition and environmental characteristics being almost constant for all the treatments in the present study, the variations in CF in the MT treated fish groups seems to reflect the effect of the hormone on the weight-length relationship of the fish.

Application of MT at a dosage of less than 10 mg in the diet improved the digestibility, gross conversion efficiency and FCR as against the significant reduction in the above parameters at concentrations exceeding 10 mg MT. The best

FCR, gross conversion efficiency and digestibility obtained at 2 mg MT indicates that at optimum levels of incorporation MT can improve the digestion and conversion of ingested food in L. parsia fry. This improvement is achieved either through stimulation of thyroid hormone (Overbeek and McBride, 1977; Hunt and Eales, 1979b) or by accelerating the activity of protease of the gut as observed by Lone and Matty (1981b) in the carp Cyprinus carpio. Yamazaki (1976) also found histological evidences for increase in the proteolytic activity of pancreas and intestine of O.masou treated with MT (10 ppm) for two weeks.

The FCR and GCE are response parameters which reflect upon the efficiency of conversion of the ingested food. In L. parsia both the factors were significantly influenced by the MT levels in the diet and the results are comparable to the best food conversion at 2.5 mg MT observed by Yu et al. (1979) in O.kisutch fry. Similarly, in the fry and juveniles, moderate improvement in FCE was achieved with 1 mg MT in the diet for O.kisutch (Fagerlund et al., 1972; Fagerlund and McBride, 1979) and with 2.5 ppm MT diet for rainbow trout (Simpson, 1976). Thus the improvement in digestibility and FCE observed in the present study suggest that MT augments

growth in fish by improving digestibility and conversion efficiency of the food.

Further results of the present study show that supra-optimal concentrations of MT besides lowering the digestibility of the nutrients in the feed significantly affects the metabolism of nutrients, resulting in poor FCE. It is likely that MT at hyper-dosages affects the secretion and activity of the digestive enzymes and also interferes with the metabolism of the assimilated nutrients.

In contrast to the present findings, significantly higher conversion efficiency was observed by Nirmala and Pandian (1983) in the adult fish Channa striatus injected with MT dosages of 5, 10, 20 and 30 mg/kg body weight. The superior performance of the above fish may be due to the larger size of the fish or the frequency of hormone administration or due to the genetic make-up of the species. Thus, it seems that the influence of MT on food conversion efficiency may be related to size, species as well as the frequency of administration.

In the present investigation, the best FCR (2.623) and PER (1.090) were recorded for the 2 mg MT treatment. These results suggest that the higher weight gains attained by Liza

parsia at lower MT dosages are associated with the improved digestibility, food conversion rate and protein utilization by the animal. Thus, oral administration of 17 α -methyltestosterone at relatively lower dosages has significant anabolic effect in Liza parsia by improving the conversion of ingested food and protein for tissue building. Gogoi and Keshavanath (1988) also observed improved conversion efficiency rate in the fingerlings of Tor khudree at the dose of 2.5 MT (3.98) as compared to the control (5.42). Similarly, the diet containing 2 mg MT, provided better FCR in adult O.tshawytscha than the control.

The best PER recorded in the present study is in agreement with that of Yu et al (1979), who have recorded the highest value for PER in 2.5 mg MT treated O.kisutch as compared to the fish fed on the steroid deficient diet. Further, higher concentrations of MT seems to have a inhibiting effect on the protein conversion. The present findings indicate that MT levels at less than 10 mg/kg acts as a growth promoter by improving the digestibility of ingested nutrients by the fish and by increasing efficiency of conversion of the ingested food and protein. The results further suggest that optimum conversion and digestibility may be obtained at a dosage of 2 mg MT.

Body composition analysis indicates that dietary MT levels has significant influence on the protein and lipid contents. The steady increase in protein (upto 4 mg MT) and lipid (upto 10 mg MT) contents in fish associated with the increase in MT levels in the diets indicate that the rate of protein and lipid deposition is regulated by the concentration of the hormone in the diet. A concomitant drop in the moisture content was noted indicating that the protein and lipid deposition has taken place by the replacement of water from the tissues.

The present results further suggest that MT has dose dependant influence on the body composition of this fish, since the highest protein (62.8%), lipid (24.23%) and lower moisture (72.22%) levels were observed at 2 mg/kg MT diet; whereas relatively low levels of protein and lipids were found in fish fed on high MT dosages. Results of similar magnitude were recorded by Lone and Matty (1980a), when fry of common carp, Cyprinus carpio, were treated with 2.5 to 10 ppm, a sharp increase was recorded in protein and lipid contents of the fish body at both the MT levels. However, the effects of MT on body composition of salmonids appears to be different from that of Liza parsia. MT exerts only minor effects on body moisture and protein content of juvenile

salmonids. Fagerlund and McBride noted that the lower doses of MT (1 ppm) decreases the lipid level (22.39%) over the control (28.45%), but increases the protein (61.5%) as compared to the control (56.04%). In contrast, when fry of coho salmon were given 0.2 and 1 ppm MT at different temperatures, protein level increased but fat decreased at 16.5° C, where as an inverse trend with decrease in protein and increase in fat was noticed at 11.5° C. Similarly, in the fry of O.kisutch fed on 2.5 mg MT, protein was not affected but lipid level in MT treated group (7.47%) was lower than the untreated fish (8.43%) (Yu et al., 1979). These results suggest that response of MT to deposition of body constituents is species, size and temperature dependant.

In the present study ash and moisture contents are significantly lower in the fish groups fed the 2 mg MT, which gained the highest weight. Ash and moisture levels were lower than the control upto 8 mg/kg for ash and upto 10 mg/kg for moisture. Above this concentration a steady increase was evident as the MT dose increased. The changes in the contents of ash and moisture can mainly be correlated to variation in protein and lipid contents rather than the direct effect of MT.

In the present study MT has shown severe effect on the

survival of the fish. The mortality was directly related to the concentration of MT in the diet. As a consequence, the treatment groups receiving higher dosages of MT (40 mg to 60 mg/kg) were terminated after 40 days. Further, continuation would have caused higher mortalities. The highest percentage (95%) of survival was observed in the control diet after 60 days. The lowest percentage of survival (53%) was observed in 60 mg MT treatment after 40 days as compared to the control (97%), while after 60 days the dose of 30 mg MT recorded 65% survival as against 95% in the control.

No attempt was made in this study to examine the reason for such high casualties. However, from the reduced food utilization in these groups it can be assumed that the fish have used a good proportion of the body nutrients to meet their energy demands to cope up with the stress resulted from the higher concentrations of steroid hormone. It is also possible that extreme stress might have induced morbid condition in the fish leading to eventual death. Further studies are necessary to know if there is any deleterious effect of high MT levels on the physiology of the fish.

Rao and Rao (1983) also observed relatively low survival in the fry of Cyprinus carpio (18.8%) given a diet with high

dosage of MT (220 ppm) as compared to the control (72.72%). In contrast, Tilapia fry, Oreochromis mossambicus did not show much difference in survival with reference to MT level of 30 ppm in the diet (89%) over the control (90%).

In the present study relatively high percentage of survival was observed among fish groups fed on the control diet, and 4 mg and 2 mg MT diets. In the fry of O.gorbuscha, Fagerlund and McBride (1977) observed relatively low or no mortality at the dose of 1 ppm MT in the diet. Thus the survival of fish fry is highly related to the MT dose in the diet.

Thus the results of the experiments suggest that (i) there is no beneficial effect of high MT dosages to L. parsia fry (ii) maximum growth could be achieved with relatively low MT doses (iii) 2 mg MT/kg diet is adequate enough to elicit maximum anabolic effect (iv) the response of the fish to MT is dose-dependant (v) high dosages (above 10 mg) are detrimental to growth and survival and results in overall depressing effect.

Response of the fish to dietary DES:-

Diethylstilbestrol (DES) has been widely used in

animal husbandry because of its ability to promote growth and improve food conversion efficiency without manifesting any renotropic or hepatotropic side effects. However, there are conflicting reports regarding action of diethylstilbestrol in fish. Ghittino (1970) reported a slight depression of body growth in rainbow trout fingerlings fed DES supplemented diets at fairly high dosages (50 - 500 mg/kg food). In contrast, Cowey et al. (1973) noted that DES, when given in low doses (1.2 mg/kg dry food) accelerated the growth rate and improve food conversion efficiency in plaice, Pleuronectes platessa, and suggested that negative growth response to oral administration of DES noted in rainbow trout by Ghittino (1970) could have been due to the use of excessive doses. In view of this argument, the growth trial presented in this study was conducted with graded levels of DES.

The results indicate that diethylstilbestrol does not promote growth or improve CF significantly when fed at even the lowest dosage of 0.3 mg/kg feed in L.parsia. DES at higher than 0.3 mg levels induced a growth depressing effect on Liza parsia. However, at low doses of 0.6 to 1.2 mg DES has been shown to accelerate growth in the fingerlings of plaice, Pleuronectes platessa, though a dose of 2.4 mg failed to promote growth (Cowey and Sargent, 1972; Cowey et al.,

1973). Similarly, DES (4 ppm), has recorded a nonsignificant weight gain (2.8%) over the control, when fed through diet in the fingerlings of Tor khudree (Shyama and Keshavanath, 1988), while the same authors have recorded a negative growth for the silver carp with DES. The present results are also in agreement with the catabolic effects of DES noted in gold fish, Carassius auratus (Hoar, 1958), channel catfish, Ictalurus punctatus (Bulkley, 1972), coho salmon (Fagerlund and McBride, 1975 b) and rainbow trout, Salmo gairdneri (Ghittino, 1970; Bulkley, 1972; Fagerlund and McBride, 1975a; Matty and Cheema, 1978). In contrast to these growth depressing results, the fingerlings of Labeo rohita showed superior growth when 3 mg/kg DES was fed through diet (Nanjundappa and Varghese, 1988). Thus different species of fish seems to respond differently to the same estrogen.

Likewise DES doses also did not improve the conversion efficiency, digestibility or protein efficiency ratio. With the exception of 0.3 mg dose, DES severely affected the digestibility and utilization of the food in Liza parsia (Table VI). Similarly, no significant difference was observed in FCE and protein conversion efficiency in coho salmon O.kisutch administered with 2.5 mg DES (Yu et al., 1979). In contrast, Cowey et al., (1973) noticed improvement

in food efficiency when Pleuronectes platessa was fed on a diet containing DES doses of 0.6 and 1.2 mg/kg dry food). Nanjundappa and Varghese (1988) also observed improved food conversion efficiency when fingerlings of Labeo rohita received 1 mg DES.

Proximate composition data indicate that body protein and lipids were increasingly catabolized for energy production rather than tissue building with the progressive increase in DES levels in the diets. Water and ash levels in the tissues steadily increased as a result of the protein and lipid catabolism. Yu et al. (1979) did not find any significant difference in protein and ash contents, though lower values for lipid were recorded in O.kisutch receiving 2.5 mg DES in the diet. Similarly, significant differences were not observed for moisture and total nitrogen in the juvenile rainbow trout when fed on diets containing 1.2 mg/kg DES (Matty and Cheema, 1978).

From the gains in weight and body protein at 0.3 mg over the control it can be inferred that DES levels less than 0.3 mg/kg may have some growth promoting effect in L. parsia fry.

In conclusion, from the response data obtained during

this study, it can be inferred that there is no advantage in adding DES to the diets of L.parsia fry. Further, the future of DES has been called into question because it is carcinogenic (Anonymous, 1972 cited by Cowey et al., 1973). Clearly, the metabolism of DES and the rate at which it is removed from the carcass during feeding on a diet free of DES will require careful investigation before its use in production diets may be contemplated.

Response of the fish to dietary 17 β - Estradiol(ES):-

The estrogen 17 β - estradiol (ES) proved to be a growth promoter for L. parsia as evidenced by the response attained in the present study. However, only moderate increase in growth (11.1%) was observed with 2 mg ES as compared to the fish fed on the hormone deficient control diet. Specific growth rate and condition factor are also found to be enhanced (Table VII) in this group. The increase in condition factor (1.180) at 2 mg/kg estradiol diet over the control (1.098) shows that the increase in growth is attained through weight increment rather than increase in length. Probably, this concentration may cause enhanced food utilisation to account for the growth increment.

The growth promotion in L. parsia is in agreement with

the observation of Yu et al. (1979), who noticed about 15% weight gain over the control in coho salmon, O.kisutch on a dry diet supplemented with 2.5 mg estradiol. However, the same species (O.kisutch) gave only 5.5% weight gain over control, when the dose of this hormone was increased to 10 mg/kg (Fagerlund and McBride, 1975 ; Donaldson et al., 1979).

The fry of the common carp seems to require relatively higher concentration of this hormone to produce weight gain compared to the present study. Rao and Rao (1983) observed an average weight gain of 50.98% in the diets with 200 mg estradiol per kg; whereas 120 mg/kg estradiol had a growth depressing effect (Jensen and Shelton, 1979). On the other hand Yamazaki, (1976) could not come to any conclusion when Carrassius auratus were treated with 1 - 10 mg/kg of this steroid. Thus the response of fish species to the estrogen, estradiol seems to be dose as well as species dependant.

Relatively better values for digestibility, food conversion rate, gross conversion efficiency and protein efficiency ratio were found for fish groups receiving 1 and 2 mg estradiol in the diet (Table VII). But, the highest feed conversion efficiency (28.1%) and PER (0.809) were recorded

for fish given 2 mg estradiol in the diet. It indicates that 2 mg estradiol is the optimum dietary level required for maximum growth in Liza parsia. Response of similar magnitude for FCE and PER were observed in coho salmon (O.kisutch) fed on diet containing 2.5 mg/kg estradiol Yu et al., 1979.

This hormone functions as an anabolic agent in Liza parsia by improving the digestibility and conversion of food and protein into tissue.

The results of proximate analysis are presented in Table (VII). ES did not have much influence on the body composition of Liza parsia. However, slightly higher values for protein and lipid as well as lower levels of moisture and ash percentages were observed in the fish treated with 1 and 2 mg estradiol. Estradiol level exceeding 4 mg/kg produced fish with relatively lower protein and lipid than the control fish suggesting catabolic effect of higher levels of this steroid on body nutrients in L.parsia. In O.kisutch, estradiol (2.5 mg/kg) diet did not induce any significant change in body composition as compared to fish receiving a steroid deficient diet Yu et al 1979.

Results of this experiment indicates that estradiol supplementation at the dose of 2 mg/kg diet can be beneficial, in that Liza parsia could be raised to desired

size in a shorter period of time, with relatively less food and better feed efficiency.

Response of the fish to thyroid hormone (T3) and MT levels and their combinations :

A) Thyroid hormone (T3)

Oral administration of thyroid hormone (T3) caused significant weight gain in Liza parsia fry. Besides, T3 hormone along with MT serves as a growth promoter in this fish. Oral administration of T3 induced significant improvements in weight gain, conversion efficiency, as well as protein and lipid contents of Liza parsia over the control.

Data shows that relatively low doses (3 and 6 mg) of T3 improves growth and condition factor in Liza parsia over the control (Table VIII). The anabolic effect may be caused by several mechanisms, operating individually or synergistically: (i) through the ability of thyroid hormone to potentiate the effects of other anabolic hormones, most notably growth hormones (Donaldson et al., 1979 ; Eales, 1979), (ii) through stimulation of DNA-dependent RNA synthesis and subsequent protein synthesis (Higgs et al., 1982), (iii) T3 may potentiate appetite and or food

utilization (Markert et al., 1977). Anabolic effect of T3 has also been reported in O.kisutch (Fagerlund et al., 1980) and Salmo gairdneri (Eales, 1979 b).

Comparing the different dosages used in the present study, superior growth has been achieved with 3 ppm T3. The dose of 9 mg T3 /kg diet seems to have a depressing effect on growth and feed utilization in L. parsia. Juveniles of Salmo salar, however, seems to require higher dosages (20 mg T3) for the optimum growth (Saunders and Henderson, 1980, cited by Higgs et al., 1982). These results suggest that the anabolic effect of T3 in fish is size as well as species oriented (Higgs et al., 1982). Besides the hormone dosages, many other factors seems to influence efficiency of T3, i.e. route of administration and nutritional status of the fish etc. In the present study, the composition of the diets used was maintained quite uniform and hence the response attained relates to that induced by T3 incorporation alone.

While the growth, FCR and PER were better for the 6 mg T3 diet the protein and lipid levels were higher for fish receiving the 3 mg T3 diet suggesting that maximum protein synthesis and lipid deposition occurs at relatively lower levels of T3 and that T3 significantly influences the protein and lipid deposition in Liza parsia fry.

The results of gross conversion and protein conversion efficiencies and digestibility coefficient suggest that growth promotion in Liza parsia is attained through the positive influence of T3 in improving utilization of food and protein. As compared to the GCE and FCR obtained at 3 mg T3 in L. parsia, Fagerlund et al. (1979), obtained better FCE and PER at the dose of 2mg T3 in the fish O.kisutch. Results of similar magnitude for FCE and PER were recorded in O. tschwytscha (Higgs et al., 1982).

The dose of 9 mg/kg resulted in poor feed utilization suggesting that T3 at supra-optimal levels affect appetite or food utilization. Similarly, Fagerlund et al. (1979) noted that the high dose (25 ppm) of this hormone results in lower food and protein conversion efficiencies in O. kisutch but in Liza parsia the food and protein utilization is affected at relatively low dosages of T3. Diet composition (Fagerlund et al., 1979 ; Higgs et al., 1982) and prevailing hormonal milieu (Fagerlund et al., 1980) are factors that are suspected to affect the potency of T3 in enhancing food and (or) protein utilization.

The fish on the 6 mg T3 /kg diet were found to be very healthy and consequently high survival rate (97%) was

recorded; whereas the fish groups receiving 9 mg/kg T3 recorded low percentage of survival (83%) suggesting that the fish may be under physiological stress induced by high level of T3 in the diets.

In mammals thyroid hormones play an important role in the regulation of appetite, digestion, nutrient absorption, protein anabolism and catabolism and in non-protein energy deposition and mobilization (Felber, 1977). There is also evidence for thyroidal involvement in all these physiological processes atleast in some fish species (Donaldson et al., 1979). In L. parsia digestibility of the ingested food has been affected by the T3 level in the diet with T3 levels exceeding 6 mg inducing a significant decrease in digestibility. These results seem to suggest that high T3 levels may interfere with the activity of some digestive enzymes. Further research is necessary to confirm this speculation. On the other hand, improvement in the utilization of food and protein for growth as evident in Liza parsia is commonly observed (Higgs et al., 1982) .

In the present study smaller gains for protein (61.5%) and lipid (23.83%) were observed in fish treated with 9 mg T3 diet as compared to the control (Table VIII). The gains

recorded for protein may be caused by the action of T3 on RNA synthesis and thereby protein or enzyme biosynthesis in the fish body (Higgs et al., 1982). In juveniles of O.kisuth the diet incorporated with 4ppm T3 showed a slight improvement in protein of the fish content (62.5%) as compared to control (60.5%) (Fagerlund et al. ; 1980). However, the lipid and moisture contents were lower in this treatment (4 ppm) than the control diet. In contrast, significantly lower values for body protein were observed in Salmo gairdneri when given T3 dosages (0-25 ppm) through diets (Higgs et al., 1982). The present results confirm the observations that deposition or mobilization of protein and lipid may depend upon hormone dose (Narayan Singh and Eales, 1975). However, circulating levels of other hormones which influence body energy reserves in fish, such as growth hormone (Donaldson et al., 1979) and prolactin (Meier, 1970) may also interact with thyroid hormones to determine the response direction.

B) Hormone combinations:-

For the first time the combinations of a steroid hormone (MT) and a thyroid hormone (T3) have been tested on Liza parsia at various concentrations. To the author's knowledge there is no other report relating to the combination of MT

and T3 to test fish growth, although several other combinations have been evaluated. However, Higgs et al. (1977) have studied the effect of combination of T4, bGH and MT as well as T4 + MT on coho salmon.

Results of the present study suggest that growth enhancement also can be achieved by the combination of MT and T3 at optimum doses over the control. For instance, maximum growth in Liza parsia occurred, when a diet with a combination of 9 mg T3 + 2 mg MT was fed. The same fish group gave the second best values for specific growth rate and condition factor amongst all the groups.

In the present study the 9 mg T3 + 2 mg MT/kg diet combination provided the highest growth upto 30 days probably due to the synergistic effect. This combination would have created an internal hormonal milieu most conducive to rapid growth of the Liza parsia. Hormone combination probably stimulate growth by improving appetite and (or) food conversion efficiency as has been shown for bGH (Markert et al., 1977) and MT (Higgs et al., 1977). The steroid (MT) would have contributed to the growth promotion by stimulating deposition of protein and lipid, while T3 may promote feed conversion ability of the fish (Higgs et al., 1982). From

studies with mammals it is known that the hormones which have the greatest influence on cartilage and bone development are the thyroid hormone, androgens and growth hormones, and the thyroid hormones and sex steroids are more active on the process of calcification and ossification (Rapport, 1975). This may also be the case in fish. Some hormone such as thyroid hormone influence somatomedin activity in mammals, which may mainly regulate chondrogenesis and hence affect the rate of growth (Gaspard et al., 1975). A similar situation may exist in fish.

The fish groups fed on the dosage of 3 mg T3 + 2 mg MT per kg and 3 mg T3 + 4 mg MT per kg gave lower growth increment over the control, suggesting that these combinations are not appropriate to promote the anabolic effect in Liza parsia. It is likely that the anabolic effect of the combination of this hormone may also be dose - dependent and that specific ratios of hormone levels may also be necessary to induce a growth promoting effect.

The increase in condition factor, noted for 9 mg T3 + 2 mg MT per kg fish group indicate that this proportion of hormones induces greater growth in weight than length in Liza parsia.

Although the combination of 9 mg T3 + 2mg MT per kg gave the second best growth promotion, the survival rate (83%) was low as compared to the control as well as the fish groups receiving individual hormones [6 mg T3 per kg (97%), 2 mg MT per kg (98%) and 4 mg MT per kg (93%)] suggesting that as far as survival is concerned, the individual treatments are better than the hormone combinations for the fry of Liza parsia.

Combinations of T3 and MT treated fish groups recorded better food utilisation than that of control, except for the fish groups receiving 3 mg T3 + 2 mg MT, and 3 mg T3 + 4 mg MT. The best FCE and Da recorded in fish fed on 9 mg T3 + 2 mg MT indicates improved assimilation of the ingested food at this level of combination, resulting in improved weight gains, food conversion and protein utilisation in the fish.

Results obtained (Table VIII) shows that, the combination of MT and T3 changes the proximate composition of Liza parsia. Most of the hormone treated fish had greater levels of protein and lipid and lower moisture and ash percentage than the controls. The highest percentage of lipid (23.76%) and protein (64.43%) were noted in fish fed on 9 mg T3 + 2mg MT. The lower contents of moisture and ash in the fish group receiving 9 mg T3 + 2 mg MT over control gives an

evidentary proof in this study that, the combination of T3 and MT at this level has stimulated the accumulation of more protein and lipid in Liza parsia.

The results suggest that hormones combination can be used to manipulate the proximate composition, food conversion efficiency and condition factor in Liza parsia. From the response of the fish to the various hormones and their combinations 2 mg/kg 17 α - methyltestosterone seems to be the best for the maximum growth promotion if the experimental duration is longer, while for shorter duration the dose of 9 mg T3 + 2 mg MT per kg would be ideal.

In Liza parsia the androgen MT seems to be more suitable than the combination of T3 + MT.

Response of the fish to MT at different dietary levels of protein:-

Since 17 α - methyltestosterone was found to be the most potent growth promoter in Liza parsia fry an experiment was conducted to ascertain whether the growth promoting effect could be achieved by a reduction in protein level by incorporating MT. Secondly, to the author's knowledge, so far, only in two species the effects of androgens on the

protein sparing action has been investigated (Matty and Cheema, 1978; Lone and Matty, 1982; Fagerlund et al., 1983). Of these, only Fagerlund et al. (1983) evaluated the protein sparing effect of MT on O.kisutch, while the other study was conducted with another androgen, ethylestrenol, on Cyprinus carpio (Matty and Cheema, 1978; Lone and Matty, 1982).

In the present study irrespective of the MT levels (1, 2 and 3 mg/kg) all the diets containing 30% protein produced superior weight gain and specific growth rate in the fish as compared to the 35% protein diet. The percent weight gain and SGR suggest that optimum dose of MT for Liza parsia is 2 mg/kg at the dietary protein level of 30%. At this combination MT proved to be most effective as a growth promoter. Likewise, 35% protein + 2 mg MT/kg also produce higher weight gain as compared to other levels of MT in 35% protein diets.

However, the minimum protein level in the diet necessary to produce maximum growth in Liza parsia fry at the dose of 2 mg/kg MT seems to be about 30% dietary protein. This is significantly lower than the protein requirement of 40% recommended for the fry of this species (Kiron, 1989) using purified diets. Thus, the inclusion of the steroid hormone,

MT, improves protein utilization significantly for promoting optimum growth.

However, Fagerlund et al. (1983) have observed higher weight gain in O.kisutch fed on diet containing higher protein level (51%) than lower protein levels (35%), though both diets had 1 mg MT/kg. The present results confirm the earlier report made on rainbow trout fry by Matty and Cheema (1978), that fish receiving a lower protein diet (35%) with 3.5 mg/kg ethylestrenol produce significantly higher weights than diets with 40 and 45% protein. The relative poor growth in 35% and 25% dietary protein levels in the present study indicates that in the presence of an anabolic agent like MT in the diets, dietary protein levels above 30% is poorly utilized for the growth. Probably some amount of protein is catabolized at this dose.

Decreasing the dietary protein content upto 30% containing MT (2 mg/kg) increased the growth in relative to length. This resulted in significant increase of the condition factor of Liza parsia. In contrast, ratio of weight to length was found to be decreasing gradually with time when O.kisutch fed on diet containing dietary levels of protein and lipid with the inclusion of 1 ppm MT (Fagerlund et al., 1983).

Besides acceleration of growth, another factor which is of considerable relevance to fish farming is the efficiency with which fish can convert food into flesh. Present findings show that the fish groups had improved digestibility, conversion efficiency, protein efficiency ratio and food conversion rate at the dose of 30% protein + 2 mg MT. The enhancement in conversion efficiency may be due to the improved intestinal absorption as reported by Habibi and Ince (1983) and Habibi et al. (1983). Therefore, assimilation and conversion of nutrients notably proteins seems to be increased in the fish body at this dose. These findings support the earlier observations of Fagerlund et al. (1983) who found PER to be inversely related to the dietary protein levels when O.kisutch fry were fed on a lower dietary protein diet with supplementation of 1 ppm MT. Thus, the results suggest that MT improves protein utilization in fish when protein content of the diets are relatively lower than the normal levels.

The results (Table IX) of body composition indicates that the changes in the protein, lipid, moisture and ash are only marginal. However, the fish fed on the diet containing 30% protein with 1 mg, 2 mg and 3 mg/kg MT recorded slightly higher protein and lipid levels than the other diets of different MT concentrations.

The highest protein and lipid content in the fish groups receiving 30% protein + 2 mg MT/kg suggests that in this treatment MT had its highest anabolic effect. Unlike the present findings the MT supplemented low protein diet (35%) did not affect the body composition of O.kisutch (Fagerlund et al., 1983). The increase in body contents of protein in Liza parsia might have occurred due to the increased incorporation of amino acids into proteins, as a result of the stimulation of RNA synthesis by MT, as observed by Matty and Cheema (1978) in Cyprinus carpio.

Survival rate was above 90% in most of the groups containing 35% and 30% protein with different concentrations of MT. Similarly, the fish groups receiving 25% protein + 1 mg MT/kg also gave 90% survival rate (Table IX), suggesting that the survival of animals will not be adversely affected by the lowering of dietary protein levels in MT supplemented diets.

Results of the present study suggest that Liza parsia fry may be reared on a 30% protein diet by incorporating 2 mg MT for achieving maximum growth, food and protein utilization. The manipulation of diet composition and MT content provides the fish culturist a means to control growth

and thus helps in attaining the optimum size with savings in cost of feed.

Response of the ovary of fish to injections of MT and Estrone:-

(i) MT Injections

The ovaries were observed at the termination of experiment, which initially were found to be immature (in oogonial stage). Ovaries of all the three groups of MT treated fish appeared normal except that some ova were slightly larger in size at all the treatments. All the MT-injected fish had ovaries, which were in the central nucleolus stage as against the oogonia of the control fish indicating that administration of MT triggers the initiation of vitellogenesis. However, all the ovaries were in similar state of development and none of the doses produced further development, after the central nucleolus stage. These results suggest that administration of MT stimulates vitellogenesis, but inhibits further ovarian development within the dosages employed in the study.

Fagerlund and McBride (1975) obtained similar results. After injecting MT doses, 10 - 50 mg, the ovary of O.kisutch

was found to be unaffected. However, when this steroid level increased to 100-500 mg/kg marked degeneration occurred in the ovary suggesting that high dosages would deleteriously affect the development of ovary.

(ii) Estrone Injections

In the present study some changes were seen in the ovaries of the estrone treated fish. The ovary of the controlled fish groups were in the oogonia stage. In contrast to the ovaries of the fish treated with 1 mg and 2 mg/kg estrone which were in the chromatin nucleolus stage, the fish receiving the injections of 3 mg/kg estrone were in perinucleolus stage of development. Thus injection of estrone stimulated the development of the ovary towards maturation with the progressive increase in dosages from 1 to 3 mg/kg of fish producing a steady advancement towards the maturation of the fish. Past experiments gave contradictory results when estrone was injected to finfish species. Estrone injection causes proliferation of oogonia in the ovary of minnow, Phoxinus laevis (Bullough, 1942), gudgeon Hypseleotris galli (Mackay, 1973); while the 45 rat unit of estrone/week did not affect the ovaries of L. reticulatus (Berkowitz, 1938) at 3,00,000 IB unit inhibited the degeneration of Labistes reticulatus ovary (Svardson, 1943).

CHAPTER V

SUMMARY

S U M M A R Y

The present study was conducted to determine the efficacy of selected steroid hormones, viz., 17α -methyltestosterone (MT), diethylstilbestrol (DES), 17β -estradiol (ES) and thyroid hormone (T3), and to find out their optimum dosages, which elicit maximum anabolic effect in the fry of the mullet Liza parsia. As a part of the study, the interaction of selected dosages of T3 + MT was also examined to find out if growth promotion could be achieved through a combination of hormones. Through another experiment the protein - sparing effect of MT was also studied with a view to improving the dietary protein utilization and reduce the protein levels in the diet without affecting the performance of the fish. Besides this, estrone and MT were administered intramuscularly to adult immature mullets to see the changes in the ovary, if any, associated with these exogenous hormones.

The duration of the feeding experiments ranged between 45 and 60 days and injection experiments 30 days depending upon the objectives of the study. All the feeding experiments were conducted in the laboratory selecting a randomized block design with three replicates for each treatment. Isocaloric and isonitrogenous compounded diets were used for most of the experiments except for the protein

- sparing experiment. Compounded diets were prepared from locally available feed ingredients like, fish - meal, groundnut oil - cake (lipid - free), tapioca, rice bran, corn oil and cod liver oil. Vitamin and mineral premixes were added in appropriate quantities as recommended by John Halver for fish. Graded levels of hormones were used for determining the optimum hormone requirement in the diet. Fish were fed on a restricted ration of 7% of the body weight twice a day.

Environmental parameters (salinity, ammonia, pH, and dissolved oxygen contents of water) were monitored regularly, and most of them were found to be within normal range.

Sampling of the animals for obtaining growth data were carried out at regular intervals and based on this, feeding rates were adjusted.

Response parameters considered included specific growth rate, condition factor, survival rate, food conversion ratio, digestibility coefficient, gross food conversion efficiency, protein efficiency ratio and proximate composition of the fish.

Standard procedures were followed for biochemical

analysis. Data were processed with suitable statistical procedures.

To find out the optimum levels of MT two experiments were conducted. The first experiment was conducted for 60 days with dietary MT doses ranging from 0 to 60 mg with an interval of 10 mg. Based on the findings of this experiment, another experiment, using 0 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg and 15 mg doses, was conducted for 45 days to find out the optimum dietary levels of MT.

Heavy mortalities in the fish groups receiving diets with MT levels above 40 mg compelled the discontinuance of the treatments after 40 days and the remaining fish groups were reared upto 60 days. The response of the fish fry to the dietary doses of MT was found to be dose - dependent. MT doses exceeding 10 mg/kg diet induced poor growth in Liza parsia. Moderate gains in weight over control was found from 2 to 8 mg MT and, therefore, doses from 2 to 8 mg seems to be anabolic, whereas MT doses above 10 mg results in a negative growth. Diets containing MT levels exceeding 30 mg has deleterious effect on the fish and induce heavy mortalities. The survival, growth, digestibility, conversion efficiency, protein efficiency ratio, food conversion rate and body

composition indicate that 2 mg is the optimum dietary level of MT for the fry of the mullet Liza parsia. There is also no advantage by using more than 2 mg MT in the diet. MT doses exceeding 10 mg/kg diet has a growth depressing effect on the fish fry.

Since diethylstilbestrol (DES) gave contradictory results in teleosts, one experiment was performed for sixty days to find out the efficacy of this steroid. In this experiment dietary levels of DES ranged between 0 to 1.8 mg, with an interval of 0.3.

The response achieved by the fish suggest that DES is not a anabolic steroid for the fish fry, within the dosages tested during this experiment. Only marginal weight gain was observed in the fish groups receiving 0.3 mg/kg DES; above this level weight gain was found to decrease steadily as dosages increased, indicating the growth depressing effect of DES. Further DES is known to be a carcinogenic steroid and hence not recommended in the diet of L.parsia.

To determine the anabolic effect of 17β - estradiol one experiment was conducted for sixty days using graded levels of this hormone, viz., 0 mg, 1 mg, 2 mg, 4 mg, 6 mg, 8 mg and

10 mg/kg. The results revealed that relatively low dosages upto 4 mg/kg, promote growth and improve the feed and protein conversion efficiency in L.parsia.

One set of experiments was conducted to test the efficacy of thyroid hormone (T3) and also to study the synergetic effect of MT and T3, if any. In this experiment selected levels of individual hormones T3 (0 mg, 3 mg, 6 mg and 9 mg/kg) and MT (0 mg, 2 mg and 4 mg /kg) and their mixtures (3 mg T3 + 2 mg MT, 6 mg T3 + 2 mg MT, 9 mg T3 + 2 mg MT, 3 mg T3 + 4 mg MT, 6 mg T3 + 4 mg MT and 9 mg T3 + 4 mg MT per kg diet) were fed to Liza parsia fry for 45 days. A hormone-free control diet was also kept.

Among the individual hormone dosages 2 mg MT showed the best anabolic effect, and of the T3 dosages, 3 mg/kg showed moderate response. Based on the survival and growth indices, the dose of 9 mg T3 + 2 mg MT proved to be the most effective in promoting growth during the first 30 days of rearing. However, at the termination of the experiment, after 45 days, the concentration of 2 mg MT/kg gave superior growth than all other treatments. Therefore, for the short rearing periods the combination of 9 mg T3 + 2 mg MT is more effective, while for the longer duration 2 mg MT is the best

anabolic agent for Liza parsia.

After noting the efficacy of MT at the dosage of 2 mg/kg diet, one experiment was performed for sixty days, to observe the protein sparing action of this hormone for the gold-spot mullet fry, if any. Compounded diets, with protein levels of 35%, 30% and 25%, and for each protein level three different MT dosages (1mg, 2mg and 3mg), were prepared and fed to the fish.

Results obtained for growth, food conversion, digestibility, protein utilization and body contents showed that the diets containing 30% protein with 1 mg, 2 mg and 3 mg levels of MT, are better than the 35% and 25% diets. However, the dose of 30% protein + 2 mg MT/kg gave the best results, thus showing that dietary protein could be better utilized and that significant savings can be made in the protein content of the diet by incorporating MT at optimum levels in Liza parsia.

Two experiments were conducted to study the changes in the ovary associated with the injections of MT and estrone. Adult immature fish were used to test the efficacy of these steroid hormones through injections. The experimental fish were fed on a isocaloric compounded diet containing 35%

protein during the experimental period.

A total of ten injections were given intramuscularly to each of the fish at the rate of 1 injection/3 days, and the doses were 0, 0.5, 1.0 and 1.5 mg MT/kg and 0, 1, 2 and 3 mg estrone/kg body weight.

Histological observations indicate that both the hormones have induced the ovary development. All the MT treated fish and the fish injected with 1 mg and 2 mg estrone were observed in central nucleolus stage of development. The 3 mg estrone treated ovary was in perinucleolus stage as compared to the oogonia stage of control ovary. The results suggest that estrone at a dosage of 3 mg/kg has significant positive influence on ovarian maturation in L.parsia.

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