Catholic University
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EFFECTS OF ISOPROTERENOL, SALBUTAMOL, FENOTEROL AND CLENBUTEROL ON BODY COMPOSITION AND GROWTH OF THE AFRICAN CATFISH, CLARIAS GARIEPINUS (BURCHELL, 1822)

A Thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Fundamental and Applied Marine Ecology

Promoter:

Prof. Dr. F. OLLEVIER
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Zoology Institute
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By:

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LIST OF ABBREVIATIONS

C : Control
DC : Diet with clenbuterol
DI : Diet with isoproterenol
DS : Diet with salbutamol
DF : Diet with fenoterol

SUMMARY

The effects of the repartioning agents or β-agonists, isoproterenol, salbutamol, fenoterol and clenbuterol on body composition and growth of the African catfish Clarias gariepinus (Burchell, 1822) were studied.
Some of these products have already been successfully tried out by several research groups in cattle, sheep, poultry and swine to partition nutrients away from fat deposition towards protein accretion. In fish however, and more so, in the African catfish, this is the first time that the effects of these β-agonists have been tested on body composition and that some positive effects were obtained. Uptil now in fish, only the effects of growth hormone, anabolic steroids and thyroid hormones - all of which mobilize lipids from lipid stores to provide energy while sparing dietary protein - have been tested by other research groups.
The effects of these β-agonists were tested during 10 weeks on two size categories of African catfish - a group of smaller fish weighing 185 ± 18 g. and bigger fish weighing 266 ± 26 g. Emphasis was laid on muscle protein and fat content.
The products seem to have acted differently on these two size categories of fish. In the group of smaller fish, only isoproterenol reduced fat levels significantly ($p < 0.05$) compared to the control fish after 17 and 80 days. A significant increase ($p < 0.05$) of protein content due to all the products compared to the control was seen after 17 days. However, isoproterenol still gave significantly higher protein levels on days 61 and 80 ($p < 0.05$) compared to the control. The specific growth rate due to all the β-agonists tested in the smaller fish was significantly higher than the control ($p < 0.05$).
Food conversion ratio and efficiency though not significantly different from the control was ameliorated.

In the bigger fish, no statistically significant difference ($p < 0.05$) was seen on protein and fat levels as well as on growth rate and feed utilisation throughout the experimental period.

It is known from the farm animal industry, that these products act rapidly : with time, their effect wanes.

We can only hypothesize that this could have possibly happened on the first 17 days on protein accretion and then with time their effect waned in the smaller fish.

The relatively more consistent significant increase in protein and decrease in fat in the muscle of smaller catfish due to isoproterenol as opposed to the rest of the other products is possibly due to its greater potency at a similar dosage than the other products. Since Isoproterenol is a mixed β-agonist, while clenbuterol, salbutamol and fenoterol are pure β_2 agonists, we can only propose a hypothesis that in our case β_1 receptors are predominant in catfish muscle. This makes the effect of isoproterenol more pronounced on body protein and fat.

These products are known to have an influence on the endocrine system of cattle, poultry and swine. The improvement in growth of the smaller fish could be due to the influence on the endocrine system by these products.

How they affect the endocrine system especially the pituitary is not yet known in the African catfish and should be an issue of further research.

Age related differences could possibly explain the overall lack of effect of these products in the bigger fish.

C. gariepinus has relatively low fat in the muscle. It might not be easy to see the influence of these products in the muscle tissue fat.

Probably in fish with a higher fat content, the effects of these products could provide more easily detectable results.

Once the use of these products is approved in the farm animal industry, then they can be tried out in aquaculture and more so in fish species with a great culture potential such as the African catfish.

These products need to be tested more closely in catfish to help identify the nature of receptors in the effective dosages as well as their direct or indirect influence on the endocrine system. This would enhance the understanding of their mode of action in catfish and other fishes. To use the words of Stiles et al. (1984) : *The understanding of β-agonists started 10 years ago. More interesting discoveries seem to lie in the future for their mechanism of action.*

INTRODUCTION

Evidence for aquaculture practice dates back as early as 2000 B.C.
Books on fish culture were written by the Chinese around 473 B.C. Oyster culture existed in ancient Rome. Tilapia culture in Egypt. The ancient Romans introduced carp from Asia Minor in Greece and Italy. In Central Europe, carp culture was already well established in the 17th century (Bardach et al, 1972, Maar et al, 1966).

Aquaculture was then practised under extensive conditions. This has hitherto improved with new technological developments over the years from ocean ranching to highly intensive cage or tank operation. New species have been added like salmon, cod, turbot, freshwater cray fish and newer candidate species like catfish among others (Otto Kine, 1980). The techniques aim at maximizing quality under conditions which minimize costs of feed, operation, construction and energy (Marine Fisheries International Magazine, 1987).

Aquaculture can make a unique contribution to nutrition in many parts of the world by virtue of its extremely high productivity in many situations and the fact that aquatic crops are primarily protein crops rather than starchy staple foods. In this regard, it should be noted that certain aquatic organisms may be better food converters of primary foods than ruminants, fowls or even pigs (Bardach, 1972).

Aquatic animals possess a number of advantages for use in husbandry. The body density of fish and swimming crustaceans is nearly the same as that of the water they inhabit. They are spared the chore of supporting their weight and may devote more energy to growth than terrestrial animals. Fish and invertebrates being cold blooded animals expend no energy on thermoregulation. This property could further enhance their

growth rate which is far more plastic than in higher vertebrates (Bardach, 1972).

The need for more interest in aquaculture is because the contribution of the world's waters is limited and wild stock production will possibly reach a ceiling. The world's aquatic production being 85 million tons (estimation for 1983) look static (Machiels, 1987). Aquaculture accounts for about 10 million metric tons of wet weight per year of the world aquatic yield. 67 % of this are finfish.

Constant international discussions about fishing rights and quota indicate that fish have become scarce and a further increase in its supply is not expected (Anonymous 1981, Otto Kine 1980, Fish Farming International Magazine, 1987).
Africa accounts for only 3 % of the total fish consumption.

Protein shortage is evident and husbandry of fish in aquaculture can help satisfy the growing need for high quality protein in human nutrition, especially in developing countries (Anonymous, 1980).

In Africa culture of fish centres around Tilapia culture. The first attempts for fish culture in modern Africa started in 1924 with Tilapia in Kenya followed by Congo (now Zaire) in 1937. In Central-East-Africa first trials of fish pond culture were made in Zambia in 1942 to be followed by Rhodesia (now Zimbabwe) in 1950 (Bardach et al, 1972, Maar et al, 1966).

Tilapia culture has been marred, though popular, with harvests of too many small size Tilapia from overpopulated ponds (Hogendoorn, 1983).
The desirable characteristics in a cultured fish are summarized as those whose female and male breeders should be induced to mature and shed their sex products in captivity allowing the culturist to exercise some control. The eggs and larvae should be hardy. They should be able to be reared under

polyculture conditions. They should be adapted to the climate, have a high growth rate, accept and thrive on cheap feeds and be able to support higher population densities (Bardach, 1972, Hogendoorn, 1983).

1. LITERATURE REVIEW

The African catfish, Clarias gariepinus, also synonymous with Clarias lazera, Clarias senegalensis and Clarias mosambicus (Teugels, 1986), stands out as a new species in aquaculture whose culture has been expanding in Africa, Asia and Europe (Britz and Hecht, 1989, Huismans and Ritcher, 1989).

1.1. Classification:

There are 2,000 catfish species in the world. This is approximately 8 % of the number of fish species.

Phylum:	Chordata
Subphylum:	Craniata
Superclass:	Gnastomata
Class:	Osteichtyes
Infraclass:	Teleostei
Superorder:	Suliroformes
Family:	Clariidae
Genus:	Clarias
Species	gariepinus

(Teugels, 1986)

1.2. Brief Biology

1.2.1. Distribution

C. gariepinus ranges from Southern Africa through Central, West and North Africa through the Middle East into Eastern Europe (Uys, 1989).

1.2.2. Habitat

It is eurytopic. It inhabits a wide range of inland waters including streams, rivers, pans, swamps, underground sink holes, shallow and deep lakes as well as impoundments (Bruton and Smith, 1986).

1.2.3. Environmental Conditions

It can stand water temperatures ranging from 9-45° C (Babiker, 1984). The water temperature range for hatching is 17 to 32° C. An optimal salinity of 2.5 ppt is appropriate though it can tolerate ranges from 0-12 ppt (Babiker, 1984). It can stand Oxygen ranges from nearly 0 till oversaturation.

1.2.4. Breeding Biology

In nature, they spawn from April to September, it could differ from region to region. The peak period for maturity is from August to October. The smallest mature female has been found at 27 cm length at the age of 8-10 months.

Sexual dimorphism is already apparent at 4 months. It is a communal spawner whose peak maturity occurs during the rainy season.
It is a very fecund species with modal size females producing 50,000 to 200,000 eggs. Fecundities in excess of one million eggs have been reported.

Spawning takes place in temporary water, often after rain. They spawn once a year with the onset of a major rainfall. Under pond conditions, the rhythm is more or less maintained and males and females mature after 7 months. Spawning takes place at night generally, typically in some cases between 20 00 H and 02 30 H.

Fertilization is external with sperm being motile for 80 to 120 seconds. Hatching is between 24 to 48 hours afterwards depending on temperature. After 35 hours larvae begin to swim and feed externally after 80 hours. After 7 days, larvae change to juveniles (when the fin rays start to appear).
In nature, the period of maturation is 1-3 years at 180-260 g body weight. The internal gonadal maturation cycle can be arbitrarily divided into:

(i) A long post-spawning period characterized by flat empty gonads in a non-secretory condition and recovering afterwards.
(ii) A pre-spawning period characterized by nearly ripe gonads preceeding spawning.

After sexual maturity the fish leave the lake and move upstreams to spawn in flooded areas of the river. Immature ones also move upstreams. After spawning, fertilized eggs are distributed over a wide area and stick to flooded edges and grass. The larvae are omnivorous.
At the size of < 50 mm TL, they prefer chironomid larvae, shrimps, small planktonic and benthic crustaceans. At < 100 mm TL, they feed on the above as well as on dragon fly nymphs,

fish fry and small crabs and plant matter. Adults feed on fish, crabs and snails.

Clarias gariepinus has high levels of amylase, protease and gastric lysozymes with wide temperature profiles which facilitate its opportunistic feeding habits and its ability to utilize a wide range of nutrients efficiently.

Its growth is varied. In natural conditions, they reach 200 to 300 mm in the first year and annual increments in the order of 80 to 100 mm. The maximum weight in most lakes and small rivers is rarely more than 20 kg. Very large specimens, > 40 kg at times may be found in large turbid rivers. A length of 130 cm is seen as the maximum (Ritcher, 1976, Babiker, 1984, Hogendoorn, 1983, Bruton and Smith, 1986, Uys, 1989, Hecht, 1985).

1.2.5. Potential for Culture

- Rapid growth, even in high density intensive culture.
- Can reach marketable size of 800-1000 g in its first year and it has an efficient feed utilization.
- Easy to reproduce in captivity.
- Can tolerate adverse culture conditions.
- Can be reared in stagnant ponds.
- Has the ability for both aerial and aquatic respiration using its epibranchial organ, epibranchial epithelium, gill fans, etc.
- Ranks high in the consumer preference list.
- Broodfish can produce eggs and sperm throughout the year.
- Has fast egg development leading to less labour costs in terms of care.
- Produce a large number of eggs, thus fewer broodstock required (Hogendoorn et al, 1983, Clay, 1977, Hogendoorn and Koops, 1983, Marine International Fisheries Magazine, 1987, Hogendoorn, 1979).

1.2.6. Nutrition (Background)

Research concerning nutrition of fishes has a relatively brief history. It has evolved from fresh abatoir-by-products and raw fish in the 1950's to moist feeds upto the current dry feeds used (Uys-1989, Hecht, 1986).

The yield of fish per pond area or per culture unit and thus the profitability of fish farming, depends to a large extent on the amount of supplementary feed used. The more intensive the aquaculture system, the greater the importance of supplementary feeding as well as the feed costs to total production costs. Feeding usually represents the single most expensive production cost in intensive culture (Collins and Delmedo, 1979, Hecht, 1986).

The cost of supplementary feeds can reach 56.3-58.8 % of the total production costs (Collins and Delmedo). When the feed conversion is high, the profitability of the fish farm will correspondingly decrease.

Weppleman (1984), gives an implication of feed cost importance in the poultry industry in the U.S.A., where approximately 70 % of the cost of producing broilers is due to the cost of feed and approximately 12.5 % to the cost of chicks. The remaining 17.5 % consists of components - those that depend primarily on maintenance (e.g. animal health care, and transportation costs). It is clear from this analysis that agents that improve feed efficiency are more valuable than those that accelerate growth without affecting efficiency.
For example, as he argues, a 10 % improvement in efficiency would result in a definite 7 % reduction in the producers costs. In contrast, a 10 % reduction in the time required to produce a marketable broiler would affect only the time dependent component on the non-feed, non-chick costs and this would result in savings of less than 1.75 %.

The most efficient level of feeding is attained only when the correct supply of energy and essential nutrients are available in the proportions required by the fish for maintenance and growth. The main nutrients utilized from tissues is fat. Glycogen reserves utilized via glycolysis provide energy from carbohydrates. Triglycerides are a major energy source for metabolism in fish muscle. The calorific value of fatty acids (9.2 Kcal/g) derived from hydrolysis of triglycerides is more than double that of carbohydrates and a considerable amount of fatty acid oxidation takes place in the red muscle (Herpher, 1988). In fish there is no distinct fatty (adipose) tissue and fat storage sometimes in large amounts is dispersed among various tissues such as muscle, intestine and its surrounding mesentery and liver (Bilinski, 1969).

Protein, the basic component of animal tissues contributes for 45-75 % of tissue dry matter (Herpher, 1988). Protein metabolism mainly in the liver and muscle can take 2 directions:

1. Anabolic, leading to growth.
2. Catabolic, involving deamination of protein molecules to produce carbon skeletons which may be utilized for either energy or lipogenesis.

The proportion of metabolized protein depends on the protein requirements of the fish, content of protein in the diet and proportion of amino acids within it, energy requirements and amount of energy available from other sources (Werner, 1989, Herpher, 1988).

1.2.7. Catfish Nutrition

Understanding of applied nutrition in *C. gariepinus*, involving fundamental aspects like feeding biology and physiological processes has received little attention (Hecht, 1986).

A better knowledge of the nutritional requirements of this species, can help in making its production even more meaningful, although it is not easy to finalize its protein and energy requirements (Machiels and Henken, 1987).

1.2.8. Nutritional Requirements of Juveniles and Sub-Juveniles

1) Vitamins:

These are non-nutritive dietary essentials required in small quantities. Their deficiency leads to poor growth, irregular bone formation and susceptibility to disease. The following table summarizes vitamin requirements:

Table 1. Tentative Vitamin premix for *Clarias gariepinus* diets, based on levels used in commercial diets for channel catfish (Robinson, 1984)

Thiamin	11 g
Riboflavine	13 g
Pyridoxine	11 g
Pantothenic acid	35 g
Nicotinic acid	88 g
Folic acid	2,2 g
B12	0,09 g
Choline	550 g
Ascorbic acid	350 g
A (IU)	4400 (IU x 1000)
D (IU)	2200 (IU x 1000)
E (IU)	55 (IU x 1000)
K (IU)	11 (IU x 1000)

2. Lipids:

Apart from supplying energy, they are a source of essential fatty acids. Polyunsaturated fatty acids of the 'Omega-3' family are most important. The optimum lipid content of the diet has been found to be 10 to 11 % for C. gariepinus. This varies depending on energy provided by carbohydrates in the diet (Hecht, 1986).

3. Carbohydrates:

The presence of amylase in catfish indicates that they can utilize starch.

Table 2. Recommended formulation of a dry feed for *C.gariepinus* larvae and early junveniles (Uys, 1989)

Torula yeast (Candida utilis)	50 %
Fish meal	43 %
Vitamin Premix	1 %
Cod liver oil	3 %
Sunflower oil	3 %

4. Minerals:

Little is known of mineral requirements of fish. Once fish are fed diets of animal origin - fish meal, animal waste - the requirement for minerals is minimized. Calcium and phosphorus can be supplemented in diets as calcium phosphate (usually about 1 %) and trace minerals like Magnesium, Iron, Cobalte, Zinc are required at levels of < 0.01 % of diet (Uys, 1989; Hecht et al, 1986).

5. Protein:

Reports of 38 to 42 % of crude protein and an energy level of 12 KJ/g calculated as digestible energy (DE) have been suggested. Since no data are available on the digestibility of feeds for C. gariepinus, digestible energy values determined for channel catfish, Ictalarus punctatus have been used.

Table 3. Recommended proximal composition for a *C. gariepinus* production feed

Dietary requirements (constraints in linear programming terms)

Crude protein	38-40 %
Total lipid	> 8 %
Digestible energy	12 kJ/g
Calcium	1,5 %
Phosphorus	0,5 %

Bounds on ingredients:	lower	upper	reason
Fishmeal	10 %		amino acids
Molasses powder	8 %	12 %	binder
Oil supplement		8 %	pelletability
Vitamin premix	0,1 %		as I. punctatus

(Source: Hecht et al, 1988)

DE values are not interchangeable between species, but until such values are established for C. gariepinus these that apply to channel catfish are most likely the best.

Table 4. Digestible energy values for channel catfish (*Ictalurus punctatus*) as given by Lovell (1984)

Ingredient	DE (kJ/g)
Wheat	10,7
Raw corn (maize)	4,6
Cotton oil cake	10,7
Soy oil cake	10,8
Fish meal	16,3
Meat and bone meal (carcass meal)	14,5
Alfalfa meal (lucern)	2,8
Fish oil	36,9

(Source: Hecht et al, 1988)

Conclusion about dietary requirements are not easy to draw because of different experimental and culture conditions (Machiels and Henken, 1987).

As this fish grows older, its relative food consumption rates decreases from approximately 10 % of body weight per day (four weeks old) to 2 % of body weight/day (10 weeks and older). Growth rate also decreases from 14 to 2 % of body weigth per day and feed conversion increases from 0.7 to 1.3 (Uys, 1989).

Improvement of growth and feed conversion as well as body composition changes are being of concern in aquaculture (Donaldson et al, 1979). Conversion of feed to body meat is crucial.

What is clear now is that high protein and low fat diets enhance growth and improve food conversion in the African catfish. Huisman and Richter (1987) have found the highest gains in growth at > 60 % protein. Degani et al (1988) using 10-12 g fish at the start of their experiment found better protein utilisation, higher protein content in the muscle,

better food conversion with high protein diets. The same was observed by Henkel et al (1986) even more so with fish meal which is more palatable (Kine, 1980) and has high feed quality (Machiels and Van Dam, 1987).

In intensive culture, where fish are fed to less than satiation, diets higher in protein are beneficial. Energy deficient diets lead to utilization of part of the dietary protein in the diet otherwise available for growth (Lovell, 1984, Degani, 1989). Since high protein requirements are expensive, several other substances are being tried out in aquaculture for enhancement of growth and improvement of food conversion (i.e. feed to body meat) (Donaldson et al, 1979). Hormones, especially growth hormones (GH), thyroid hormone (T_3 and T_4), anabolic steroids as well as carnitine are being tried out.

Wilson (1988) using recombinant bovine growth hormone at 10 µg per day and per gram injections found 44.8 % larger weights than control and an improvement in feed efficiency in the channel catfish.
Treatment of salmonids with purified mammalian GH depresses condition factor, an index of the fatness or leanness of fish. Induced titre of GH increases growth relatively more in length than weight.
The food intake of hypophysectomized Fundulus heteroclitus is increased after bovine growth hormone (bGH) treatment (Pickford, 1957).

Market et al (1977) observed that the amount of dry food or protein feed per gram gain in weight of yearling Coho salmon at 10° C was less for bGH-treated fish relative to that of controls at two ratio levels. He outlined possibilities of action of GH as (i) stimulation of fat mobilization and oxidation. The percentage of lipid in muscle of Coho salmon (Oncorynchus kisutch) declined following treatment with bGH (Higgs et al, 1976, Higgs et al, 1975, Lone, 1989).

Clark, (1976) found that bGH treatment decreased whole body lipid (expressed as % of dry weight) of underyearling Coho and Sockeye salmon. In this case, more of the amino acids would be available for growth since lipid would be preferentially used as an energy source (Frye, 1971).

(ii) GH's influence on the rate of protein synthesis and breakdown.

Higgs (1975, 1976) estimated that the mean amount of protein in bGH treated intact underyearling or yearling Coho salmon was substantially higher than that of control fish.

The estimated ratio of protein to dry weight was higher for bGH-treated fish relative to those of control fish. Thus GH promotes a positive nitrogen balance in fish because of a stimulatory influence on protein synthesis. He suggested also that GH could influence the synthesis and release of insulin and hence improve protein conversion.

Thus more of the energy from ingested food would be available for growth (Market et al, 1977).

Anabolic steroids affect appetite and food conversion efficiency. This was seen in underyearling Coho salmon by Fagerlund et al (1978). Diets containing 1 ppm of 17 α-methyl testosterone were used. Food conversion efficiency of groups fed to satiation and restricted ration increased by 10.0 and 22.1 % respectively. The whole body fat content of fish fed hormone-supplemented diets at either ration was significantly decreased.

The demonstrated lipid mobilization could spare protein for growth.

Yamazaki (1976) using Carassius auratus, Onchorynchus myksis and Onchorynchus nerka with methyl testosterone in the diets at 1 ppm found a 10 % increase in growth acceleration rate over the control.

The effect of thyroid hormone is not clear cut as its mechanism is not clear. It may enhance growth of teleosts by increasing voluntary food intake (appetite) and/or gross food conversion efficiency (Donaldson et al, 1979).
Jackim and La Roche (1973) observed that T3 and T4 stimulated in vivo incorporation of L-(1-^{14}C) leucine into muscle proteins of killifish.

Narayansingh and Eales (1975a) reported that physiological doses of T4 increased in vivo incorporation of L-(1-^{14}C) leucine into liver and gill protein of rainbow and brook trout.
On lipid metabolism, Barrington et al (1961) noted that rainbow trout treated with T4 had less abdominal fat.
Also, Narayansingh and Eales (1975b) found decreased hepatic and visceral lipid reserves in brook trout after T4 treatment. These findings suggest that thyroid hormones stimulate lipid mobilization in teleosts.
If thyroid hormone administration normally does lead to mobilization of visceral lipid reserves in teleosts, and this is to furnish more energy, then more protein would be spared in the diet (Donaldson et al, 1979).

The action of carnitine is slightly different in that it carries activated long chain fatty acids across the inner mitochondrial membrane and enhances Beta oxidation providing energy more readily. It has been tried out in seabass (Dicentrachus labrax) diets (Santulli et al, 1986) with high dietary fat (Santulli and D'amelio, 1986a, Santulli et al 1986b) to study its role in stimulation of lipid oxidation while imparting a protein sparing action. In all the cases, increased growth, or reduced muscle lipid was observed.

Carbohydrates and fat spare protein in the diet (Phillips, 1984). Mobilization of stored lipid in skeletal muscle, liver, and mesenteric fat layer after a brief exposure to

agents stimulating their breakdown could avail extra substrate with a protein sparing action (Rudman and D'Girilamo, 1967).

The products being tested in this experiment - salbutamol, clenbuterol, isoproterenol and fenoterol, stimulate lipid and carbohydrate mobilization. They can also influence protein accretion, improve growth and food conversion (Johnson, 1987).

1.3. The Products (A brief review)

They are pharmacologically classified as 'Beta' agonists (An agonists is a compound that occupies a receptor and mimics the activity of a natural, biological mediator, usually in a more potent manner than the endogeneous mediator) (Fiems, 1987). Structurally, they are like subtsituted catecholamines.

The receptors they act on are the hypothetical structures or systems located in or near the muscle or gland affected by epinephrine and are called beta-receptors.
They are located in the immediate vicinity of adrenergic nerve terminals in peripheral target organs or at postjunctional regions relatively remote from sites of release of norepinephine thus they can be stimulated by circulating catecholamines (Stiles et al, 1984).

The adrenergic receptors constitute a family of closely related proteins. Functionally and structurally they are related to receptors of a wide range of other hormones and neurotransmitters that are coupled to G-proteins (Lefkowitz and Hoffman, et al, 1989).

There are two distinct types of adrenotropic receptors as determined by their relative responsiveness to the series of racemic sympathomimetic amines most closely related struc-

turally to epinephrine. Alquist (1948) divided them into α and β-types. Lands et al (1967), divided the β-receptors (our interest here) into β_1 and β_2 depending on their action in the myocardium and smooth muscle respectively.

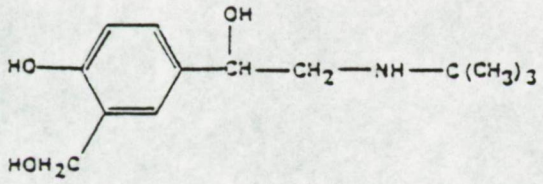
The Beta agonists possess many similar structural and functional features to the natural catecholamines epinephrine and norepinephrine whose action they mimic by binding to the beta-receptors initiating an intracellular response via cyclic 3'5' Adenosine monophosphate (C-AMP) (Lefkowitz et al, 1985).

Structures and Chemistry of the Substituted Beta Agonists

Ref. to figs. 1 and 2

L-phenylethylamine can be viewed as the parent compound consisting of a benzene ring and an ethylamine side chain.
Substitutions on the α and β carbon atoms and the terminal amino group yields a variety of compounds with sympathomimetic activity (Weiner, 1985).
Increase in size of the alkyl substituent increases β-receptor activity e.g. Isoproterenol (Hoffman and Lefkowitz, 1988).
Selective β-receptor stimulants require a large amino substituent, but depend on other substitutions for their selectivity for β than α-receptors.
Hydroxy-groups in position 3 or 5 confer β-receptor selectivity on compounds with large amino substituents, e.g. Fenoterol.
Salbutamol, a selective β_2-receptor stimulant has CH_2OH substituent on position 3 and is an important exception to the general rule of low β-activity (Hoffman, Lefkowits, 1988).
Compounds with a Bulky nitrogen attached moiety exhibit relatively greater β-adrenoreceptor selectivity than compounds with smaller nitrogen moieties e.g. Fenoterol.

Salbutamol



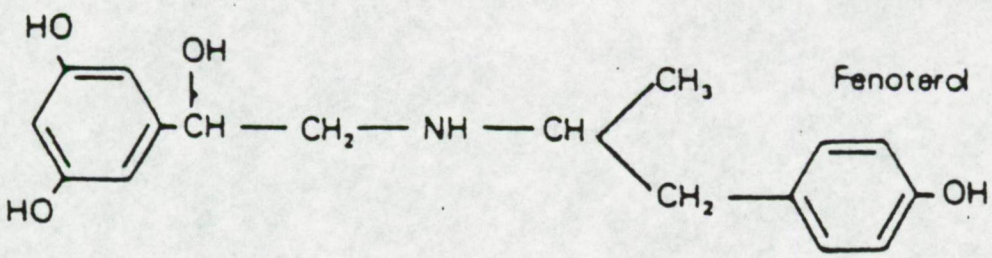
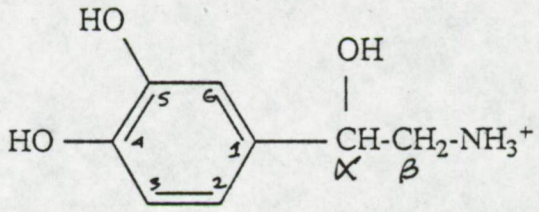
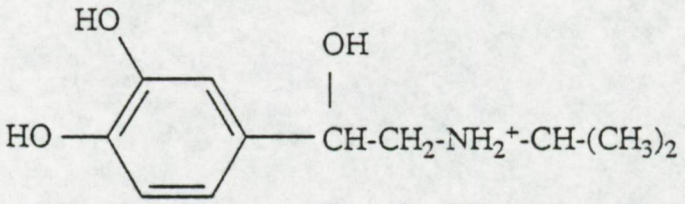


Figure 1.

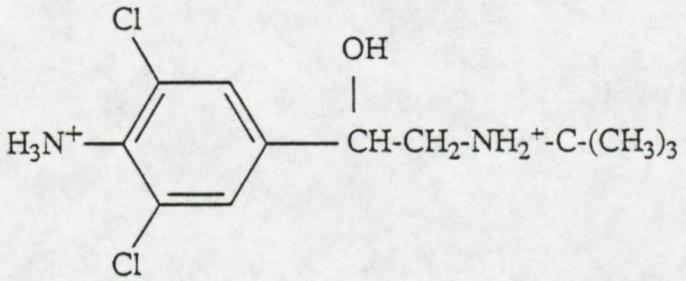
norepinephrine



isoproterenol



clenbuterol



epinephrine

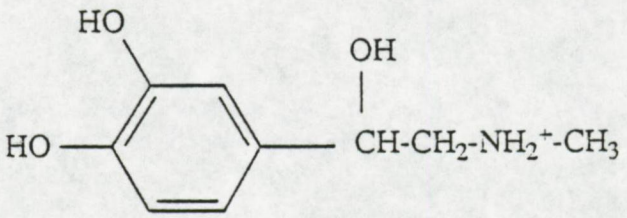


Figure 2.

Affinity for β_2-selectivity may be related to the size of the nitrogen-attached moiety on a catechol or resorcinal base (Heel et al, 1978).

Modification of β-adrenergic agonists have resulted in drugs with lower rates of metabolism and enhanced oral bioavailability compared to natural catecholamines. Modifications have included placing the hydroxy groups in the third or fifth position of the phenyl-ring or at the third position. This has yielded drugs such as salbutamol that are not substrates for the enzyme catechol-O-methyl-transferase (COMT) involved in the breakdown of catecholamins in the liver.
Bulky substituents on the amino group of catecholamines contribute to β-selectivity alright and to a decreased metabolism by mono amine oxidase (MAO) in outer mitochondrial surface as well as a decreased activity at alfa-adrenergic receptors (Hoffman and Lefkowitz, 1988).

1.3.1. Mode of Action

All β-agonists bind to β-adrenergic receptors and stimulate adenylate cyclase (AC) via a G protein named Gs. This leads to:

(i) an accumulation of c-AMP
(ii) an altered function of numerous cellular proteins as a result of their phosphorylation
(iii) Activation of c-AMP-dependent protein kinase (Berschauer and Bayer, 1989; Lefkowitz, 1981).

Brighenti et al (1987) and Triner et al (1970), using catfish hepatocytes and uterus of virgin rats respectively have observed an increase in levels of c-AMP with catecholamines. Birnbaum et al (1976) observed an increase of c-AMP levels using epinephrine and Isoproterenol.

The enzyme AC exists as a tetramer (R_2C_2) consisting of two regulatory (R) and two catalytic (C) sub-units.

Binding of c-AMP causes dissociation of the R-sub units with the resultant activation of the catalytic sub-units. Phosphorylation of various cellular proteins then causes responses that are characteristic of those produced by β-adrenergic agonists. When the stimulus is withdrawn, the situation returns to normal (Stiles et al, 1984, Fain and Garcia-Sainz, 1983).

A well defined example of these mechanism is the activation of hepatic glycogen phosphorylase - the enzyme that catalyzes the rate limiting step in glycogenolysis. c-AMP-dependent protein kinase catalyses the phosphorylation of phosphorylase kinase there by activating it.
The phosphorylase kinase then phosphorylates and activates phosphorylase.
Concurrent with activation of hepatic phosphorylase, c-AMP-dependent protein kinase also catalyzes the phosphorylation and inactivation of another enzyme glycogen synthase decreasing the net rate of synthesis of glycogen from glucose. Similar reactions result in the activation of triglyceride lipase in the fat cells (whether in liver, adipose tissue or intramuscular) resulting in the release of free fatty acids.
The lipase is activated when it is phosphorylated by c-AMP-dependent protein kinase. This dual effect of c-AMP enhances conversion of glycogen to glucose, decreases synthesis of glycogen from glucose, with an increased output of glucose from the relevant organ or tissue. It also enhances release of free fatty acids (FFAS) and decreases fat synthesis. There is thus an increased supply of substrate-FFA's and glucose for oxidative metabolism by this mechanism (Lefkowitz et al, 1989; Turtle and Kipnis, 1967; Fredrickson and Stralfots, 1981; Campbell and Scares, 1985).

To explain broader effects of these β-agonists, a multiple response mechanism has been proposed. They are schematically shown in figs. 3, 4 and 5.

Adrenergic activation cascade

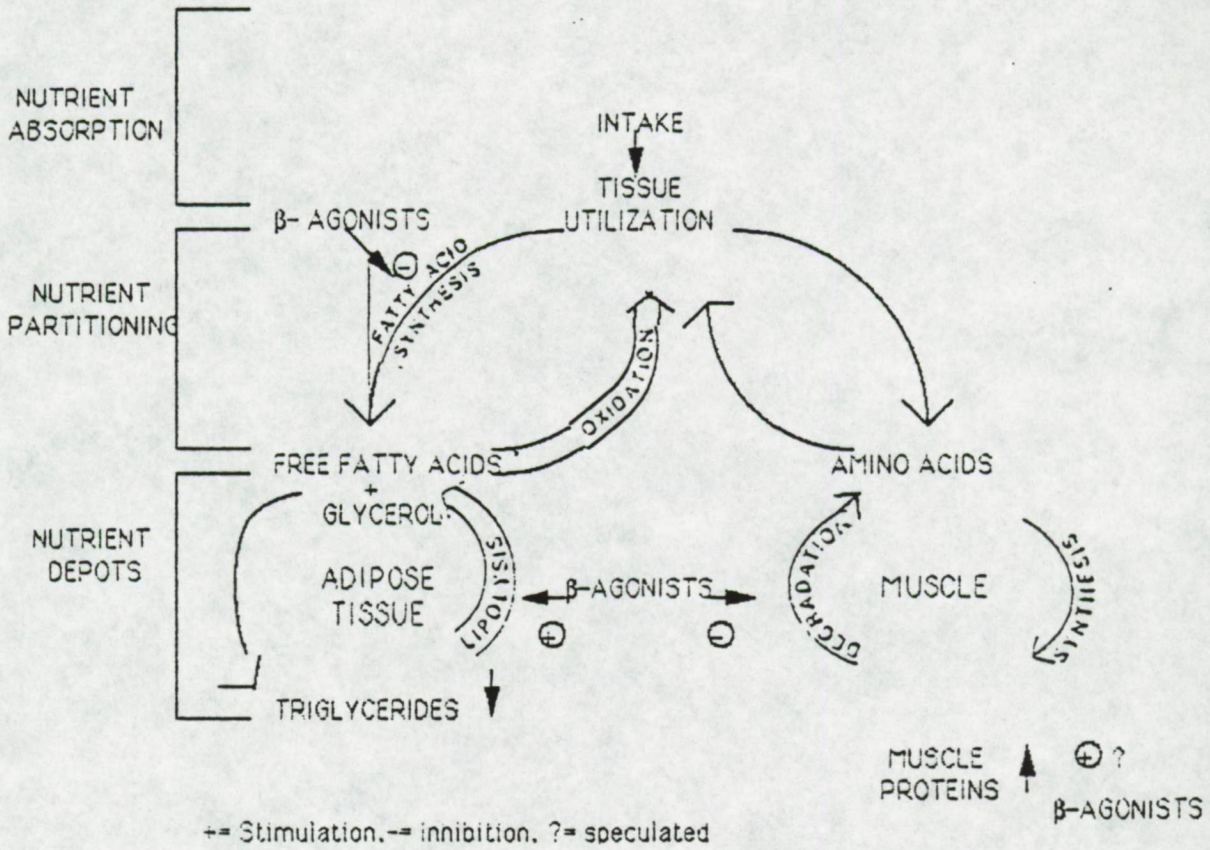
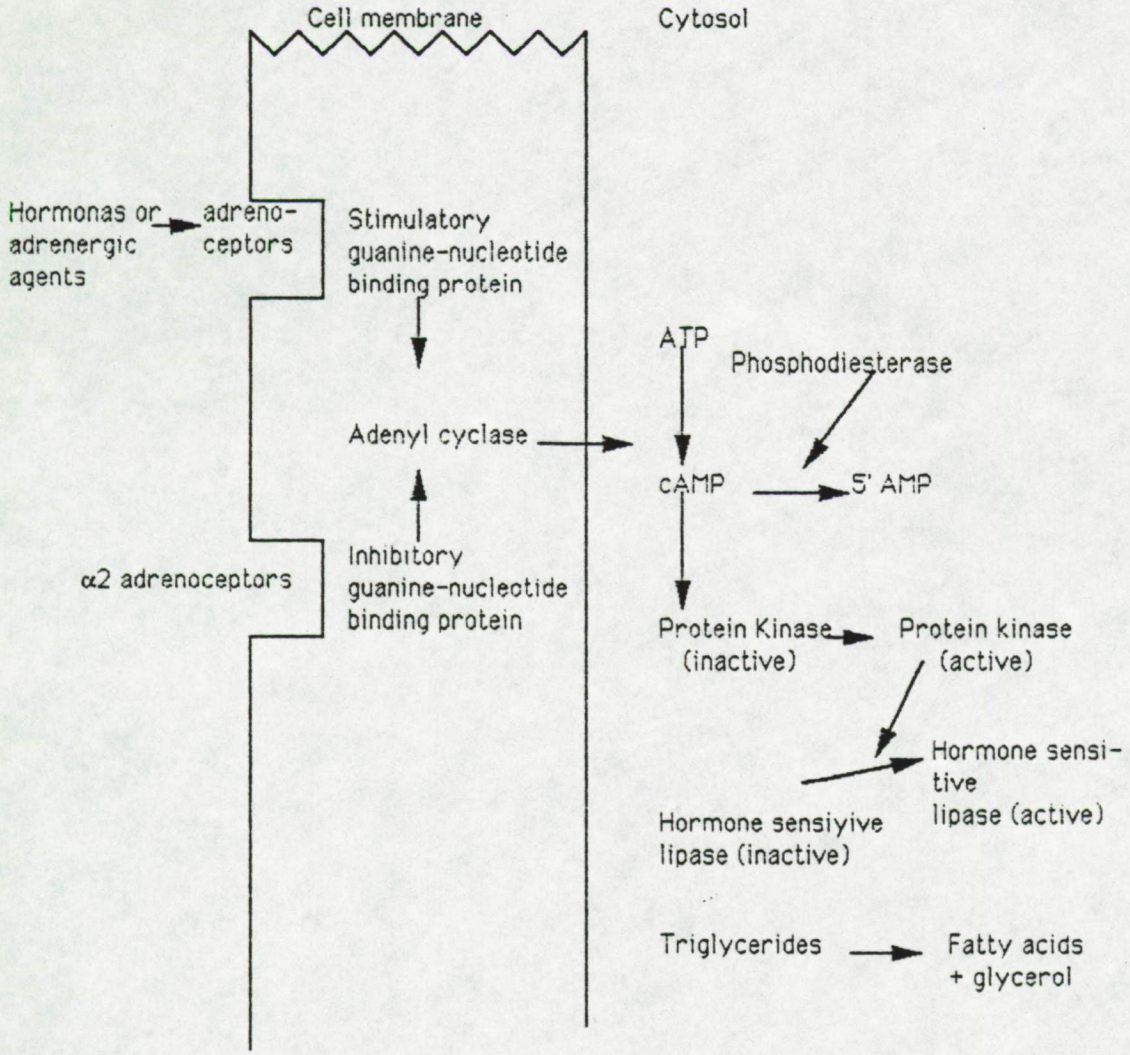


Figure 3.

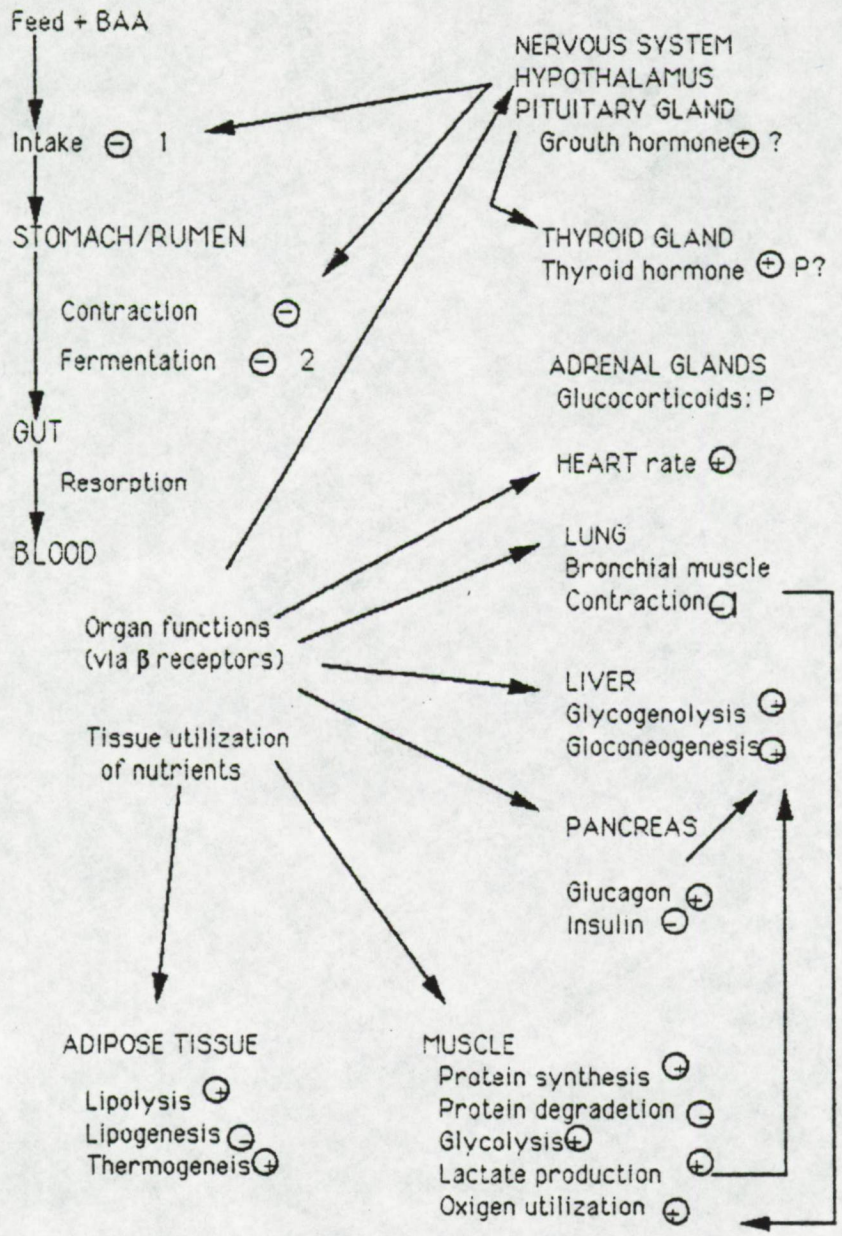
(from Ricks et al., 1984)



Regulation of lipolysis in adipose tissue.

Figure 4.
(from Stiles et al., 1984)

βAGONISTS IN ANIMAL PRODUCTION



⊖ : inhibition by (BAA), ⊖ : stimulation by BAA, ? : synergistic consecutive effect not yet clear, 1 : species dependent, 2 : dose dependent, P : permisive effect on β-agonists.

Figure 5.

(from Fiems; 1987)

In all, they stimulate lipolysis, inhibit lipogenesis, stimulate glycogenolysis, stimulate glycolysis and prevent protein degradation (Williams, 1987, Berschauer and Bayer, 1981; Fain et al, 1983; Blum et al 1982).

The use of these beta agonists has recently received closer attention in various farm animals where they are also called repartitioning agents. More energy is required to produce 1 kg of adipose tissue than is for the same quantity of muscle. A change in composition without markedly changing food intake would yield significant inprovements in food efficiency as energy is diverted away from fat deposition towards protein accretion (Hanrahen, 1987).

Weppleman (1984), used clenbuterol in poultry. Clenbuterol in steer feeds at 0, 10 and 500 mg per head per day caused an increase of 13 and 14 % more protein than control and 30 and 10 % less fat in carcass with lower blood urea levels (less loss of protein) and better gain (Ricks et al, 1984).

A decrease in fat in pigs and an improved food conversion has been observed by Jones et al (1985) and Darylymple et al (1984a) using cimaterol and AC 263,780 respectively. Antilipogenic effects in rats by Duquette and Muir (1985) using clenbuterol and isoproterenol. Alteration in body composition was observed by Emery et al (1984) in rats using fenoterol and clenbuterol. The effect on protein metabolism in wether lambs (Baker et al, 1984) was observed using clenbuterol. Their effect in growth is not consistent, though Darylymple et al (1984c) have observed significant growth at 1 ppm cimaterol in pig diets.

Most of the products in these studies are administered in days to weeks due to their supposedly rapid action (Hanrahan, 1987). In fish however, very little work has been done related to their effects on body composition changes and growth.

Most work in fish has involved the use of these analogues in specific organs/tissues - liver, plasma, muscle over a short time scale rarely exceeding hours and in vitro mostly.
Brighenti et al (1987) used isolated catfish hepatocytes to investigate glycogenolysis in vitro. They used epinephrine, isoproterenol and norepinephrine. All the catecholamines stimulated phosphorylase activity accompanied by a decrease in glycogen content in cells and by an increase in glucose output into the medium. De Ross and De Ross (1978) looked at in vivo glucose level changes due to catecholamines. Their work involved monitoring glucose levels in plamsa of shark (Squalus acanthius) due to epinephrine and observed an increase in glucose levels in plasma after 30 minutes with a decline after 24 H. Ince and Thorpe (1977) monitored plasma insulin and glucose responses due to glucagon and epinephrine in the European silver eels Anguila anguila). They observed a potent hyperglyceamic effect due to epinephrine. Larson (1973) observed hyperglyceamia and an increase in plasma free fatty acids in the eel (Anguila anguila at high doses of 5.0 mg/kg. In muscle and liver of catfish, glycogenolysis was observed by Ottolenghi et al (1986). A significant increase on the levels of phosphorylase activity was seen in both muscle and liver of Ichtalurus melas. Sheridan (1987) observed lipolysis due to isoproterenol, epinephrine and norepinephrine in liver slices of Coho salmon (Onchorynchus kisutch) in vitro and he suggested a β-adrenergic mediated pathway for this process.

All the workers have been able to observe lipolysis and glycogenolysis due to natural catecholamines and some analogues via c-AMP accumulation. Storage of lipids in fish is largely in the form of triglycerides in the liver, skeletal muscle and mesenteric fat layer (Greene and Selivonchick, 1987; Rudman and D'Girolamo, 1967). Carbohydrates are stored as glycogen in skeletal muscle (Johnston, 1982) and liver (Ottolenghi et al, 1981). High fat diets do not have a satisfactory protein sparing action in catfish since it results in excess fat deposition and does not improve growth (Watanabe,

1982; Huisman and Ritcher, 1987; Machiels and Henken, 1985). Controlled mobilization of these fuels - carbohydrates, and fat from their depots by repartitioning agents would avail oxidative substrate - free fatty acids from lipolysis, glucose from glycogenolysis. The substrates could possibily spare dietary protein on one hand, and reduce body muscle fat and inhibit protein degradation on the other hand.

The aim of this experiment is to check the effect of the beta agonists - Isoproterenol, clenbuterol, fenoterol and salbutamol - on the body composition of fish. Special emphasis is put on the muscle protein and fat of the African catfish - C. gariepinus, i.e. if they can partition nutrients away from fat deposits towards protein accretion and possibly enhance growth.

2. MATERIALS AND METHODS

2.1. Fish

In October 1990, a total of 450 unsexed African catfish, C.gariepinus, were selected from an initial population of fish that were reared at the Laboratory of Ecology and Aquaculture in Leuven originating from the broodstock. They were divided into two weight categories by hand-grading. The mean weights were 185 g ± 18 (range 150-200 g) and 266 ± 26 (range 220-300 g).
The former were called small and the latter big fish. Both groups were divided over 10 tanks. Each tank contained 45 fish. They were held there for two weeks prior to initiation of the experiment for acclimatization. The fish were fed at 2 % of their body weight on a commercial diet TROUVIT TR2.KO three times a day at 09 00, 13 00 and 17 00 H.

2.2. System of Culture

This was a flow through system with tap water.

2.3. Experimental Conditions

These were maintained as similar as possible in all the tanks from the beginning of the acclimation period up to the start of the experiment which finally ran for ten weeks.
The temperature was kept at 27° C ± 0.5° C by submersed heating elements in the main water inlet tanks.

2.4. The flow rate

This was adjusted to 300 ml per minute. Each tank contained about 70 l of aerated water. The tanks were covered with wire gauze with wooden frames on the sides to prevent fish from escaping.

The photoperiod was maintained on a 12 H light/day cycle. Levels of total ammonia and nitrite were kept below 4 ppm and 0.1 ppm respectively on a daily basis and any anomaly was corrected for by adjusting the flow rate and the amount of food given accordingly.

2.5. Diet Composition

The diet type was Trouvit crumble 'O' with the following proportions of various nutrients:

Crude protein	55 %
Crude fat	13 %
Cellulose	2 %
Crude ash	11 %
Moisture	10 %

2.5.1. Diet Preparation

This was done in stages to incorporate the products - Salbutamol, Fenoterol, Clenbuterol and Isoproterenol. In the first stage, 2 kg of 'trouvit diet crumble zero' still unpelletised was mixed with 20 mg of the products. They were mixed together using a Kenwood mixer (KENWOOD MAJOR) for 30 min and put in plastic bags.
In the second stage, the rest of the calculated diet estimated for the whole experiment period was mixed at the Laboratory of Agriculture at the Catholic University of Leuven in a particu-

lar mixer used for the preparation of porcine feeds for 30 min following their standard procedure. (The quantity of products being small, it was not possible to use a marker to ensure that the products were distributed in the diet).
In the third stage, the whole mixture was pelletized. The final concentration of the products in the diet was 1 ppm.

2.6. Source of the Products

Salbutamol, Isoproterenol, Fenoterol were purchased from local drugstores. Clenbuterol was provided by Sigma Chemicals.

2.7. Distribution of Fish

The fish were removed from the tanks and maintained in large plastic containers with submerged heaters.
The small fish being held separately from the big ones. They were mixed and 45 fish taken randomly and placed in each of the tanks which had been cleaned and desinfected with dettol, filled with water and treated with 1 g chloramphenicol 24 hrs earlier.

Each aquarium was assigned a particular diet in a non-random manner. The seventh, eighth, ninth, tenth, eleventh and twelfth at the top were assigned control, Clenbuterol, Isoproterenol, Salbutamol, Fenoterol for the small fish and the last one, Clenbuterol for the big fish. The last four aquaria on the bottom bench were assigned Isoproterenol, Salbutamol, Fenoterol and control for big fish

2.8. Beginning of Sampling

2.8.1. Baseline data

Two fish were removed randomly from each of the tanks to represent an initial population from which base line data for body composition and hepatosomatic index would be based. They were pooled into two categories. The fish were anaesthesised in excess phenoxyethanol, their lengths and weights determined to the nearest .1 g and 0.1 cm. The following parts were excised/removed and kept for further analysis: Muscle, liver and blood.

2.8.1.1. Blood Plasma

Blood was removed from the caudal vein using 2 ml syringes with heparinized needles and transferred to centrifuge tubes, immediately centrifuged at 0°C for 15 minutes at 3,000 rpm (Santulli et al, 1986a) in Centrifuge IEC Centra-4R MODEL. The supernatant was removed using teat pippetes stored in plastic vials and kept in the freezer at - 30° C till analysis.

2.8.1.2. Liver

The liver was excised, weighed, packed individually in aluminium foil, wrapped in plastic bags and stored at - 20° C till analysis.

2.8.1.3. Muscle

The muscle was dissected on both sides along the dorsal line from the area just behind the head region to the tail with fillet coming out on either side of the vertebra and avoiding any bones as much as possible. The skin was removed, the muscle homogenised intermittently for 1-2 minutes using a kitchen blender TYPE-320 from MIRULINEX.
The samples were wrapped in aluminium foil then put in plastic bags and stored at - 20° till analysis.

2.9. Feeding

The mean weights of 25 fish per tank were used at the beginning of the experiment to calculate the amounts of feed per tank on the basis of 2 % of body weight on wet weight basis.

2.10. Daily Measurements

2.10.1. Temperature

Thermometers (mercury) were stuck to the sides of the aquaria and readings taken every morning

2.10.2. Nitrite

This was measured using a TETRATEST NITRITE KIT. 5 ml of water sample was put in a graduated cell. 5 drops of Tetra nitrite 1 were added; then 5 drops of Tetra nitrite 2; the colour was allowed to develop and readings taken against a standard Tetra kit colour wheel in ppm.

2.10.3. Ammonia

This was measured using a Hach DR/3 spectrophotometer. 25 ml of demineralized water was transferred in a clean graduated cylinder to the 25 ml mark.
25 ml of water sample was transferred to a clean sample cell. 1.0 ml Nessler Reagent was added to each cell using a 1.0 ml celibrated dropper suited to mix. A yellow colour developed. The sample cell containing the demineralized water was put in the cell holder and wavelength adjusted to 425 nm. The prepared sample was placed into the cell holder and the ammonia nitrogen was read off (Water Analysis Handbook; Hach company, DR/3 and DREL/5, 1985).

2.11. Weekly Measurements

2.11.1. Weight and length of the fish

At least every week 25 fish from each tank were weighed to the nearest 0.1 g on a "Sartorius balance 1203MP" and their length determined from the tip of the snout to fork or notch end of the tail. To ease catching of fish the water level in the aquarium was lowered nearly by half using the drainage pipe. The fish were transferred to a bucket of water, and anaesthesised with phenoxyethanol at 1 ml per 5 l and dried with tissue paper to remove excess water.
Prior to the weighing day the fish were starved for 24 hours to avoid regurgitation of food during handling. 25 fish were weighed each time until the number of fish remaining dropped below 25 due to dissection in which case all the fish in the tank were weighed.

2.11.2. Ration

The ration was adjusted according to the new mean weights of the fish in each tank at every weighing interval and calculated at 2 % of wet body weight as follows:
Mean weight in the tank X
Total weight in the tank X*N
2 % of this ..... (X*N) x 2 % of diet for one week fed per day where N is the number of fish in the tank.

2.12. Fortnightly Measurements

At least every two weeks, five fish were removed randomly from each tank after lowering the water level. They were anaesthesised in phenoxyethanol and blood, liver and muscle removed. The samples were handled in the same way as for baseline data till analysis.

2.13. Chemical Analysis

2.13.1. Muscle

These were the parameters determined:

1) Crude protein
2) Total fat
3) Ash
4) Moisture

The frozen samples were thawed for 4 Hrs at 3° C prior to analysis.

2.13.1.1. Protein

Analysis of protein in the muscle was carried out using the Kjeldahl method.

Principle: The product is digested with concentrated sulphuric acid using copper sulphate ($CuSO_4$) as catalyst to convert organic Nitrogen to ammonium ions. Alkali is added and the liberated ammonia distilled into an excess of boric acid solution. The distillate is titrated with standard acid - H_2SO_4 or HCl to determine the ammonia absorbed in the boric acid.
The method followed was as specified in the manual for Tecater KJELTEC System, 1990.

Digestion: 1 g muscle samples in duplicates were weighed and transferred directly to the digestion tubes.
12 ml of concentrated H_2SO_4:H_3PO_3 mixture ratio of 950:50 v/v was added.
Then 10 ml 30 % H_2O_2 was added and finally 10 g of catalyst mixture $CuSO_4$:K_2SO_4 in the ration of 0.1:5 g was added. The digestion unit TECATOR DIGESTION SYSTEM 6,1007 DIGESTOR with the temperature preset at 420°C was used and the digestion time lasted one hour.

Distillation: Once the digest was allowed to cool, the distillation of ammonia was carried out in the automatic pre-programmed distilling unit - KJELTEC SYSTEM, TECATOR 1026 using 40 % NaOH and collecting the distillate in 25 ml 4 % boric acid with bromocresol green, methyl red indicator prepared in absolute ethanol (100 mg/ml) of each in the ratio of 10:7 v/v.

Titration: The end point was determined by colour change from green to purple and at a pH of 4.8.

2.13.1.2. Fat

This was analysed in two stages.

1) Acid Hydrolysis

The hydrolysing unit used was TECATOR SOXTEC SYSTEM 1047

Principle: The sample is boiled in dilute HCl to free the occluded and bound lipid fractions and is subsequently extracted with petroleum ether. The method used is as stated in the accompanying manual by Tecator Soxtec Sytem. About 2 g of muscle was weighed and transferred to the hydrolysing/digestion tubes in duplicate. Approximately 2 g celite was added. Then 100 mls 4N HCl was added, the heating knob switch set to maximum and hydrolysis commenced for one hour. 100 mls of cold distilled water was added. The hydrolysate was sucked to the thimbles. 250 mls distilled water at 50°C was used to rinse the residue, sucked and the thimbles dried in a microwave oven for 30 minutes (ZANKER COMPACT MICROWAVE OVEN). The remaining liquid in the hydrolysing tubes was recovered by cotton wool wetted in petroleum in acetone and transferred to the thimbles once cooled.

2) Fat Extraction

The SOXTEC SYSTEM HT,1043 extraction unit was used.

Principle: The fat is extracted with petroleum ether from the sample either dried from moisture determination or from acid hydrolysis. The solvent is removed by evaporation and fat residue weighed. The method used is as stated in the manual of Tecater Soxtec. The metallic recipients were dried in the oven (HVL TYPERB 500) at 100°C, transferred to a dessicator to cool. They were then weighed in a balance SARTORIUS 1207MP 2

to the nearest 0.001 g. 50 mls of petroleum ether boiling point 40-60°C was added. The thimbles were placed in position in the extractor and temperature set to 110°C. The extractor was set at 50 minutes in boiling position and 50 minutes rinsing position and 15 minutes evaporation position. The recipient and the fat residue was transferred to the oven at 100°C for 30 minutes, placed in a dessicator and weighed.

2.13.1.3. Moisture

Principle: The sample is dried to a constant weight in an air oven.

Procedure: Empty porcelein dishes were dried in the oven HVL TYPE RB 500 for 30 minutes, transferred to a dessicator to cool for 30 minutes. The dishes were weighed empty. About 2 g of homogenised muscle was put in the dishes and the whole weight determined. The dishes with their contents were placed in the oven, preset at 102°C and left overnight. The dishes were removed from the oven, transferred to a dessicator to cool and weighed. Drying was continued for a further period of less than an hour to ensure achievement of constant weight.

2.13.1.4. Ash

This method is applicable to samples containing less than 50 % fat. Empty porcelein dishes were dried in the oven as for moisture for 30 minutes, cooled in a dessicator and weighed to the nearest 0.001 g. About 2 g of homogenised muscle sample was transferred to the porcelein dish and the whole weight determined. The whole set was transferred to the furnace (THERMOACTIVE Ltd MODEL 021>-5 PD) with the temperature being adjusted slowly first at 200°C then 400°C after 2 hours then finally 550°C overnight. The dishes were transfer-

red to the dessicator to cool for 30 minutes then weighed when cold.

2.13.1.5. Plasma

Time could not allow plasma samples to be analyzed further.
Due to unavoidable circumstances replicates of fish could not be maintained.

2.13.1.6. Wing Tests

One reference fish was killed and each time fresh solutions were prepared or analysis done for the day, its muscle was analysed for the above parameters to check consistency of results (Data not shown).

3. BIOTECHNICAL CALCULATIONS

3.1. Protein

$$\% N = \frac{14.01 \times (ml\ of\ acid - ml\ of\ blank) \times normality\ of\ acid}{10 \times weight\ of\ sample}$$

% Protein = N x 6.25
where N = Nitrogen

3.2. Fat

Weight of recipient (g) = W
Weight of recipient and fat (g) = W1
Weight of fat = W1 - W

$$\% fat = \frac{W_1 - W}{weight\ of\ sample} \times 100$$

3.3. Moisture

Weight of sample (g) = Y
Weight of dish and sample before drying (g) = Y_1
Weight of dish and sample after drying (g) = Y_2

$$\% moisture = \frac{Y_1 - Y_2}{weight\ of\ sample} \times 100$$

3.4. Ash

Weight of sample (g) = A

Weight of ash (g) = A_1

% Ash = $(A_1/A) \times 100$

3.5. Food Conversion Ratio:

$$\frac{\textit{Wet Weight Gain}}{\textit{Food given dry weight}}$$

3.6. Food Efficiency Ratio:

$$\frac{\textit{Food given dry weight}}{\textit{wet weight gain}}$$

3.7. Hepato-Somatic Index:

$$\frac{\textit{Liver weight}}{\textit{wet weight of fish}} \times 100$$

3.8. Specific growth rate:

$$\frac{\ln W_t - \ln W_{t_o}}{t} \times 100$$

W_t = Weight at time 't'

W_o = weight at time zero

t = time in days

4. STATISTICAL ANALYSIS

Anova Model I, Bonferroni (Dun) T-tests for variables as well as Least Square Means were used to test for effect due to treatments and time on growth as well as on body composition changes.

5. RESULTS

5.1. Protein

The group treated with isoproterenol showed a significantly ($P < 0.05$) higher content on days 17, 61 and 80 compared with the control for the group of smaller fish. The other products, clenbuterol, fenoterol and salbutamol showed a significantly higher difference only on day 17 compared with the control (table 5 and figure 6) and a rapid increase as such.

There was no net effect on protein accretion for the group of bigger fish (table 6 and figure 7) throughout the experimental period when sampling was done ($P < 0.05$) with respect to the control. A correlation between protein vs. weight of big fish for both groups did not yield any statistically significant difference for the whole experimental period ($P < 0.05$) (tables 7 and 8). In fact, for the group of bigger fish, clenbuterol even showed a statistically significant ($P < 0.05$) lower protein-weight relationship. On day 61, isoproterenol's effect on protein content was significantly higher than clenbuterol. It's effect was significanlty higher ($P < 0.05$) than salbutamol on day 80. Clenbuterol gave a higher protein level ($P < 0.05$) than fenoterol on day 46. Salbutamol also gave a higher amount of protein on day 80 than fenoterol ($P < 0.05$).

5.2. Fat

Lower fat levels were observed for isoproterenol than the rest of the products on day 17 and 80 and with respect to the control ($P < 0.05$), for the group of smaller fish (table 9). On day 17, compared to salbutamol and fenoterol, isoproterenol gave a significantly lower fat volume ($P < 0.05$); also clenbuterol gave lower fat levels ($P < 0.05$) than fenoterol. Fenoterol did not seem to have lowered fat throughout the sampling

periods. On table 10, a correlation between fat and weight for the group of smaller fish did not give any significant difference ($P < 0.05$) with respect to one another due to the treatments and from the control. The same applied to the group of bigger fish (table 11), although all gave a negative correlation.

For the whole experimental period, a correlation between protein and fat was not significant ($P < 0.05$) for all treatments including the control for both groups of fish (tables 12 and 13). But a regression for protein and fat gave a negative relationship for the group of smaller fish due to isoproterenol. The rest gave a positive relationship. For the group of bigger fish all the products showed a positive relationship (figures 8 and 9). Isoproterenol for some unknown reason gave a positive correlation between protein and fat for the group of bigger fish and it was significant compared to the control ($P < 0.05$). For the group of bigger fish, there was no significant (with respect to the control) differnce ($P < 0.05$) due to the treatments on the fat content (table 14).

5.3. Moisture Content

For the group of smaller fish, all the products gave a higher moisture value at day 31 only ($P < 0.05$) with respect to the control (table 15). For the group of bigger fish, no difference was seen ($P < 0.05$) (table 16).

A correlation between protein and moisture was also not significant ($P < 0.05$) due to all the treatments (table 17), although isoproterenol gave a positive but not significant difference.

For the group of bigger fish, isoproterenol gave a negative correlation between protein and moisture ($P < 0.05$) (table 18). The fat vs. moisture relationship was negative for all the treatments for the group of smaller fish but not significant ($P < 0.05$) (table 19). For the group of bigger fish, the

control differed instead significantly ($P < 0.05$) from the rest (table 20).

5.4. Ash Content

This did not differ ($P < 0.05$) for both smaller and bigger groups of fish compared with the control (tables 21 and 22).

5.5. Growth

Growth due to the products for the group of smaller fish was significantly higher ($P < 0.05$) compared with the control (table 23).
No differences in growth rate ($P < 0.05$) were observed in the group of bigger fish due to the products (figure 11, and table 24).

The hepato-somatic index on tables 25 and 26 indicated no significant difference for both groups of fish ($P < 0.05$) due to treatment and time. The regression (table 30) was not significant except for fenoterol and clenbuterol which was even significantly higher for the group of bigger fish. For the group of smaller fish, only fenoterol gave a significantly ($P < 0.05$) higher difference from the control.

The results on figure 10 and table 24 indicate that the slopes of the growth rate (versus time) for the group of smaller fish was significantly different ($P < 0.05$) from the control, except for salbutamol. But for the group of bigger fish (figure 11, table 24) no significant difference in growth rate was observed with respect to the control at $P < 0.05$.

5.6. The Weight-Length Relationship

There was no statistical difference ($P < 0.05$) observed compared with the control for both smaller and bigger fish groups (figs 12 and 13). The condition factor was also not different statistically ($P < 0.05$) for both groups of fish (table 29).

The food conversion ratio and food efficiency ratio was not statistically different ($P < 0.05$) (table 27). A marked improvement was seen expressed as a percentage of the control (table 28).

TABLE 5. % PROTEIN FOR GROUP OF SMALLER FISH
$P < 0.05$
Superscripts-differences due to Products
*$P < 0.05$, mean ± s.d.
Superscripts a, b, c, d, e f
Differences in efficacy of the products themselves $P < 0.05$
($n = 5$)
a = clenbuterol from isoproterenol
b = clenbuterol from fenoterol
c = clenbuterol from salbutamol
d = salbutamol from fenoterol
e = salbutamol from isoproterenol
f = fenoterol from isoproterenol

	DAYS				
	17	31	46	61	80
C	17.97 ± 0.43	18.89 ± 0.38	18.99 ± 0.14	18.77 ± 0.42	18.89 ± 0.47
DI	*19.10 ± 0.73	19.29 ± 0.18	18.99 ± 0.07	[a]19.59* ± 0.34	[e]19.63* ± 0.75
DC	*18.74 ± 0.33	18.94 ± 0.29	[b]19.10 ± 0.62	18.92 ± 0.39	[e]19.63 ± 0.39
DF	*18.82 ± 0.37	18.62 ± 0.52	18.42 ± 0.11	19.45 ± 0.47	[d]19.63 ± 0.17
DS	*18.89 ± 0.34	18.74 ± 0.74	18.70 ± 0.49	18.97 ± 0.76	18.77 ± 0.30

TABLE 6. % PROTEIN FOR GROUP OF BIGGER FISH
* $P < 0.05$ from control, $n = 5$

	DAYS				
	17	31	46	61	80
C	18.93 ± 0.57	18.93 ± 0.41	18.61 ± 0.57	18.72 ± 0.28	18.71 ± 0.20
DI	18.98 ± 0.34	18.69 ± 0.28	19.08 ± 0.33	18.80 ± 0.54	19.05 ± 0.47
DC	19.13 ± 0.30	18.75 ± 0.12	18.75 ± 0.77	18.74 ± 0.32	18.88 ± 0.74
DF	19.51 ± 0.33	18.93 ± 0.32	18.75 ± 0.15	19.06 ± 0.46	18.35 ± 0.35
DS	19.51 ± 0.33	19.01 ± 0.55	18.82 ± 0.76	19.11 ± 0.40	18.90 ± 0.63

TABLE 7. PROTEIN-WEIGHT CORRELATION FOR GROUP OF SMALLER FISH
n = 23

	CORR. COEFF.	PROBABILITY (P < 0.05)
C	0.12	0.60
DC	0.14	0.55
DI	- 0.05	0.86
DS	- 0.26	0.22
DF	0.33	0.11

TABLE 8. PROTEIN-WEIGHT CORRELATION FOR GROUP OF BIGGER FISH
n = 23

	CORR. COEFF.	PROBABILITY (P < 0.05)
C	0.027	0.90
DC	- 0.41	0.04*
DI	- 0.27	0.20
DS	- 0.316	0.15
DF	- 0.136	0.54

TABLE 9. % FAT FOR GROUP OF SMALLER FISH
n = 5, *P < 0.05

	Days 0	17	31	80
C	1.60 ± 0.31	1.78 ± 0.43	2.14 ± 0.48	1.68 ± 0.24
DI	1.60 ± 0.31	[f,e]1.34 ± 0.16	[f]1.69 ± 0.21	[f]1.22* ± 0.06
DC	1.60 ± 0.31	[e,c,b]1.49 ± 0.33	1.54 ± 0.28	1.67 ± 0.52
DF	1.60 ± 0.31	2.18 ± 0.21	2.26 ± 0.55	1.88 ± 0.41
DS	1.60 ± 0.1	[c]1.99 ± 0.47	[d]1.68 ± 0.47	1.62 ± 0.47

TABLE 10. FAT-WEIGHT CORRELATION FOR GROUP OF SMALLER FISH
n = 13 (P < 0.05)

	CORR. COEFF.	PROBABILITY
C	- 0.11	0.70
DC	0.28	0.34
DI	- 0.018	0.95
DS	- 0.16	0.59
DI	- 0.35	0.19

TABLE 11. FAT-WEIGHT CORRELATION FOR GROUP OF BIGGER FISH
n = 13, (P < 0.05)

	CORR. COEFF.	PROBABILITY
C	- 0.23	0.46
DC	- 0.14	0.64
DI	- 0.089	0.75
DS	- 0.15	0.57
DF	- 0.46	0.04

TABLE 12. PROTEIN-FAT CORRELATION FOR GROUP OF BIGGER FISH
n = 15, (P < 0.05)

	CORR. COEFF.	PROBABILITY
C	- 0.220	0.51
DC	- 0.165	0.61
DI	0.61	0.019*
DS	- 0.21	0.48
DF	0.21	0.49

TABLE 13. PROTEIN-FAT CORRELATION FOR GROUP OF SMALLER FISH
n = 15, (P < 0.05)

	CORR. COEFF.	PROBABILITY
C	- 0.290	0.41
DC	- 0.003	0.99
DI	- 0.27	0.41
DS	- 0.466	0.126
DF	- 0.38	0.17
START	0.066	0.77

TABLE 14. % FAT FOR GROUP OF BIGGER FISH
n = 5, *P < 0.05

	Days 0	17	31	80
C	1.82 ± 0.35	1.99 ± 0.37	1.73 ± 0.39	1.64 ± 0.70
DI	1.82 ± 0.35	1.82 ± 0.39	[e,f,a]1.11 ± 0.28	1.81 ± 0.70
DC	1.82 ± 0.35	1.99 ± 0.45	1.73 ± 0.39	1.64 ± 0.37
DF	1.82 ± 0.35	2.09 ± 0.34	2.43 ± 0.45	1.31 ± 0.27
DS	1.82 ± 0.35	1.80 ± 0.34	1.92 ± 0.42	1.27 ± 0.55

TABLE 15. % MOISTURE FOR GROUP OF SMALLER FISH
n = 5, *P < 0.05

	Days 0	17	31	80
C	79.11 ± 0.46	76.66 ± 0.59	*76.27 ± 0.34	76.97 ± 0.84
DI	79.11 ± 0.46	77.98 ± 0.57	*77.22 ± 0.21	77.79 ± 1.44
DC	79.11 ± 0.46	77.62 ± 0.87	*78.51 ± 0.51	77.80 ± 0.18
DF	79.11 ± 0.46	77.01 ± 0.68	*78.10 ± 0.84	77.19 ± 0.70
DS	79.11 ± 0.46	77.92 ± 0.46	*77.91 ± 0.35	78.03 ± 0.21

TABLE 16. % MOISTURE FOR GROUP OF BIGGER FISH
n = 5, *P < 0.05

	Days 0	17	31	80
C	78.83 ± 0.60	77.32 ± 0.81	*77.45 ± 0.85	77.74 ± 0.81
DI	78.83 ± 0.60	*76.98 ± 1.71	*78.80 ± 1.20	77.16 ± 1.76
DC	78.83 ± 0.60	77.48 ± 0.87	77.46 ± 0.43	76.73 ± 1.24
DF	78.83 ± 0.60	77.11 ± 1.18	77.19 ± 1.02	76.97 ± 1.18
DS	78.83 ± 0.60	77.17 ± 0.59	77.18 ± 0.64	76.52 ± 1.7.

TABLE 17. PROTEIN-MOISTURE CORRELATION FOR GROUP OF SMALLER FISH, n = 15, (P < 0.05)

	CORR. COEFF.	PROBABILITY
C	- 0.29	0.41
DC	- 0.003	0.99
DI	0.070	0.82
DS	- 0.47	0.126
DF	- 0.43	0.057

TABLE 18. PROTEIN-MOISTURE CORRELATION FOR GROUP OF BIGGER FISH, n = 15, (P < 0.05)

	CORR. COEFF.	PROBABILITY
C	0.29	0.38
DC	0.02	0.94
DI	- 0.59	0.03*
DS	0.12	0.74
DF	0.26	0.36

TABLE 19. FAT-MOISTURE CORRELATION FOR GROUP OF SMALLER FISH, n = 15, ($P < 0.05$)

	CORR. COEFF.	PROBABILITY
C	- 0.289	0.41
DC	- 0.252	0.42
DI	- 0.097	0.77
DS	- 0.176	0.545
DF	- 0.227	0.410

TABLE 20. FAT-MOISTURE CORRELATION FOR GROUP OF BIGGER FISH, n = 15, ($P < 0.05$)

	CORR. COEFF.	PROBABILITY
C	- 0.70	0.016*
DC	- 0.16	0.60
DI	- 0.245	0.393
DS	- 0.205	0.48
DF	- 0.497	0.070

TABLE 21. % ASH FOR GROUP OF SMALLER FISH
n = 5, *$P < 0.05$

	Days 0	17	31	80
C	1.02 ± 0.01	1.26 ± 0.09	1.58 ± 0.12	1.53 ± 0.11
DI	1.02 ± 0.01	1.31 ± 0.15	1.68 ± 0.12	1.58 ± 0.11
DC	1.02 ± 0.01	1.07 ± 0.32	1.44 ± 0.22	1.32 ± 0.14
DF	1.02 ± 0.01	1.25 ± 0.17	1.56 ± 0.18	1.56 ± 0.23
DS	1.02 ± 0.01	1.41 ± 0.14	1.45 ± 0.19	1.26 ± 0.21

TABLE 22. % ASH FOR GROUP OF BIGGER FISH
n = 5, *P < 0.05

	Days 0	17	31	80
C	1.11 ± 0.1	1.23 ± 0.16	1.45 ± 0.21	1.44 ± 0.16
DI	1.11 ± 0.1	1.15 ± 0.10	1.51 ± 0.14	1.15 ± 0.10
DC	1.11 ± 0.1	1.37 ± 0.10	1.46 ± 0.21	1.31 ± 0.11
DF	1.11 ± 0.1	1.14 ± 0.22	1.43 ± 0.20	1.31 ± 0.22
DS	1.11 ± 0.1	1.33 ± 0.19	1.32 ± 0.21	1.09 ± 0.19

TABLE 23. SPECIFIC GROWTH RATE FOR BOTH GROUPS OF FISH
n = 9, *P < 0.05

GROUP	SMALLER FISH	BIGGER FISH
C	0.42	0.43
DI	*0.78	0.42
DC	*0.59	0.41
DF	*0.75	0.45
DS	*0.82	0.31

TABLE 24. SLOPES FOR WEIGHT-TIME RELATIONSHIPS (y = a + bx) FOR SMALLER AND BIGGER FISH
n = 25, *P < 0.05

GROUP	SMALLER	BIGGER
C	0.00481	0.005219
DI	0.00660*	0.006133
DC	0.00591*	0.005813
DF	0.0069*	0.006419
DS	0.0060	0.003576

TABLE 25. HEPATOSOMATIC INDEX FOR GROUP OF SMALLER FISH
n = 9, *P < 0.05

	Days					
	0	17	31	46	61	80
C	1.15 ± 0.18	0.94 ± 0.05	0.79 ± 0.08	0.88 ± 0.15	0.88 ± 0.09	0.68 ± 0.15
DI	1.15 ± 0.18	0.96 ± 0.11	0.92 ± 0.07	0.96 ± 0.08	0.92 ± 0.12	0.74 ± 0.32
DC	1.15 ± 0.18	1.01 ± 0.24	0.90 ± 0.19	0.90 ± 0.15	0.90 ± 0.09	0.68 ± 0.15
DF	1.15 ± 0.18	1.02 ± 0.16	0.95 ± 0.11	0.96 ± 0.21	0.87 ± 0.11	0.86 ± 0.13
DS	1.15 ± 0.18	0.95 ± 0.14	0.93 ± 0.10	0.93 ± 0.15	0.82 ± 0.27	0.82 ± 0.14

TABLE 26. HEPATOSOMATIC INDEX FOR GROUP OF BIGGER FISH
n = 9, *P < 0.05

	Days					
	0	17	31	46	61	80
C	1.14 ± 0.10	0.89 ± 0.23	0.88 ± 0.11	0.86 ± 0.13	0.90 ± 0.07	0.80 ± 0.13
DI	1.14 ± 0.10	0.97 ± 0.12	0.96 ± 0.32	1.03 ± 0.05	0.97 ± 0.32	0.82 ± 0.18
DC	1.14 ± 0.10	0.95 ± 0.14	0.88 ± 0.11	0.89 ± 0.15	0.89 ± 0.12	0.89 ± 0.19
DF	1.14 ± 0.10	0.97 ± 0.21	0.85 ± 0.11	0.91 ± 0.11	0.79 ± 0.19	0.81 ± 0.10
DS	1.14 ± 0.10	0.78 ± 0.19	0.94 ± 0.07	0.90 ± 0.08	0.94 ± 0.07	0.94 ± 0.20

TABLE 27. OVERALL FOOD CONVERSION RATIO FOR THE WHOLE EXPERIMENTAL PERIOD COMPARED WITH CONTROL FOR SMALLER
n = 9, *P < 0.05

	SMALLER FISH		BIGGER FISH	
GROUP	FCE	FCR	FCE	FCR
C	0.31 ± 0.32	3.31 ± 0.91	0.31 ± 0.21	3.20 ± 0.9
DI	0.47 ±0.21	2.00 ± 1.26	0.34 ± 0.32	2.31 ± 0.90
DC	0.50 ± 0.20	2.14 ± 0.80	0.43 ± 0.11	2.70 ± 1.00
DF	0.49 ± 0.15	2.04 ± 0.71	0.25 ± 0.11	2.47 ± 0.70
DS	0.49 ± 0.07	2.08 ± 0.81	0.40 ± 0.21	3.94 ± 1.20

TABLE 28. % IMPROVEMENT OF FCR, FCE OVER CONTROL FOR WHOLE EXPERIMENT FOR BOTH BIGGER AND SMALLER FISH

	SMALLER FISH		BIGGER FISH	
GROUP	FCE	FCR	FCE	FCR
DI	51.01	31.82	38.70	27.81
DC	61.61	36.10	3.00	9.31
DF	58.06	33.82	9.00	22.81
DS	58.06	34.82	-6.00	-23.15

TABLE 29. CONDITION FACTOR FOR BOTH SMALLER AND BIGGER FISH DURING THE WHOLE EXPERIMENT

GROUP	SMALLER	BIGGER
C	1.00 ± 0.04	1.00 ± 0.004
DI	1.01 ± 0.005	1.00 ± 0.005
DC	1.00 ± 0.004	1.00 ± 0.001
DF	1.00 ± 0.00	1.00 ± 0.004
DS	1.00 ± 0.00	1.00 ± 0.003

TABLE 30. REGRESSION EQUATIONS FOR HEPATO-SOMATIC INDEX FOR BOTH GROUPS OF FISH
(*P < 0.05), n = 5

	Group of smaller fish	Group of bigger fish
C	y = 0.972 - 0.0029 ln x	y = 0.924 - 0.0011 ln x
DC	y = 1.024 - 0.024 ln x	y = 0.936 - 0.0001 ln x
DI	y = 0.99 - 0.0028 ln x	y = 1.054 - 0.0023 ln x
DS	y = 1.015 - 0018 ln x	y = 0.806 - 0.002 ln x
DF	y = 0.99 - 0.0019 ln x *	y = 0.954 - 0.0019 ln x *

Biotechnical Calculation for Condition Factor:

$$\log W' = \log a + b \log L$$

$$Condition\ factor = \frac{W}{W'}$$

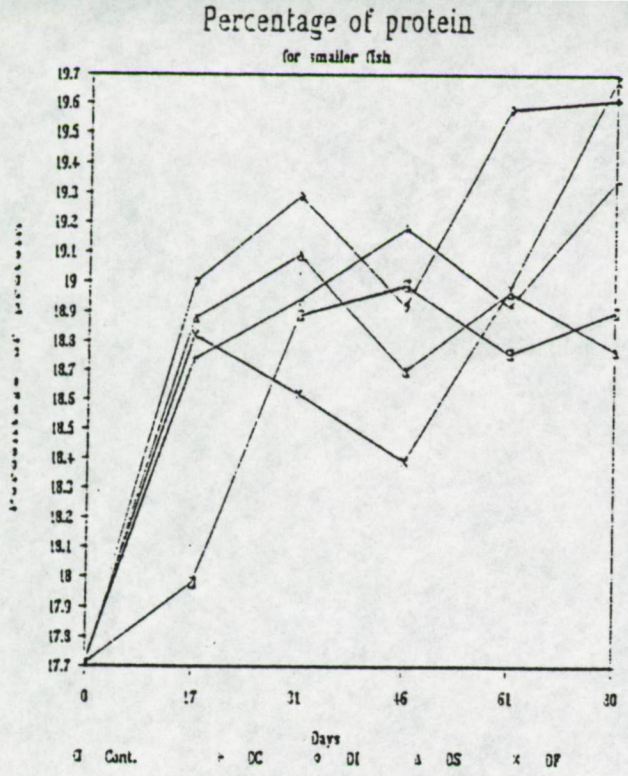


Figure 6.

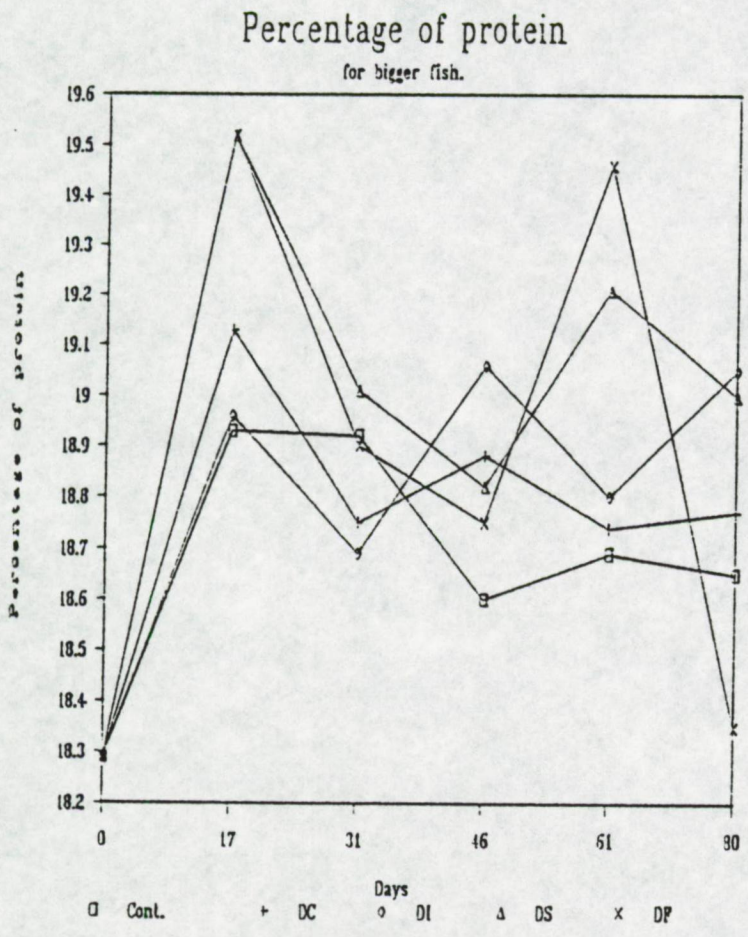
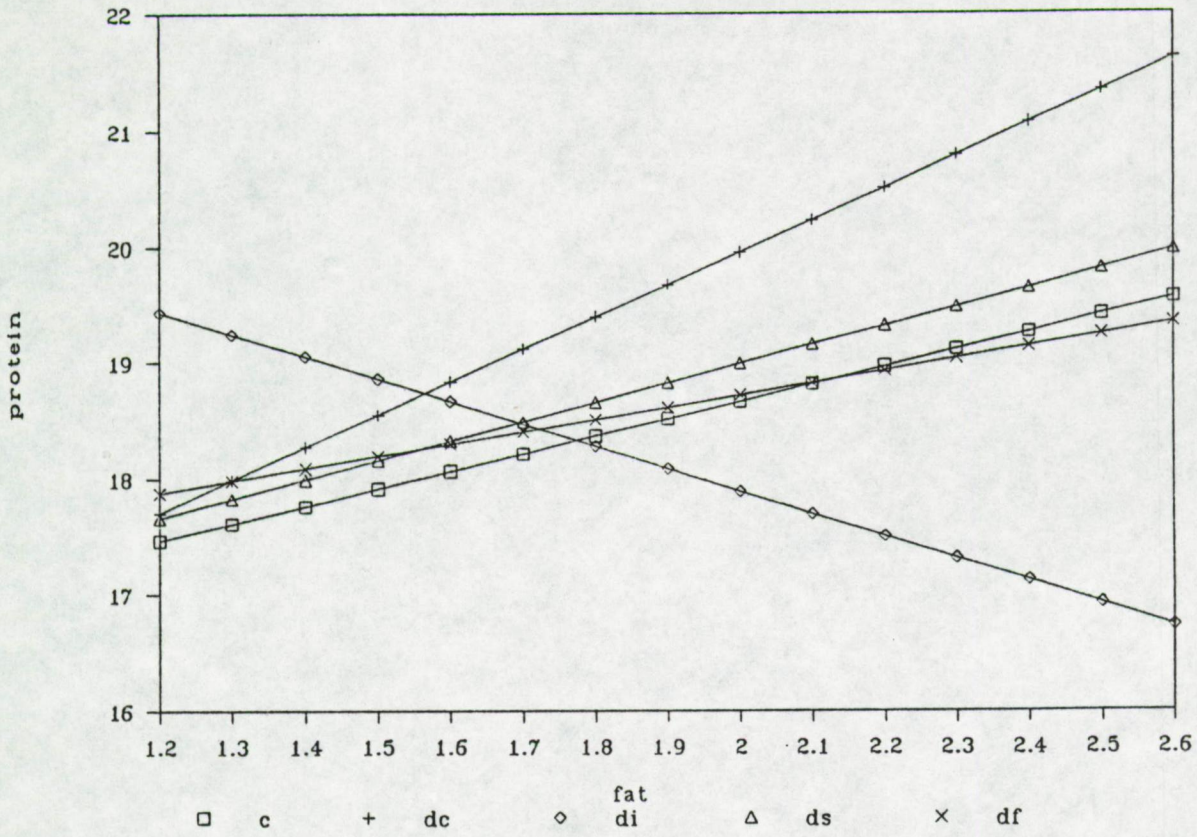


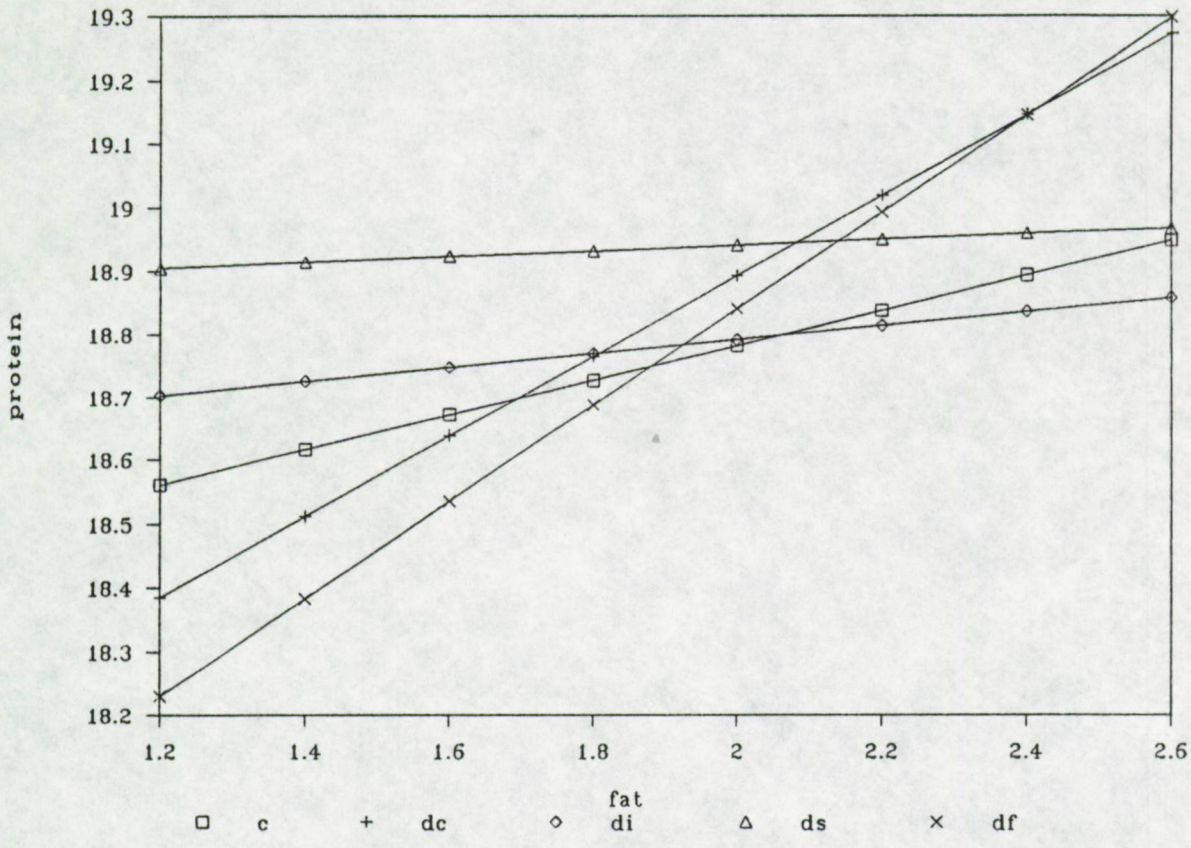
Figure 7.

Protein _ Fat ralationship (Small fish)



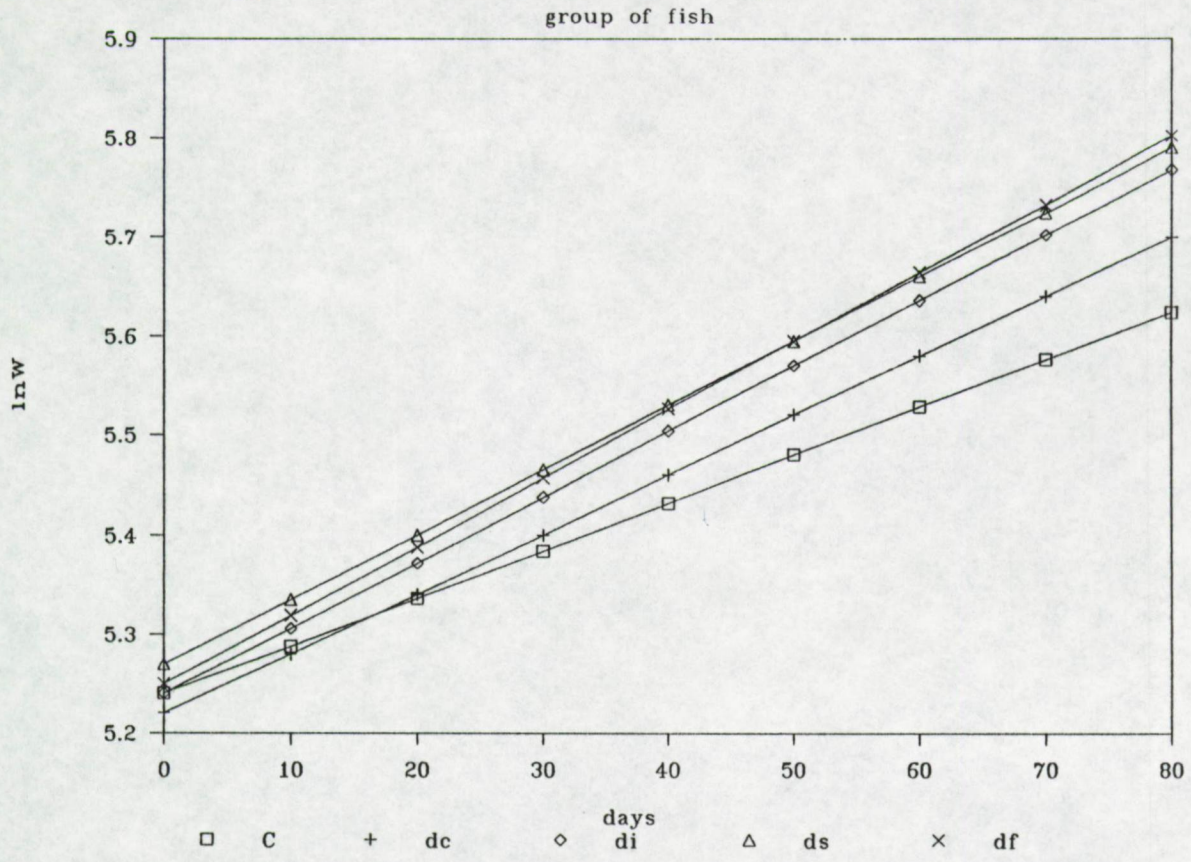
		R^2
C	y = 15.68 + 1.50 x	0.33
DC	y = 14.35 + 2.80 x	0.07
DI	y = 21.75 - 1.93 x	0.25
DS	y = 15.68 + 1.65 x	0.30
DF	y = 10.63 + 1.04 x	0.16

Figure 8.



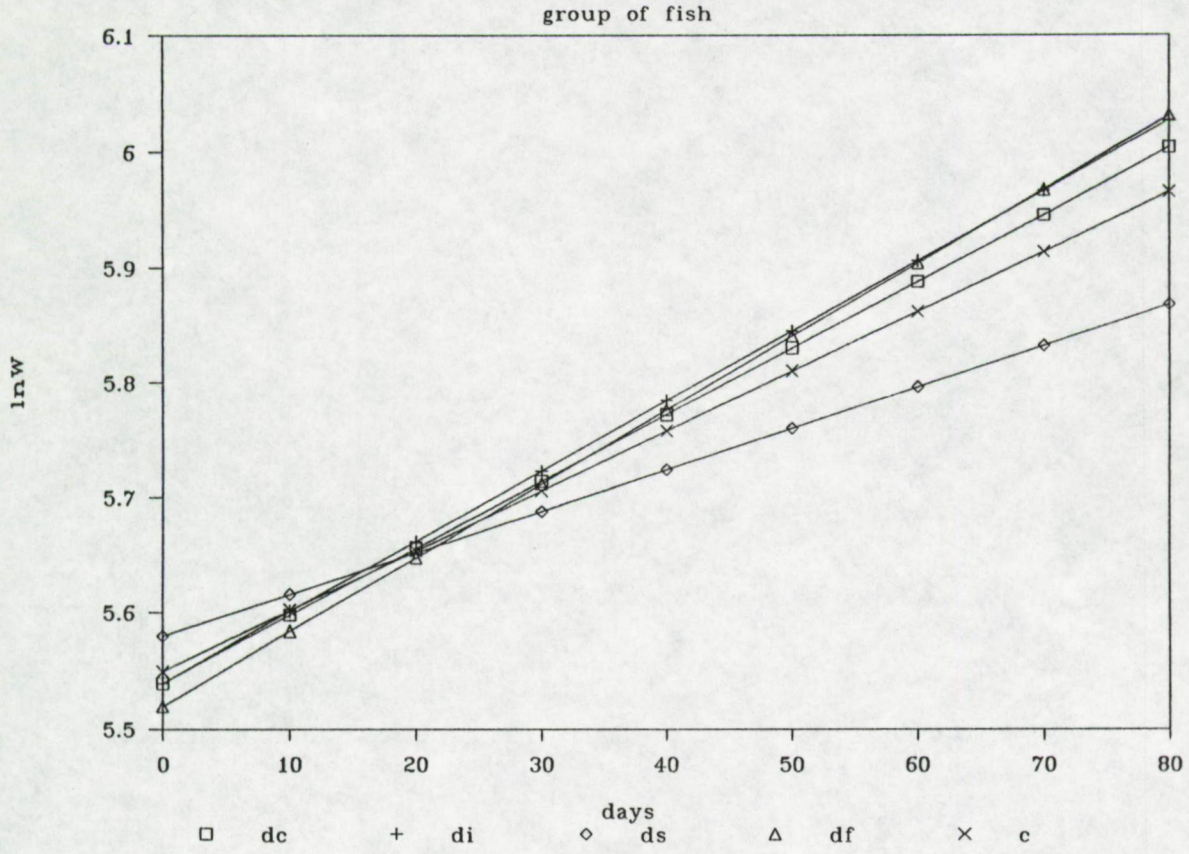
		R^2
C	y = 18.23 + 0.271 x	0.02
DC	y = 17.62 + 0.635 x	0.07
DI	y = 18.57 + 0.11 x	0.01
DS	y = 18.85 + 0.046 x	0.007
DF	y = 17.31 + 0.736 x	0.40

Figure 9.



	R^2
C = lnW = 5.24 + 0.0048 Days	0.5096
DC = lnW = 5.22 + 0.0060 Days	0.4726
DI = lnW = 5.24 + 0.0066 Days	0.4858
DS = lnW = 5.27 + 0.0065 Days	0.5350
DF = lnW = 5.25 + 0.0069 Days	0.5127

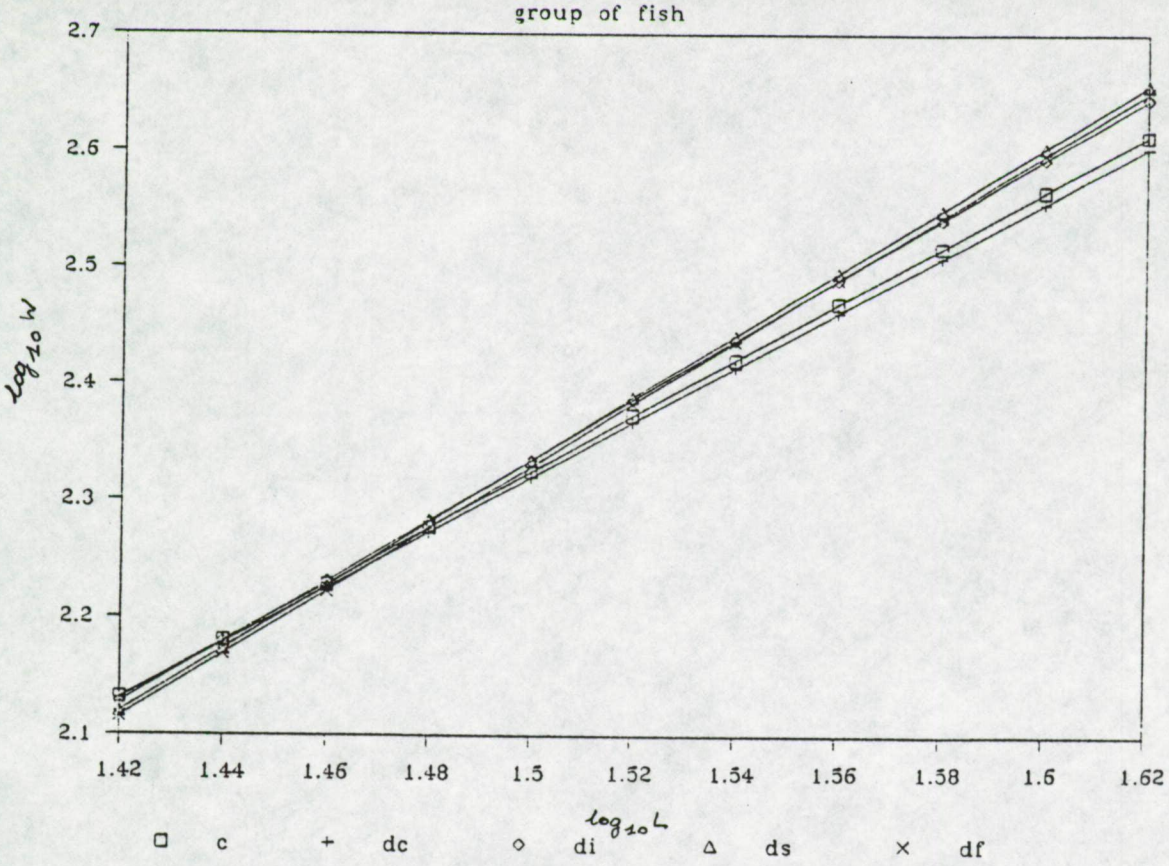
Figure 10.



		R^2
C	ln W = 5.55 + 0.0052 Days	0.4209
DC	ln W = 5.54 + 0.0058 Days	0.4158
DI	ln W = 5.54 + 0.0061 Days	0.4415
DS	ln W = 5.58 + 0.0036 Days	0.5157
DF	ln W = 5.55 + 0.0052 Days	0.4029

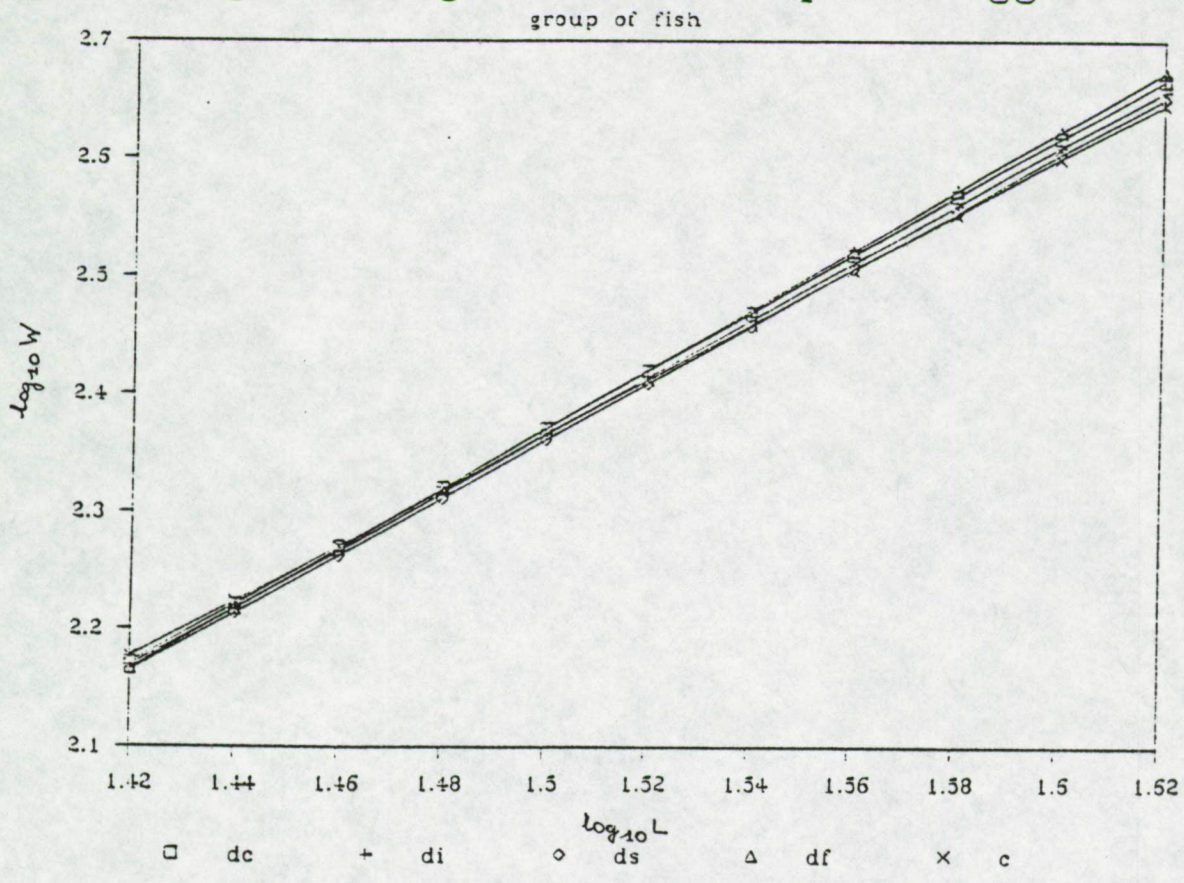
Figure 11.

Length -weight relationship for smaller group of fish



		R^2
C	$\log_{10}W = -1.29 + 2.41 \log_{10}L$	0.8424
DC	$\log_{10}W = -1.22 + 2.36 \log_{10}L$	0.8548
DI	$\log_{10}W = -1.55 + 2.59 \log_{10}L$	0.8634
DS	$\log_{10}W = -1.70 + 2.69 \log_{10}L$	0.9046
DF	$\log_{10}W = -1.69 + 2.68 \log_{10}L$	0.9112

Figure 12.



		R^2
C	$\log_{10}W = -1.16 + 2.35 \log_{10}L$	0.8641
DC	$\log_{10}W = -1.35 + 2.48 \log_{10}L$	0.8568
DI	$\log_{10}W = -1.31 + 2.45 \log_{10}L$	0.8518
DS	$\log_{10}W = -1.30 + 2.44 \log_{10}L$	0.8449
DF	$\log_{10}W = -1.44 + 2.54 \log_{10}L$	0.9147

Figure 13.

6. DISCUSSION

Effects of exogenous Epinephrine analogues are difficult to predict because of the multitudinous possibilities for physiological and metabolic regulation. There is also divergent receptor sub-type distribution, specificity and density in individual tissues within a species and between species within a given time.
Species specificity for pharmacodynamics and pharmacokinetics including absorption, distribution, metabolism and excretion of exogenous analogues can further modify effects observed in a given species (Mersman, 1987) as well as structural differences.

Work by Ottolenghi et al (1981) and Brighenti et al (1989) in catfish hepatocytes in vitro has revealed the basic mechanistic processes also predicted to act in higher animals. Farkas, 1969, however, working with lower vertebrates has his reservations in metabolic similarity between higher and lower animals.

Mersman (1987b), who has used these repartitioning agents in pigs, in vitro concedes that interpretation of any animal in vivo is complex and requires caution.

The overall effects of these products administered to fish do not seem to compare well with the results achieved in cattle, sheep, swine, chickens and rats, although there, variation also exists. The lacklustre effect seen on body composition due to the products could be due to any of the following reasons:
(i) receptor density; (ii) ages of the fish; (iii) receptor sub-type; (iv) selectivity; (v) refractoriness or tachiphylaxis; (vi) dosage; (vii) leaching. Although there is speculation about mechanisms yielding changes in muscle composition or compartimentation in response to Beta adrenergic agonists (Ricks et al, 1984), there is essentially no evidence that

these compositional changes in vivo directly result from changes in adipose tissue or muscle anabolism and/or catabolism.

6.1. Effects on Fat and Protein Content

Isoproterenol significantly reduced the fat content ($P < 0.05$) at most times it was tested i.e. after 17 and 80 days compared to the control in small fish. It also showed a negative relationship between fat and protein. This could be due to receptor density sub-type. An important factor in the response of any cell or organ to sympathomimetic amines is its density and proportion of β-subtype of receptors and the selectivity of the agonist used (Hoffman and Lefkowitz, 1988). Isoproterenol is known to be a potent non-selective β-adrenergic agonist. Carlson et al (1972, 1974) have provided evidence for the existence of both β_1 and β_2 receptors in the same tissue or organ and in some cases a single type of receptor. Wankova et al (1976) in a study involving attributes and intrinsic activities of salbutamol and Isoproterenol on human and rat adipose tissue provided more evidence for receptor sub-type induced response. Isoproterenol with both β_1 and β_2 activity was more potent as a lipolytic agent on both rat and human adipose tissue while salbutamol was only potent on rat. They suggested receptor sub-type as a major contributor. Since the rest of the products used in this study are β_2 specific, in case there is a varied proportion of β_1 and β_2 receptors on catfish adipocytes, with more β_1 then it would be reasonable to suggest that the predominant β_1-subtype receptor in catfish muscle is the main reason for the lipolytic or anti-lipogenic activity due to Isoproterenol. What was not studied here was the nature/density of the receptor sub-types so finalizing conclusion based on the facts remains elusive. Since Isoproterenol seems more efficacious, the nature of receptors if predominantly of the β_1-subtype in catfish would give Isoproterenol an edge over the rest of the products.

Case studies of potency and efficacy of Isoproterenol as a strong lipolytic agent have been studied by Jolly et al (1978), also by Kather and Simon (1980). The former used isolated rat adipocytes to compare the efficiacy of Isoproterenol over salbutamol. Comparing lipolytic effects of salbutamol and isoproterenol, they found a similarity in overall effect though at a higher dose for salbutamol. The type of receptors in rat adipocytes, they said were β_2 predominantly.
In the latter study, using the same products on human adipocytes, which has predominantly β_1-receptors, isoproterenol was efficacious and not salbutamol. Thus the receptor sub-type effect was once seen.

The concentration or dosage of the products used is also important. It is not known whether all the products were within the effective dose range for catfish. It could have been too high or too low for the two classes of fish.

A typical dose-response curve has a no-effect portion, an increasing effective portion, a plateau, and a decreasing effective portion and finally a no-effect of less than control response (Stiles, 1984). The concentration of the products fed in this study may not have been correct because they could have fallen in any of the several portions of the dose-response curve for either classes of fish. Mersman (1987b), in a study using a pig model in vivo, monitored plasma free fatty acids and glucose levels after treatment with fenoterol, clenbuterol and isoproterenol among other products.
He used a low dose range or a high dose range of the products. In all the studies at low dose, isoproterenol was administered at 0.0625 μg base kg^{-1} min^{-1} while the rest were at 1.0 μg base kg^{-1} min^{-1}. At the high dose range, he used 10 times more concentration of the other products than Isoproterenol. That is when he achieved the same effect. Thus, it would be reasonable to assume that isoproterenol is much more potent than fenoterol and clenbuterol. It is not surprising that in the event like in our study where isoproterenol and the rest of

the products were used at the same dosage (1 ppm), isoproterenol is seen to be a more effective lipolytic or antilipogenic agent.

Heel et al (1979) used isoproterenol, salbutamol and fenoterol and still isoproterenol was more effective and the same was observed by Mersman (1984) using isoproterenol, salbutamol and fenoterol. Whereas he used cross-bred pigs of 12-14 kg and male rats weighing between 275-325 g and also different regions as source of his adipocytes (epidydimal) fat pads for rats and subcutaneous fat deposits for pigs, still, isoproterenol was more potent and efficacious at the same dose than the other two ! A similar observation has been made by De Leen (1982).

The effect of age could also be an alternative hypothesis to the lack of effect seen for all the products on the big category of fish. In studying the effect of age on isoproterenol-stimulated bronchial smooth muscle, Anderson et al (1982) found that young animals responded much more dramatically to catecholamines than older ones. They did not however measure the β-adrenergic receptor number in the tissues and so the mechanisms of reduced sensitivity in older animals remained unknown.

A clinical study by Vestal et al (1974) demonstrated in normal male subjects aged 21-74 years, that the dose of isoproterenol required to increase the heart rate by 25 beats/minute was increased with age. This work confirmed observations (made previously) that sensitivity to β-adrenergic receptor adenyl cyclase system decreases with age although changes in pharmacokinetics or drug distribution cannot be excluded.

Again, no quantitation of β-adrenergic receptors was performed thus the mechanism for the decreased sensitivity remains enigmatic. Giudicelli and Pecquery (1978) found that the lipolytic response of rat adipocytes to catecholamines

begins to decline before the animals reach full maturity and at a time when the number of β-adrenergic receptors and catecholamine-stimulated adenyl cyclase activity is still increasing. This suggests that decreased sensitivity to catecholamines is related to changes in one or more steps of the lipolytic pathway beyond the β-adrenergic adenyl cyclase system. The number of receptors did fall however so maybe the decrease in number could explain the sensitivity decrease.

It is not known for sure however that in catfish, during growth or due to size differences, there could be reduction in response comparable to the studies above. What is known for fish however is that during growth, they tend to deposit more fat. An increase in fat cells would indirectly suggest an increase in receptor number thus higher dosage would be required to elicit an effect.

Nutrient leaching during feeding is an inevitable event accompanying dry feeds in aquaculture. In all animal species studied, most of the products fed are mixed with any feed and fed thus. No provision was made to account for the effect of leaching of the products reducing further their concentration. This effect however is not uniform owing to possible differences of solubility of the products. The solubility of the products in water is in the order clenbuterol ≥ isoproterenol > fenoterol > salbutamol (Parfitt et al, 1989, Budavari et al, 1989).

Chronic exposure of β-adrenergic agonists to the receptors leads to desensitization (tachyphylaxis or refractoriness), which is the tendency of biological responses to wane over time despite the continuous presence of a stimulus at constant intensity. It is observed in all organisms. It could be due to a reduction in the rate of c-AMP sythesis, enhancement of c-AMP degradation and egress of c-AMP from the cell. It involves down-regulation of receptors or their sequesteration from the cell surface as well on functionally

covalent modifications of receptor and/or guanine nucleotide regulatory proteins (Lefkowitz et al, 1985; Benovic et al, 1988; Benovic et al, 1986: Sibley and Lefkowitz, Campbell et al, 1985).
The period of the experiment going by this possibility could lead to desensitization (Fiems et al, 1989; Kim 1989).

Specific cases of desensitization have been observed in human peritoneal adipose tissue - from patients undergoing elective abdominal surgery - preincubated with isoproterenol for three hours ! (Burns et al, 1982).
Strasser et al (1984) using β-agonist isoproterenol, in vivo on rat lung, observed desensitization after 5 minutes, a maximal effect after 10 minutes and a complete reversal by 2-3 hours. They observed a 40 % reduction after reversal in quantity of the receptors from plasma membrane.
Other workers have observed the same, e.g. Lefkowitz and Williams (1978) using frog erythrocytes. Whether this happened in the catfish is not clear but the ever strong lipolytic or antilipogenic effect due to isoproterenol declined with time and after 80 days of the experiment, the effect due to the products was not quite evident, except for isoproterenol. For the rest of the products, especially in the group of bigger fish, after 31 days the fat was lower than in the control, for isoproterenol-treated fish though it was not significantly different. The period of the experiment could allow tachyphylaxis to occur.

Isoproterenol's effect on protein metabolism looked more obvious than the rest for the small fish. However, after the first 17 days all had a greater amount of protein ($P < 0.05$) than the control. For the big fish, no statistically significant difference was observed. Few beta-agonists affect body-protein levels.
Reeds et al (1988) using rats with clenbuterol and salbutamol in the diets observed for the first 7 days protein accretion reducing 21 days later. Salbutamol was seen not to be as

effective as clenbuterol though: They interpreted the transient nature of the effect of clenbuterol on muscle protein increase to be due to tachyphylaxis. This explanation might help to account for the rapid increase in protein due to the products in the first 17 days because afterwards, only isoproterenol showed an increase in level of protein above the control ($P < 0.05$) on days 61 and 80 where the receptors possibly reappear in the smaller fish. The apparent lack of effect on protein metabolism in the big fish could be due to size-related increase in metabolic demands with time in which case, the effective dose required would play a role (Phillips, 1984). Nutting (1982) observed a dose-response and time dependent desensitization of amino acid and glucose uptake in rat diaphragm muscle due to catecholamines.
Reduced protein degradation due to clenbuterol and fenoterol has been observed by Emery et al (1984) in rat skeletal muscle in an experiment restricted to 15 days with highest changes of protein levels compared with the control being observed after 7 days.

Shuliman (1974), quoted by Wetherly (1976) has associated growth of fish primarily to be protein growth and muscle mass is most important. Protein growth of young fish is directed wholly to building up body proper whereas in adults, fish protein growth is largely associated with gonad development.
Two types of muscle - slow and fast have different β-receptor density. The slow muscle is known to hypertrophy due to β-agonists (Williams, 1984; Bohorov, 1987), principally because of its high receptor β-density and shows greater protein accretion. Johnston (1982) has confirmed the existence of slow muscle and fast muscle in hatchery reared fish. The amount of protein accretion seen depends on the different levels of these muscle types. This could still be true for catfish in that if it has less slow muscle type, then protein accretion or differences in protein due to β-agonists would not be so obvious.

Protein accretion in vitro has been elucidated for isoproterenol to suggest an increased uptake of alanine mediated via β-adrenergic pathway and an increased conversion of glutumate to glutamine. Changes in the amino-acid levels in the perfusion system used by Li and Jefferson (1977), after incubation of isoproterenol led them to suggest increased protein synthesis. Maybe due to the potency of isoproterenol as well, this process proceeds throughout most of the experimental period. This can of course be said assuming similar action in catfish.

6.2. Effect on Moisture

The expected relationship for moisture where these products have been used is to increase its level (Emery, 1984; Ricks et al, 1984). This however was not seen in this case constantly. The significant difference observed at day 31 for the group of smaller fish was the only positive result. The regression results were mixed for the products for protein and fat. This could suggest that they do not influence moisture in phase with fat and protein as would be expected. Normally with increased protein, moisture increases and with fat decreases. It could be hypothesised that this anomaly could be associated with lower fat levels in the muscle of catfish such that detectable effects become enigmatic.

6.3. Effect on Ash

The changes in ash content were also not significant for both groups of fish. The amount of organic matter does not change much, as confirmed by lack of change in protein levels.

6.4. Feed Utilization

A marked improvement of food conversion and feed efficiency is in smaller fish compared with the control for all the products. Almost the same is observed for big fish except for the salbutamol treated group which is lower than control. Efficiency improvements result from the energy spared when depositing muscle instead of fat. Inspite of the approximately equal amount of energy required to synthesize fat and protein (Van Es, 1977), there is a considerable net savings in energy when comparing adipose tissue versus muscle tissue due to the fact that a unit of muscle tissue contains 70 to 75 % water while an equal weight of adipose tissue contains less than 20 %. Further more, a unit of fat contains trice the calories of a unit of protein. Based on this, Dalyrumple and Ingle (1986), stated that it is no wonder that animals fed repartioning agents are more efficient at converting feed to weight gain.

Improvements in feed efficiency have been reported by Dalrymple et al (1984b) using poultry, fed clenbuterol incorporated in the diet. Jones et al (1985) in swine, Hanrahan (1986) observed an improvement as high as 30 % in cattle compared to the control.
In catfish, this also holds true although it is much more pronounced. It is not statistically significant ($P < 0.05$) for both groups of fish. Henken et al (1986), however, observed a difference in feed utilization in Clarias gariepinus due to sex. They reported that males are better converters of feed than females and they used a similar weight range in this experiment though without the products. They reported feed conversion between 1-3. Since the fish were not sexed, it would be difficult to eliminate the possibility of females dominating in the bigger fish group fed salbutamol.

Variation in products and species response to feed utilization exists. Reeds et al (1988) observed no improvement in

rats, Baker (1984) observed no improvement in steers' feed conversion though they observed both protein accretion as well as fat reduction. This discrepancy is explained by Ricks et al (1984). The central nervous system (CNS) is implicated in food intake - at the appropriate dose, the β-agonists can stimulate the CNS to influence to food intake. Peter (1979) has suggested strongly the involvement of the CNS in teleosts, especially the lateral hypothalamus in control of food intake. Whether the β-agonist stimulation of food intake can apply to catfish as well is not verified.

Ricks et al (1984) suggest rumen inhibition mobility to be another reason for reduced feed intake due to β-agonists. It is not easy to say whether this could hold for salbutamol's lack of stimulation of feed utilization in bigger fish.

6.4.1. Effect on Growth

An improvement of growth was seen in the group of smaller fish compared with the control. This difference in growth rate was statistically lower for salbutamol. The growth performance among the big fish parallelled the food conversion efficiency and thus salbutamol treated fish in the bigger category of fish were even lower than control group in growth. Alongside feed utilization, Henken et al (1986) also noted a better growth rate due to male than female Clarias gariepinus using the same weight range as in this experiment. A higher proportion of females could possibly explain the performance due to salbutamol on growth due to the big fish. But this is not adequate. Growth is multidisciplinary due to various factors involved, i.e. intersexual differences, ration eaten, digested and assimilated (Weatherly, 1976; Peter, 1979).

Overall size increase is the main constituent of growth, i.e. other organs included. Tissue or organ growth cycles can range all the way from being completely in phase, to being completely out of phase with each other.

The role of these β-agonists on growth is not clear for bigger fish and for salbutamol in smaller fish and thus coupled with the complex nature of growth in its own right, interpretation of growth effects becomes difficult.
It is not known whether they could assert their influence on growth via the endocrine system. Gundogdu et al (1979) studied the level of GH in human plasma and even observed a decrease. Choku et al (1989) observed no influence due to β-agonists on insulin levels. Mitsuyau and Gerick (1982) observed insulin suppression in rats. Emery (1984), using clenbuterol and fenoterol saw no influece on insulin, T_3 and GH and fenoterol even reduced insulin levels; and Nutting (1982) also observed no influence of the endocrine hormones. This could partly explain their failure to stimulate growth in fish in case the mechanisms, not known yet can be the same for salbutamol.

For the rest of the small fish, involvement of GH cannot be ruled out though contradictory results have been found. Welsh et al (1987) studied the direct effects of clenbuterol on growth hormone secretion in cultured bovine adenohypophyseal cells, and suggested that elevated GH secretion may, in part, be responsible for the alterations in growth elicited by β-adrenergic agonists.

Fiems et al (1989) also suggested that the repartitioning effects of β-agonists may be in part mediated indirectly through an alteration in the levels of GH in cows. Ghigo et al, 1990 however observed a blunting of growth hormone releasing hormone by salbutamol in humans. It could be argued that the other products, fenoterol, clenbuterol and isoproterenol are possibly enhancing growth in catfish via GH going by the argument and that salbutamol probably blunts release of GH thus having a less effect on growth as seen from the results. What we must not rule out is the difference between humans and fish.

The hepatosomatic index was calculated to indirectly determine any changes in levels of glycogen and lipids in the catfish liver. The effect was not seen over the weight range although the HSI decreased with time. The rate of change in time for HSI was not different from the control ($P < 0.05$), so probably their effect in the liver was already desensitized. In fact clenbuterol and fenoterol did not affect the rate as they were even higher than the control. The studies which were carried out by De Ross and De Ross (1978) could probably explain this refractoriness due to over-exposure of products to the receptors.

No changes were observed either on condition factor for catfish which is normally 1. Thus there was no difference in length-weight relationship overall for the whole experiment. Leanness of fish is determined by condition factor and if depressed, it suggest fat mobilization (Higgs et al, 1976).

From the available data in the experiment, what is clear is that conclusions based on receptor type or density cannot be obliterated. What has a positive effect, however convincingly, is the potency of isoproterenol itself over the rest of the products. It is more lipolytic or antilipogenic than the rest and is more potent in the direction of protein accretion than the rest. The effect of leaching is ruled out because isoproterenol and clenbuterol are more soluble. The effect of age may not be critical because the ranges though there, might not be vast.

7. CONCLUSIONS

The following conclusions can be derived from this study in which some repartioning agents were mixed in African catfish (Clarias gariepinus) diets to test their effects on body composition and growth.

(i) Isoproterenol, a mixed β-agonist tends to be a stronger lipolytic or antilipogenic agent on muscle fat of small catfish compared with the other products clenbuterol, salbutamol, fenoterol.

(ii) Isoproterenol still showed the greatest potency towards protein accretion on muscle protein for the small fish. The effect of other products on protein accretion was evident after 17 days but there after, their effect waned. This was probably due to the length of the experiment which allowed tachyphylaxis to occur because of chronic exposure of the receptors to the β-agonists, as is already described for higher vertebrates.

(iii) The effects of the products on the growth rates of small fish are probably due to their influence on the endocrine system. This could also be the case for the amelioration of food conversion and feed efficiency.

(iv) In the big fish, the lack of effect by these products on body composition, growth rate and feed

utilization is probably due to age related differences in response.

(v) Some of the unexpected results such as lack of significant correlation between protein, moisture and fat could be issues for further research.
Further research is also needed for the identification of the β-receptor subtype in the African catfish muscle. The most effective dosage for the above mentioned products should also be studied.

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