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PHOSPHORUS SUPPLEMENTATION IN LOW POLLUTION DIETS

Submitted in Completion of MSc Thesis in Aquaculture

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ABSTRACT

A feeding experiment was conducted to determine the need for phosphorus supplementation with inorganic P in fish meal based diets, and in the formulation of a low P diet in which fish meal was gradually replaced with maize gluten as main source of protein.

A 3×3 experimental design was used testing 9 diets: three levels of corn gluten meal (0, 19, 38 % of dietary protein), with three levels of phosphorus supplementation (0, 0.2, 0.4 %) achieved by the inclusion of different amounts (1.1, 2.2 %) of mono calcim phosphate, available P ranged from 0.43 % to 1.2 % (1.1 % to 2 % total P).

In the experiment, duplicate groups of 30 individually identified rainbow trout *Oncohynchus mykiss* with initial mean weight of 170 g ± 70 were fed *ad libitum* one of the nine test diets for a period of 9 weeks.

It is concluded that P supplementation of the fish meal based diets was not required under the given conditions. The basal level of 1.2 % met all the requirements. Maize gluten meal was a good protein feedstuff at 19 % inclusion, but appeared suboptimal at 38 %. This was partly but not completely corrected by P supplementation. A level of dietary available P of 0.43 % is not sufficient for maximum growth and feed efficiency.

Body proximate analysis revealed no diferences between diets. Digestibility results revealed that rainbow trout might only use P equivalent to requirement, and excrete excess amounts. The level of Ca in the diet is also of major importance for the reduction of the excreted phosphorus, due to is influence in P availability.

RESUMO

Esta experiência foi efectuada com intuito de avaliar a necessidade da supplementação em fósforo inorganico em dietas à base de farinha de peixe, bem como numa dieta menos poluente na qual a farinha de peixe foi gradualmente substituida por gluten de milho como principal fonte proteica.

Assim, nove dietas foram testadas utilizado-se um desing experimental 3×3, com três níveis de gluten de milho (0, 18.75% e 37.5 % como fonte proteica) e três níveis de suplementação em fósforo (0, 0.2% e 0.4 %) obtida através da incorporação na dieta de diferentes quantidades (1.1 e 2.2 %) de mono-fosfato de cálcio. Os montantes de fósforo disponível variaram entre os 0.43 % e os 1.2 % (1.1 e 2 % P total).

Foram utilizados grupos duplicados de 30 trutas arco-iris (*Oncohynchus mykiss*) individualmente identificadas com um peso médio de 170 g ± 70 aos quais foi ministrado alimento num regime ad libitum durante o periodo de 9 semanas.

Foi concluido que nas condições existentes, a suplementação em fósforo não era necessária nas dietas à base de farinha de peixe. Um nível de 1.2% (P total) na dieta satisfaz todas as necessidades. O gluten de milho revelou-se uma boa fonte proteíca a um nível de inclusão de 19 %, sendo no entanto deficiente quando perfaz 38 % da fonte proteica na dieta. A suplementação em fósforo veio melhorar esta deficiencia mas não na sua totalidade.

Verificou-se que um nível de fósforo disponível de 0.43 % é insuficiente para a obtenção the uma taxa máxima de crescimento e eficiencia alimentar.

As análises da composição corporal revelaram a inexistência de diferença entre as dietas. Os resultados obtidos para a digestibilidade demonstram que a truta arco-iris poderá utilizar apenas a quantidade de P que necessita e excretar o excesso. Verificou-se ainda que o nível de Ca e a relação Ca/P existente na dieta são igualmente de vital importância para a redução da excressão em fósforo, dado o seu efeito na disponibilidade do P para os peixes.

INDEX

Page

1 - INTRODUCTION

The global aquaculture industry has grown at a significant rate over the last two decades. During this period it has begun to be transformed from an art to a science, and even to this date this transition remains far from complete.

During the early stages of development, aquaculture was seen as nonpolluting, an environmentally non-degrading activity. However, in the 1980s, the consequences of environmental damage and manipulation become a global concern, and aquaculture became considered to be a potential polluter (DE SILVA & ANDERSON, 1995).

Self-polluting problems in aquaculture attracted early attention, particularly in cage culture, in which uneaten food and faeces accumulated beneath the cages.

The present tightening of environmentally related restrictions to the aquaculture industry make further developments of both feed and farming techniques necessary. The major developments with respect to finfish diets are likely to be directed towards minimising the environmental degradation that is brought by uneaten food and faecal material. Already in Scandinavian countries, legal restrictions have been imposed on the quality of feeds, and such restrictions are likely to become more and more stringent. For example, in Denmark, since January 1992, the phosphorus content in fish feeds is not allowed to exceed 1 % of the total dry matter of the feed, and in Finland fish feeds used in farms situated on lakes must not contain more than 0.75 % total phosphorus (KIAERSKOU, 1991)

An important aspect of feed related pollution is the leakage of phosphorus to the water, as this element often is regarded as limiting the process of algal, and phosphorus released from uneaten food and excreta will stimulate the algal growth, causing eutrophication of the environment, mainly in fresh water, where it is considered to be main limiting element (KETOLA, 1985; WIESMANN *et al.* 1988; STEFFENS, 1989; TROJANOWSKI, 1990; BERGHEIM *et al.* 1991; FOY & ROSELL, 1991a, b; BEHMER *et al.*, 1993; PHILLIPS *et al.*, 1993; KETOLA, 1994; RICHIE & BROWN, 1996; KIBRIA *et al.*, 1996).

Phosphorus is an element required by all fish for normal growth, but several studies have shown that it is included in excess in all salmonid diets (TACON & DE SILVA, 1982), a situation which inevitably results in fish farms discharging phosphorus to the environment (KETOLA, 1985; KETOLA, *et al.*, 1990; KETOLA, *et al.*, 1991; FOY & ROSELL, 1991a; PHILLIPS, *et al.*, 1993; KETOLA, 1994; KIBRIA *et al.*, 1996). The phosphorus discharge can be reduced by reducing output, e.g.

collecting waste feed and faecal matter, reducing input through improved feed efficiency or reduction of excess dietary P (KETOLA, 1985; WIESMANN *et al.*, 1988; LALL, 1991; ÅSGÅRD & SHEARER, 1996).

Dietary P requirements for optimum growth, feed utilisation and bone mineralization ranging from 0.4 to 0.8 % have been reported for rainbow trout, Atlantic salmon, chum salmon, carp, catfish and red sea bream (KETOLA, 1975; OGINO & TAKEDA, 1976; LALL & BISHOP, 1977; SAKAMOTO & YONE, 1978; LALL, 1979; WATANABE *et al.*, 1980; VIOLA *et al.*, 1986; LOVELL, 1987; STEFFENS, 1989; DOSDAT, 1992; KETOLA & RICHMOND, 1994; DE SILVA & ANDERSON, 1995; ÅSGÅRD & SHEARER, 1996; RICHIE & BROWN, 1996). It has also been reported that the phosphorus requirement does not change when anadromous salmonids gradually adapt to sea water (LALL & BISHOP, 1977). However, the amounts required are normally exceeded during formulation.

Commercial feeds have excess of total phosphorus, with a relatively high proportion in unavailable forms. The term unavailable phosphorus refers to forms of phosphorus that are not readily digested and absorbed by the fish. Preliminary research has showed that the retention of phosphorus from salmonid practical diets is often below 35 %, indicating that most of the ingested phosphorus is not retained by the fish (WIESMANN et al. 1988; HARDY & SCOTT 1991). Ketola, (1992) reported retentions of dietary phosphorus in salmon carcass ranging from 14 to 22 %, signifying that about 78-86 % of the dietary phosphorus had been discharged to the surrounding waters. The feed type could also determine the level of phosphorus loss since a much higher phosphorus loss could result if trash feed is supplied to fish compared to dry and moist feed (WARREN-HANSEN, 1982).

Therefore, a possible means of reducing the amount of dietary phosphorus discharged into the environment would be to increase the proportion retained.

The form in which phosphorus is discharged from fish farms may have a direct influence on the enrichment of the aquatic environment and the growth of algae (FOY & ROSELL, 1991b). Generally, it is excreted in soluble and particulate forms. The soluble forms, consisting of organic P and inorganic PO_4^{3-}, affect water quality directly and are normally regarded as the most important fraction affecting water quality, because this fraction is most available for phytoplancton growth, whereas the particulate form settles to the bottom of the tank or accumulates in bottom sediment, where in time, it will gradually dissolve from anoxic sediment or sludge and become available to plants and bacteria (STEFFENS, 1989; ACKEFORS & ENELL, 1990; LALL, 1991; TROJANOWSKI, 1991; PHILLIPS, *et al.*, 1993; DE SILVA & ANDERSON, 1995).

PHILLIPS *et al.* (1993) suggest that leaching from feed and faeces may contribute up to 10 % of total phosphorus loadings, with dissolved reactive phosphorus accounting for 50-60 % of total phosphorus losses from feed and 30-40 % from faeces. However, release to the environment of phosphorus from food and faeces depends on physico-chemical characteristics of the environment such as pH, temperature, oxygen, turbulence and microbial activity (PERSSON, 1988). Person (1991) estimated that the average fraction of organically bound phosphorus in feed and faeces released to the water was approximately 80 % and 60 %, respectively. It is therefore, not surprising that interest in dietary phosphorus has recently shifted focus, from concern about dietary requirement toward reducing dietary phosphorus discharge into surrounding waters.

After calcium, phosphorus is the most abundant mineral element in the tissues of bony fish. Approximately 86-88 % of the total body phosphorus is found in fish bones, teeth and scales, where it exists as calcium phosphate (mainly in the form of tricalcium phosphate (SATOH *et al.*, 1987c)), and hydroxyapatite. The remainder is found in the cells and extracellular fluids as organic phosphoric acid esters, phosphoproteins, phospholipids and inorganic phosphate ions, $H_2PO_4^-$ and HPO_4^{2-} (TARR, 1967; LOVELL, 1987, BIRD, 1991; LALL, 1991; DOSDAT, 1992; DE SILVA & ANDERSON, 1995). The phosphorus in bone tissue is used as repository for maintaining P content in cells and fluids (KOLSÄTER & KÄLVELID, 1994).

The concentration of minerals in the body of aquatic organisms depends on food source, environment, species, stage of development and physiological status of the animal (LALL, 1989). According to several authors, whole body P content of fish is approximately 0,4 to 0,5 % body weight (LALL, 1979; STEFFENS, 1989; DAVIS & GATLIN, 1991; LALL, 1991; KOLSÄTER & KÄLVELID, 1994).

Phosphorus, along with calcium, is required for the development and maintenance of the skeletal system and in several physiological processes. The vertebrae obtain their rigidity from a solid phase of calcium phosphate. Phosphate is an important constituent of nucleic acids (DNA and RNA) and cell membranes, and it is an essential factor in all energy-production cellular reactions. It plays an important role in carbohydrate, lipid and amino acid metabolism and in muscle and nervous tissue metabolism (LALL, 1989; STEFFENS, 1989; DAVIS & GATLIN, 1991; BIRD, 1991; LALL, 1991; DOSDAT, 1992; KETOLA & RICHMOND, 1994; DE SILVA & ANDERSON, 1995). Inorganic phosphates also serve as important buffers to maintain normal pH of intra and extra-cellular fluids (DAVIS & GATLIN, 1991)

Absorbed phosphorus accumulates at first in the soft tissues (liver, kidney, muscle, blood, etc.) and deposition in skeletal tissues is relatively low (LALL, 1989; LALL, 1991; DOSDAT, 1992).

The regulation of phosphate is considered more critical than that of calcium, because fish must effectively absorb, store, mobilise, and conserve phosphate in both freshwater and sea water environments. TOMIYAMA *et al.* (1956) (in LALL, 1979), estimated that 90 % of the phosphate lost from the body was excreted via the kidney. In order to replace this loss and to supply the various structural and physiological needs of the body, phosphate must be absorbed from both food and water (DAVIS & GATLIN, 1991; LALL, 1991).

Due to the low concentration of phosphorus in natural waters (0,02 mg/L HILTON, 1989), the absorption of significant amounts of phosphorus from fresh and saltwater is unlikely, making the dietary phosphorus the main source of phosphate, required for growth and metabolism (LALL, 1979; LALL, 1989; STEFFENS, 1989; DAVIS & GATLIN, 1991; LALL, 1991, DOSDAT, 1992).

The P requirements in fishes vary depending to a large extent on the structure of the digestive tract and the nature of the P source. Species with a stomach are better able to absorb poorly-soluble phosphates than agastric species. Differences in requirements might also be expected for various sizes and growth rates of fish, according to the divergent rates of development of organ systems (bones, scales, digestive glands, reproductive organs, etc.) (VIOLA *et al*, 1986; DAVIS & GATLIN, 1991; LALL, 1991; DOSDAT, 1992).

The amount of phosphate absorbed from the food is affected by the level of phosphate in the blood (LALL, 1979). The mechanism of phosphorus absorption and transport in fish has not been well studied. In higher vertebrates, intestinal phosphorus absorption is dependent on a sodium gradient caused by the active transport of sodium, i. e. transport of phosphorus from the lumen into the cells through the brush border membrane is secondary to that of sodium (DOSDAT, 1992). Nakamura (1985) reported a similar sodium-dependent absorption of inorganic (PO_4^{3-}) in carp intestine.

Bone phosphorus and bone ash have been used along with weight gain to determine the phosphorus requirements of fish (LOVEL, 1978; WILSON *et al.* 1982; ROBISON *et al.* 1987; KETOLA & RICHMONT, 1994; LI & ROBINSON, 1996).

Clinical signs of P deficiency in most fish include poor growth, poor feed efficiency and poor bone mineralization, and also increased fat contents (TACON, 1985; STEFFENS, 1989; HARDY *et al.*, 1991; LALL, 1991; KOLSÄTER & KÄLVELID, 1994). In carp, phosphorus deficiency increases the activity of certain gluconeogenic enzymes in the liver, increases carcass fat, with decrease in carcass

water content, and reduces blood phosphate levels (OGINO & TAKEDA, 1976; TAKEUCHI & NAKAZOE, 1981; TACON, 1985; LOVELL 1987; DOSDAT, 1992). In red sea bream a low phosphorus intakecauses curved, enlarged vertebra, increased serum alkaline phosphatase activity, higher lipid deposition in muscle, liver and vertebrae, and reduction in liver glycogen content (SAKAMOTO & YONE, 1980, LOVELL, 1987). On the other hand, overdoses of P (51 g/kg dry diet) substantially depressed the growth of *Oncorhynchus tshawytscha* fry (STEFFENS, 1989).

Despite the importance of P to a wide range of metabolic processes, dietary phosphorus deficiency has not been associated with high mortalities. Dietary ingredients, such as fish meal, provide the required phosphorus in most commercial salmonid feeds (HARDY *et al.*, 1991).

The bioavailability of phosphorus, defined as the fraction of the element in the diet that is absorbed from the gut when the dietary concentration is less then requirement (SHEARER, 1995), may differ markedly among feed ingredients and inorganic phosphorus supplements. Feedstuffs of animal origin contain the highest concentrations of phosphorus. Among common feedstuffs used in fish feed formulation, fish meal (1,5 to 3,2 % P), and meat and bone meal (3,5 to 5,5 % P) are the richest sources. Bone tissue contributes a significant proportion of this element to these Feedstuffs in an inorganic form (hydroxyappatite) and the remainder as phosphate complexes of protein, lipid and carbohydrate. In these forms, phosphorus is more available to fish than as plant protein supplements (LALL, 1991; DE SILVA & ANDERSON, 1995; RICHIE & BROWN, 1996).

Phosphorus in cereal grains, and vegetable protein concentrates, may range from 0,3-0,4 % and 0,5-1,4 % respectively. Plants store phosphate in seeds as phytates, i. e. salts of phytic acid (inositol hexaphosphoric acid). Phytate, which accounts for up to 70% of total phosphorus in plant protein sources, is unavailable or has very low availability in non-ruminant animals, including fish (LALL, 1991; RICHIE & BROWN, 1996). Phytin-P is apparently very-poorly utilised by fish on account of a lack of an endogenous enzyme (phytase) in the gastrointestinal tract that catalyses phytic acid to its moieties (ANDREWS *et al.* 1973; KETOLA, 1975; LALL, 1979; OGINO *et al.* 1979; LALL, 1989; LALL, 1991; RICHIE & BROWN, 1996).

Phytic acid may also form complexes with mineral elements present in feed ingredients as cations, resulting in reduced absorption of zinc, copper, calcium and iron (LALL, 1991). However, phytase is commercially available, and the addition of phytase to fish diets may improve P availability from protein Feedstuffs (BROWN, 1993).

In general bioavailability of phosphorus (and minerals in general) has been found to be positively correlated with the solubility of the mineral in water (DAVIS & GATLIN, 1991). The

phosphorus digestibility in a diet will depend on the phosphorus source. The major phosphorus source in most fish diets is fish meal, from which 40 to 60 % P is digested by salmonids. Digestion of phosphorus from alternative sources may vary from 0 % (vegetable sources) to above 80 % (inorganic phosphorus). Thus, an increase in phosphorus digestibility will reduce both the phosphorus discharge to the environment and the need for phosphorus in the diets.

Inorganic phosphates are among the products with highest levels of phosphorus availability, but there are wide differences in availability among the variety of inorganic salts: the more soluble the salt the higher the availability of P. Thus, monobasic calcium phosphate and also monosodium and monopotassium phosphates are readily utilised (90-95%) by rainbow trout (OGINO *et al.*, 1979), channel catfish (LOVEL, 1978), red seabream (SAKAMOTO & YONE, 1978) and carp (OGINO *et al.*, 1979), while dibasic and tribasic calcium phosphates, are less readily available, 71 and 64 % for rainbow trout (OGINO *et al.*, 1979). Defluorinated rock phosphate is another inorganic source of P. It has a low solubility in water and seems to be readily digested in the stomach, possibly because of the hydrochloric acid secretions that solubilize and aid in its digestion (KETOLA, 1994).

In addition to the chemical form (solubility) affecting mineral availability, several other factors including the total content in the feed, and its interaction with other nutrients may affect the amount of P biologically available to the fish (TACON, 1985; HILTON, 1989; LALL, 1989; DAVIS & GATLIN, 1991). Although the gastric stomach generally increases the availability of minerals, after solubilization in the stomach some minerals may interact and form precipitates after being released into the basic intestine. Large intakes of iron, aluminium and magnesium interfere with the absorption of phosphorus by forming insoluble phosphates (DAVIS & GATLIN, 1991). The excess amount of P in the diet (mainly tricalcium phosphate, present in fish bones) affects zinc availability leading to poor growth, eye lens cataracts and short body dwarfism (SATOH *et al.*, 1987b; SATOH *et al.*, 1987c; PORN-NGAM *et al.*, 1993; SATOH *et al.*, 1993).

It appear that calcium levels may also affect P availability, and that calcium levels in excess of 2.5 % should be avoided. Although there does not appear to be a fixed Ca:P ratio that will produce optimal results, Porn-ngam et al., (1993) suggests a ratio of 1:1 (Ca:P).

The species, the physiology and the health condition of the fish also influence deposition (HILTON, 1989; DAVIS & GATLIN, 1991; LALL, 1991; KOLSÄTER & KÄLVELID, 1994).

Although, reduction of the phosphorus content in fish diets has been suggested as a way of reducing phosphorus discharge, adjustments must be made for FCR (food conversion ratio) and phosphorus digestibility. Today, mean P digestibility in salmonid diets is approximately 50 %, and

under this condition, a reduction of P contents of the diets below a certain level may actually cause a reduction of fish growth and increase in FCR. This may be counterproductive by increasing the amount of feed spoilage (KOLSÄTER & KÄLVELID, 1994).

At present, fish meal is the most important feed ingredient in the fish feed industry, and the formulation of low P diets will depend largely on the ability to spot fish meal batches low in P. Phosphorus in fish meal exists mainly in the form of insoluble hydroxyapatite from hard tissues such as bones and scales, major contributors to the ash content of meal. Thus, to some degree, P-content can be predicted from the ash content of fish meals, and low ash content fish meal should be selected in the production of low P diets. Fig. 1 shows the correlation found for ash and P content in fish meals of different origins.

Another line of approach is directed towards finding alternative protein sources which, either alone or supplemented with appropriate amino acids, can totally or partially replace fish meal.

In commercial salmonid rations fish meal commonly varies between 25 % and 65 % by weight (mean value 40-50 %), with higher levels being used in starter and fingerling rations. On the other hand, fish feed is not only the major nutrient, but also the most expensive component in the diet. Good quality fish meals of relatively constant chemical composition have a high cost, which can sometimes amount to 40-60 % of total feed costs (TACON *et al.*, 1985; MORALES *et al.*, 1994; DE SILVA & ANDERSON, 1995).

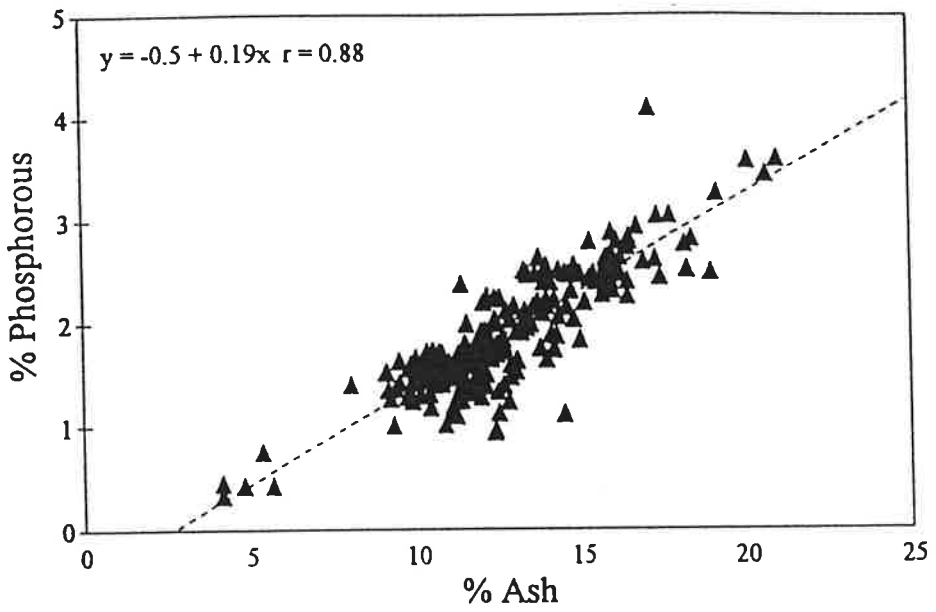

Fig.1 - Correlation between ash-content and P-content in fish meals (NUTRECO study)

Table I lists some alternative protein sources and their P contents. However, alternative protein sources have met with variable success, and have generally led to reduced feed efficiency and growth. These proteins have generally been termed secondary protein sources, and as such, are commonly incorporated at low levels in practical fish feeds (5-15 % individual inclusion level). Collectively these secondary protein sources may account for up to half the total protein in a commercial feed, with the remainder being fish meal (TACON *et al.*, 1985).

Apart from fish meal there are no animal or plant feed proteins available to the fish feed industry with an essential amino acid (EAA) profile approximating the dietary EAA requirements of farmed fish. Compared to fish meal, which has a well balanced amino acid profile, the majority of the alternative protein sources, are either deficient in a specific amino acid or suffer from an imbalance of amino acids (TACON *et al.*, 1985; DE LA HIGUERA & CARDENETE, 1987; NRC, 1993; DE SILVA & ANDERSON, 1995; GOMES & KAUSHIK, 1995). This imbalances arise from the presence of disproportionate levels of specific amino acids, and may include leucine/isoleucine antagonism, and arginine/lysine antagonism (TACON *et al.*, 1985).

However, despite this inherent amino acid imbalances some researchers have had particular success in utilising these feed proteins simply by complementing them with other protein sources, so as to obtain the required EAA profile for fish (KETOLA, 1982; TACON *et al.*, 1985; SENERICHES & CHIU, 1988).

Supplementation of amino acid deficient feed proteins with exogenous crystalline amino acids has also been used to obtain the desired EEA profile (TACON *et al.*, 1985).

In addiction to amino acid profiles that are often imbalanced, endogenous anti-nutritional factors are a major factor limiting the use of plant Feedstuffs in fish feeds at high levels. These include, protease inhibitors; phytohemaglutinins; glucosinulates; cyanogens and lathyrogens; anti-vitamin factors; anti-enzyme factors; estrogenic factors, cyclopropenoic fatty acids and gossypol; phytic acid and mycotoxins. Although these endogenous factors vary in their individual toxicity to farm animals, and differ widely in chemical complexity, many of them can be inactivated by moist heat treatment (TACON *et al.*, 1985; DE LA HIGUERA & CARDENETE, 1987; NRC, 1993).

Among the plant protein sources, soybean meal, the most tested, as been shown to possess an acceptable amino acid profile for growth of many fish species (HARDY, 1989; LOVELL, 1989; NRC, 1993; MORALES *et al.*, 1994), including salmonids, if supplemented with lysine and methionine (DE LA HIGUERA & CARDENETE, 1987; DE LA HIGUERA *et al.*, 1988)

Nevertheless, soybean meal is not a local protein source for many countries and market competition for soybean meal, as well as fish meal, has emphasised the need for evaluating new alternative protein sources.

Among these products is corn (maize) gluten meal, a by-product of the wet milling of corn. This ingredient consists of a high protein feed component (about 60 % crude protein), with protein digestibility (93-96 %) higher or similar to that of fish meal (HASTINGS, 1966; CHO *el al.*, 1982; HARDY, 1989; ANDERSON *et al.*, 1992; MORALES *et al.*, 1994), although with inferior protein quality, low lysine, tryptophan (GROPP *et al.*, 1976; FAUCONNEAU, 1988; SENERICHES & CHIU, 1988; DE SILVA & ANDERSON, 1995). isoleucine and arginine levels (SENERICHES & CHIU, 1988; ANDERSON *et al.*, 1992). Nevertheless, GROPP *et al* (1976), ALEXIS *et al.* (1985) reported that rainbow trout tolerate and utilise corn gluten meal amounting to 25 % of the dietary protein, with very good result. Morales *et al.* (1994) reported a SGR (specific growth rate) of 1.88 and a FCR of 1.05 in trout fed with a diet containing 23,4 % maize gluten and a gross energy of 20.7 (Mj/Kg). ALAVA *et al.* (1988) also found good performances for milkfish (*Chanos chanos*) using diets including 20 % maize gluten meal.

Table I - Protein and Phosphorus levels in some alternative ingredients for fish feed (Adapted from Lovell, 1989).

Ingredient	Crude Protein dry basis (%)	Total Phosphorus % dry basis
Blood Meal spray dehy.	93.0	0.26
Corn gluten meal	60.0	0.5
Peanut meal, mech. extr.	52.0	0.61
Poulty byproduct meal	62.8	1.96
Poultry feather meal, hidr.	91.3	0.72
Soybean meal, solv. extr.	49.9	0.7
Soybean concentrate	91.9	0.74
Sunflower extr.	49.8	0.98
Mineral Suplements		Available P % DB
Calcium phospate		20.3
Dicalcium phosphate		13.7
Monosodium phosphate		22.05

In addition, several other authors confirm that corn gluten is one vegetable protein source with high potential for utilisation in fish diets, because of high digestibility values (NRC, 1981; CHO *et al.*, 1982; FAUCONNEAU, 1988; CHO & KAUSHIK, 1990; ANDERSON *et al.*, 1992; GOMES *et al.*, 1995).

ANDERSON *et al.*(1992) explained this high digestibility by the wet milling process, which leads to an increase in the availability of amino acids, and by the heating of the meal that removes a large percentage of the starch and increases the digestibility of the remaining starch.

As regards the anti-nutritional factors present in corn, TACON & JACKSON (1985) refer to several, namely: protease inhibitors; phytic acid; ostrogenic factors; invertase inhibitor and possible mycotoxin (aflavotoxin) contamination. However, and as has been mentioned, many of these anti-nutritional factors can be inactivated by moist heat treatment (TACON *et al.*, 1985; DE LA HIGUERA & CARDENETE, 1987).

Therefore, the challenge for the fish feed industry is now the development of low pollution diets with good performances as regards fish growth and food conversion.

As far as reduction of phosphorus output from aquaculture is concerned, selection of fish meal and feed ingredients with low amounts of phosphorus, and the development of feed formulas containing low levels of fish meal could, together, result in a significant reduction of phosphorus output from uneaten food and excretory products. Selection of feed ingredients and of phosphorus supplements with high phosphorus bioavailability are of similar importance.

The objective of the present experiment was to study the need for phosphorus supplementation with inorganic P (monobasic calcium phosphate) in fish meal based diets, and in the formulation of a low P diet in which fish meal was gradually replaced with maize gluten as main source of protein.

2 - MATERIALS AND METHODS

2.1 - Biological Material

The fish used for this experiment were fresh water adapted rainbow trout (*Oncorhynchus mykiss*), of average weight 170 ± 70 g. These fish had hatched in May of 1994 at the NLA - KYRKSÆTER ØRA STATION, and were bought by the Lerang Research Station in July of 1994, at which time, the average weight was 15 g. Until the beginning of the trial the fish were fed on Skretting feed, the meals adjusted to water temperature.

In order to adapt the fish to the trial conditions, they were stocked in the tanks 3 weeks before the experiment. During this acclimation period the fish were fed automatically with Skretting commercial diet n° 2, at a feeding rate of ≈ 2 % body weight per day.

In order to follow individual growth, each fish was identified by a numbered tag, attached to the muscle under the dorsal fin (fig. 2). The marks were introduced with the help of the tagging device Tag -Fast® II (Dennison) after the fish had been anaesthetised with 50 mg/L Metacain.

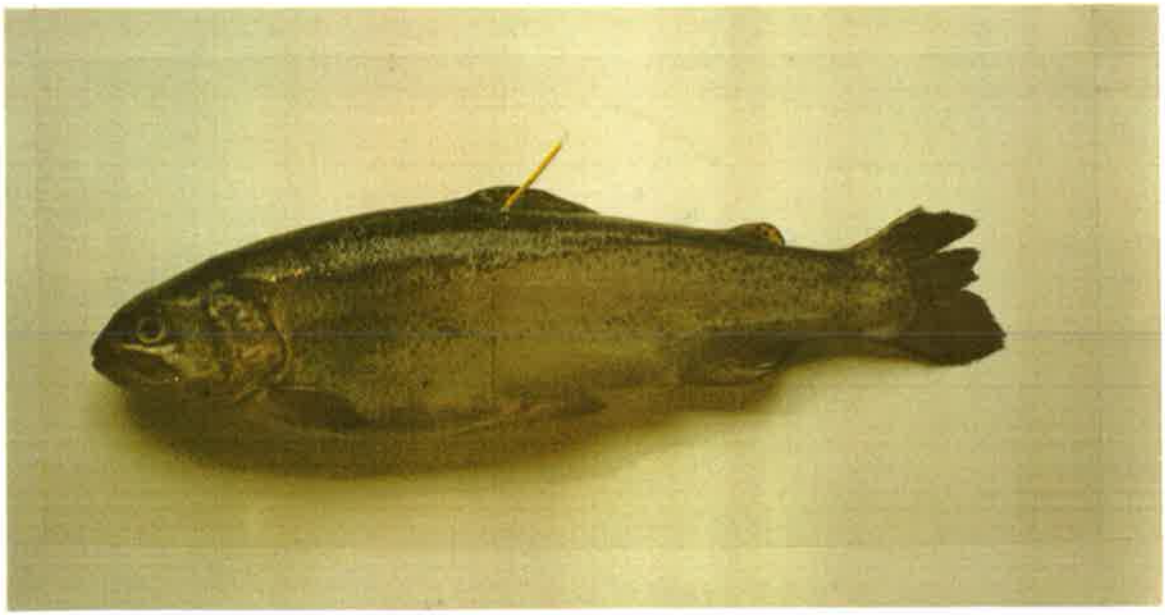

Figure 2 - Example of a tagged fish

2.2 - Trial Conditions

The experiment was carried out indoors, using fibreglass 1 square metre tanks with a capacity of 700 L (fig. 3). The water entered at the rear of each tank, via a vertical (PVC) pipe with eight evenly spaced 5 mm diameter holes orientated to produce clockwise flow, and exited through

a central bottom drain, 4 cm in diameter, covered by a metal net with 2 cm drain holes. A 5 cm diameter outside standpipe attached to a flexible rubber pipe allowed the control of water depth and also the exchange rate in each tank (fig. 3). At the end of the outlet system an 1 mm sieve was placed, to collect the uneaten food. Water flow per tank averaged 20 l/min, using a flow through system.

Duplicate groups of 30 fish were randomly assigned with the nine experimental diets, for a period of 9 weeks (Tab. II).

During the trial the fish were exposed to a photoperiod of 24 hours light, provided by a 75 W lamp (Philips-Energy saver- PL*Electronic/c), located 50 cm above each tank. The water temperature was not controlled, and changed according to the environmental temperature. During the trial the temperature ranged from 12.2° to 17.8 °C, with monthly means of 13.7°, 16° and 17.3 °C, with the sum of daydegrees during 61 days being 952,4 °C.

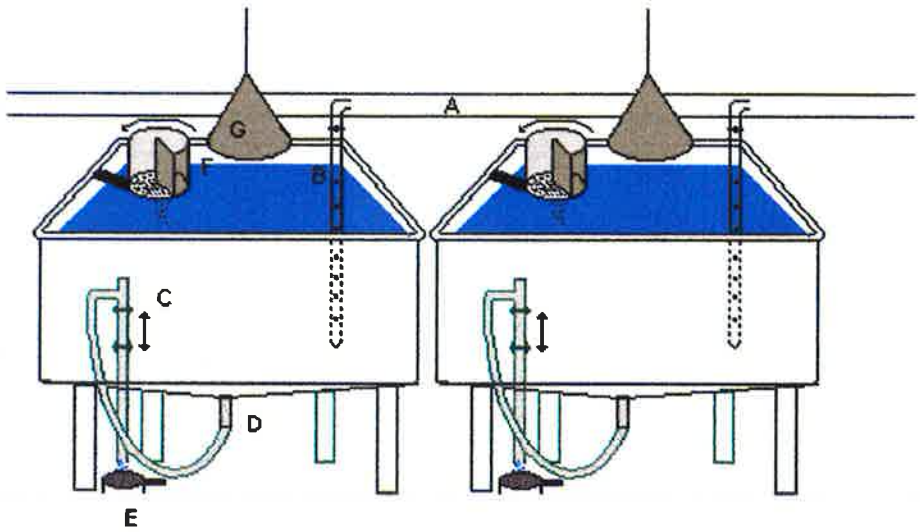

Figure 3 - Squematic representation of the tanks used in the trial. A- main distribution pipe; B - tank distribution pipe; C - outlet water control system; D - bottom drain pipe; E - 1 mm sieve; F - automatic feeder; G - Illumination.

Table II - Technical data of the dietary trial

Nº of Diets		9
Feeding regime		to satiation
Groups per diet		2
Trout per group		30
Initial live weight (g/trout)		170 ± 70 g
Experimental days		63
Feeding days		60
Water temperature (°C) mean		15,67
	range	12,2-17,8
O2 (mg/l)		> 7

The DO level was monitored every day with an Oxygen meter (Oxyguard), and when levels decreased to values near 7 ppm, the water was oxygenated by introducing air into the reservoir tank, or in most extreme cases (DO< 7) directly into the tanks.

2.3 - Feed and Feeding

A 3×3 experimental design was used testing 9 diets: three levels of corn gluten meal (0, 18.75, 38,5 % of dietary protein), with three levels of phosphorus supplementation (0, 0.2, 0.4 %) (Tab. III). The P supplementation was made with the inclusion of different amounts (1.1, 2.2 %) of monobasic calcium phosphate $Ca(H_2PO_4)2$.

Table III - Experimental design for the study: P-Supplementation; Maize Gluten inclusion.

	Phosphorus supplementation		
Maize gluten inclusion	0%	0,2 %	0,4 %
0%	Diet A	Diet B	Diet C
20%	Diet D	Diet E	Diet F
40%	Diet G	Diet H	Diet I

The diet formulations presented in Table IV, were produced by Skretting AS, in the form of 4 mm extruded pellets.

During the trial the fish were fed in excess *(ad libitum)* by a combination of the automatic feeders, and hand feeding twice a day (7.00 A.M. and 15.00 P.M.). The outlet of each tank was screened (with a 1 mm sieve) to collect uneaten feed. This uneaten food was collected every day after the automates were empty, and weighed after dried in a drier (WTB-Binder) at 105 °C for at least 20 h. The amount of food to be given every day, was calculated according to the amount of food not eaten collected two days before. Overfeeding was at least 15 % to assure that all fish were satiated.

2.4 - Sampling

2.4.1 - Growth

The sampling for growth measurements was performed every third week, during a total of nine weeks. One day before the sampling the fish were starved, in order to empty their stomach. During the sampling procedure the fish were anaesthetised in two groups, in a bucket with 10 l of water containing 50 mg/l Metacain. When the fish were anaesthetised their length was measured to

the mm, with an ictiometer, and weight was measured to the nearest gram with a balance (Mettler Toledo PB3001). After the measurements the fish were returned to the tanks, and only fed in the day after.

Table IV - Diet code and composition

	Diet code %								
Ingredients	A	B	C	D	E	F	G	H	I
LT fish meal	56,50	56,50	56,50	41,00	41,00	41,00	26,00	26,00	26,00
Bloodmeal	5,00	5,00	5,00	5,00	5,00	5,00	5,00	5,00	5,00
Maize gluten	0,00	0,00	0,00	18,75	18,75	18,75	37,50	37,50	37,50
Capelin oil	19,00	19,00	19,00	20,00	20,00	20,00	21,00	21,00	21,00
Wheat	15,42	14,32	13,18	11,17	10,07	8,93	6,42	5,32	4,18
Premix	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Ca(H2PO4)2	0,00	1,10	2,20	0,00	1,10	2,20	0,00	1,10	2,20
Edelbind	3,00	3,00	3,00	3,00	3,00	3,00	3,00	3,00	3,00
Carophyll Pink	0,0345	0,0345	0,0690	0,0345	0,0345	0,0690	0,0345	0,0345	0,0690
Rovimix stay-C (25%)	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Yttrium oxide	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Total	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00

CALCULATED CHEMICAL COMPOSITION

Diet code	A	B	C	D	E	F	G	H	I
Moisture	6,31	6,17	6,02	6,36	6,22	6,07	6,38	6,24	6,09
Protein	46,27	46,15	46,02	46,05	45,93	45,80	46,13	46,01	45,88
Fat	23,87	23,84	23,82	23,88	23,86	23,83	23,92	23,90	23,88
Ash	6,65	7,73	8,80	5,25	6,33	7,41	3,90	4,98	6,06
Carbohydrate (by difference)	16,91	16,11	15,33	18,46	17,67	16,89	19,67	18,88	18,10
Gross energy Kcal/g diet[a]	5,56	5,52	5,48	5,62	5,57	5,53	5,67	5,63	5,59
Available Phosphorus[b]	0,60	0,80	1,00	0,46	0,66	0,86	0,33	0,53	0,72
Total Phosphorus[b]	1,20	1,42	1,64	0,97	1,19	1,41	0,75	0,97	1,19

[a] Based on 5.64 Kcal/g protein; 9.44 Kcal/g fat and 4.11 Kcal/g carbohydrate (NRC, 1993)
[b] calculation was made based on the following parameters:
Fish meal 2.4 % P (analysed) with 50% digestibility (LOVELL, 1989)
Maize gluten - 0.27 % P (analysed) - 25% digestibility (LOVELL, 1989)
Blood meal - 0.3% - 50 % digestibility (LOVELL, 1989)
CaP - 20% - 90 % digestibility (LOVELL, 1989)

2.4.2 - Faeces

The collection of faeces for the digestibility analyses took place 4 and 8 days after the last growth sample, to achieve a minimum of 35 g per tank. The method used for this procedure was the stripping method that consists in applying a slight pressure upon the ventral side between the ventral fin and the anus.

After collection, 1,5-1,7 ml deluded HCl (1:10) was added every day to the tray with the faeces to avoid loss of ammonia. At the end of the collection period the aluminium boxes containing the faeces were weighed, closed and deep-frozen at -20 °C, until analysis.

2.4.3 - Fish Sampling

At the end of the trial two different batches of five and four fish were randomly selected for whole-body proximate analysis and whole-body mineral analysis, respectively. All the fish were killed with an excess of metacain. After death, the fish were vacuum packed and stored at - 25 °C, until the analyses.

2.5 - Sample preparation and Analyses

Proximate analyses to determine protein, lipids, ash, and moisture of fish and feed, were conducted using the conventional procedures of the ARC laboratory, awarded with the ISO 9001 quality certificate.

The whole-body mineral analyses were performed at the National Marine Fisheries Service, Seattle, USA.

2.5.1 - Grinding

Before the performance of the analysis and in order to get homogeneous samples, all fish and feed samples had to be grinded.

2.5.1.1 - Fish - The fish for mineral analyses were ground individually while frozen, as whole fish samples, first in a mincer (Sirman Mod 22) and then in a food processor (Philips robot compact HR 2892) for 30 sec at high speed. Fish for body composition analyses were ground as pooled samples in the mincer and then in a meat mill (Robot Coupe) for 30 sec. After drying, the fish had to be ground in a mortar because of the high fat content. The grinding with the mortar was not very effective, also because of the fat content.*

* Footnote - To achieve a better grinding of high fat samples in the future, and when homogeneity is of main importance, we should freeze-dry the samples or store them at -80 °C before grinding, to enable the use of the coffee grinder.

2.5.1.2 - Feed - Before grinding, the samples were stored at -20 °C. For the grinding process, first about 100 g of frozen sample was ground in a Waring Blender for 30 sec (high speed), and manually homogenised. Afterwards, about 35 g of the ground sample were transferred to a coffee grinder and ground for 5-6 sec., then stored in a re-sellable plastic bag.

2.5.1.3 - Faeces - After freeze-drying, the samples were ground for 20-30 sec. in the coffee grinder, and then sieved through 1,0 mm sieve to remove fish scales from the faeces. After sieving, the faeces were stored in a plastic bottle with screw cap until analyses.

2.5.2 - Moisture

For determination the water content in the samples, about 10 g of the sample was weighed on a pre-weighed petri dish, and dried in an oven (TERMAKS) at 103 °C for 16 h, after which was cooled in a dessicator and re-weighed. The water content in the sample was calculated from the loss of sample weight. No more than 0,5 % difference was accepted between replicate samples.

$$\% \text{ water} = \frac{w2 - w3}{w2 - w1} \times 100$$

w1 = weight of empty dish

w2 = weight of dish + sample before drying

w3 = weight of dish + sample after drying

2.5.3 - Ash

The ash content of the samples was determined by combusting a known weight (fish 2,5 g ± 0,05; faeces 1 g ± 0,05; feed 2 g ± 0,05) contained in a small glass vial, in an oven (Nabertherm) at 540 °C for 16 h. The amount of ash as a % of dry weight was calculated by subtraction.

$$\% \text{ water} = \frac{w2 - w3}{w2 - w1} \times 100$$

w1 = weight of empty vial

w2 = weight of vial + sample before combustion

w3 = weight of vial + sample after combustion

2.5.4 - Protein

The method used to determine the protein content in the samples, was the Kjeldahl method, in which, crude protein is determine as Kjeldahl-nitrogen, multiplied by a protein factor, 6.25. After digestion in sulphuric acid, released ammonium is determined. Distillation and titration, after adding excess of NaOH, was done in Kjeltec Auto, Tecator, 1030 analyser. The difference between two replicates should not exceed 0,7 %.

2.5.5 - Fat

The method used for fat analyses was the Soxtec-method which is a modified version of the Soxhlet-method. In this method the sample is ground and the fat is extracted with dichloromethane during heating. After which, the dichloromethane is evaporated and the fat content determined by weighing. These analyses were performed in a Soxtec System 1040 extraction unit and Soxtec System 1041 service unit (water bath), Tecator. The difference between two replicates should not exceed 0,7 %.

2.5.6 - Digestibility

The method used to evaluate the in vivo fish digestibility of fish feed involved the use of a marker, in this case yttrium oxide, added to the feed during manufacturing (0.01%). The apparent digestibility coefficient (ADC) was measured by determining the proportions of marker and nutrients in feed and faeces, followed by calculations of how much nutrients were retained, or digested, by the fish, using the equations:

$$\% \text{ ADC} = 100 - (100 \times (\frac{\% \text{ Yttrium in diet}}{\% \text{ Yttrium in faeces}}) \times (\frac{\% \text{ Nutrient in faeces}}{\% \text{ Nutrient in diet}}))$$

2.5.7 - Minerals

A maximum of 1.5 g sample of dried whole fish was used for elemental analysis. After dry weight was determined, the samples were charred and then incinerated at 550 °C during 16 h. The ash was then dissolved in a mixture of equal parts of concentrated HCl and HNO_3 and appropriately diluted with deionized water so that elemental concentrations remained within the analytical capabilities of the measuring instruments, an inductively coupled argon plasma emission spectrophotometer (Jarrell-Ash AtomComp, Fisher Scientific, Waltham, MA).

Elements selected for analysis included one nonessential element, Sr, five major elements Ca, K, Mg, Na and P, and five trace elements Cr, Cu, Fe, Mn, and Zn. The Y content was also analysed for the mineral digestibility calculations All concentration are reported as milligrams per gram wet weight.

2.6 -Data calculation

FCR (Feed conversion ratio) = weight gain (g) wet weight/ feed intake (g) dry weight

SGR (Specific growth rate) = (ln final weight - ln initial weight) / n° of days

Feed Efficiency = feed intake (g) dry weight / weight gain (g) wet weight

Condition Factor (k) = (weight/lenght^3)*100

GF3 (Growth Factor 3) =[(Final weight $^{1/3}$ - start weight $^{1/3}$) × 1000] / Total sum temperature

The Growth Factor (GF3) is one better alternative to calculate and estimate growth more accurrently, developed by Akvaforsk and Nutreco ARC.

So far, has been used the term specific growth rate (SGR), where daily growth is calculated as a percentage of fish weight (% growth /day). However it is rather difficult use recorded daily growth rates in one period to make prognoses for future growth without considering the size of the fish or the temperature (HOLMEFJORD *et al*, 1995a).

One alternative to calculating daily growth rate with varying temperatures is to look at the growth directly in relation to the temperature sum (expressed in day degrees). Several models have been presented to account for fish size, WAMA & TAUTZ (1981), CHO (1992), and using these HOLMEFJORD *et al* (1995) established the formula for a growth factor, which they have termed GF3.

GF3 represents the product of several factors affecting growth. This factors can be divided into 3 main groups:

GF3 = Growth potential × feeding × environment

The effect of reducing one of these factors by, for example, 15 % is that GF3 will also be reduced by 15 % (HOLMEFJORD *et al*, 1995b).

In ours calculations, weights are in grams and temperature sum in day degrees, i.e. °C× total days. The spreadsheet formula for estimated new weight according to a given total around the clock temperature is:

Final weight = (Start weight$^{1/3}$ + GF3 × daily temp. total ÷1000)^3

2.7 - Statistics

Analysis of variance (two-way ANOVA) and Duncan`s multiple range were used to evaluate the treatment effects on growth (SGR, GF3, weight gain) and whole body P. Differences were considered significant at $P < 0.05$. Tests of multiple linear regression were applied when appropriate. Some correlation analysis were also performed. The statistical analyses were performed in UNISTAT and Excel 5.

3- RESULTS

3.1 - Feed

The analyses performed for proximate composition of the different experimental diets (tab V) revealed some differences between the expected during diet formulation (tab. III), and the true values.

In fact, the diets with highest levels of maize gluten (G, H, I) showed clear differences from all the others. Their fat content was 2-3 % lower than proposed, and the values for protein were approximately 4 % higher than the other diets.

It was also clear that the ash % of the different diets followed their mineral content, being higher in diets with higher mineral contents.

Table V - Proximate analyses of experimental diets.

Diet	Moisture % Dry Matter	Protein % Dry Matter	Fat % Dry Matter	Ash % Dry Matter
A	4,9	49,8	24,9	9,4
B	5,8	50,1	24	10,6
C	5,7	49,3	24,6	11,7
D	6,9	51,5	24,2	7,9
E	6,4	50,2	24,3	8,5
F	6,2	50,8	24,3	9,5
G	7,9	54,6	20,9	5,6
H	7,8	53,8	21,3	6,5
I	7,4	54,3	22,1	7,5

The analyses performed for mineral contents in the diets (Tab. VI), revealed that all mineral requirement were satisfied by the diets.

P supplementation was accurate, but overall values were higher (± 0.4 % total P) than expected, reducing the possibilities of investigating P deficiency problems.

Diets containing higher percentage of fish meal were the ones with higher mineral contents, especially in Ca, Mg, K and Na, not considering the P due to the supplementation. The Ca:P ratio ranged from 1.6 in diets with higher levels of fish meal (A, B, C), 1.5 in diets D, E, F (19 % maize gluten) and 1.3 for the diets with less amounts of fish meal (G, H, I).

Regarding the composition of main raw material the analyses performed showed a higher content in protein, fat and ash than the expected for fish meal. It was also found that P values for

fish meal (Tab. VII) were 0.3 % higher that the estimated during diet preparation. The maize gluten meal on the contrary showed P levels lower than expected (0.5 mg/g).

Table VI - Mineral composition of experimental diets, expressed in mg/g dry weight

Diet	Ca	P	Zn	Mg	K	Fe	Na	Cr	Cu	Mn	Sr	Y
A	25,75	15,99	0,28	2,24	6,62	0,36	9,84	0,008	0,005	0,0423	0,053	0,1046
B	30,24	18,95	0,28	2,34	6,68	0,44	9,71	0,0143	0,0054	0,0465	0,057	0,1052
C	32,45	20,65	0,26	2,28	6,36	0,52	9,42	0,0302	0,0055	0,0492	0,057	0,1012
D	20,11	13,20	0,25	1,78	4,64	0,42	7,31	0,021	0,0062	0,0304	0,040	0,103
E	23,93	15,76	0,26	1,87	4,20	0,43	8,20	0,0192	0,0064	0,043	0,043	0,1019
F	27,31	17,96	0,26	1,86	4,71	0,44	7,66	0,0192	0,0069	0,0469	0,044	0,103
G	14,70	11,00	0,26	1,40	3,55	0,33	6,19	0,0059	0,0079	0,0377	0,029	0,1122
H	17,00	12,54	0,25	1,36	3,25	0,35	5,83	0,0072	0,0079	0,0396	0,029	0,1077
I	21,43	15,30	0,26	1,41	3,23	0,38	5,77	0,008	0,0082	0,045	0,0314	0,108

Table VII - Chemical composition of main raw materials used in the diets.

Raw material	Protein %	Fat %	Moisture %	Ash %	P %
Fish meal	69,3	9,4	8,1	14,3	2,4
Maize gluten	61,4	5,2	11,9	1,2	0,27

3.2 - Growth

Statistical testing on the results obtained for 456 of the 540 fish tested on trial (84 lost their identification) (Tab. A, Annex 1), showed that after 9 weeks the different P contents of the diets had no effect ($P \leq 0.05$) on the growth differences between each diets (Tab. VIII, IX).

The fish fed on the diet containing the lowest available P (diet G), exhibited the lowest performance in growth after 9 weeks, while diet A revealed the best results (table VIII, IX).

Table VIII - Mean weight gain and percentage of growth during the trial period (9 weeks).

Diet	Maize gluten %	P. Supl. %	Mean initial weight (g)	Mean final weight (g)	Weight gain (g)	Growth %
A	0	0	165,3	476,65	311,4	188,4
B	0	0,2	172,25	488,4	316,2	183,5
C	0	0,4	171,3	473,95	302,7	176,7
D	18,75	0	178,7	476,85	298,2	166,8
E	18,75	0,2	172	462,4	290,4	168,8
F	18,75	0,4	180,3	466,4	286,1	158,7
G	37,5	0	177,45	389,45	212,0	119,5
H	37,5	0,2	185,9	447	261,1	140,5
I	37,5	0,4	173,95	414,65	240,7	138,4

Regarding the different levels of maize gluten and is affect on the fish growth after the 9 weeks experiment, the statistical test performed (tab. X) showed a significant ($P<0.05$) reduction in

the growth of fish fed diets with higher inclusion of maize gluten (37.5 %) comparing to the other levels of inclusion (0; 18.75).

Table IX - Mean growth performance, feed efficiency, feed intake and condition index of trout fed the experimental diets.

Diet	M. G %	P Suppl. . %	SGR	GF3	FCR	Feed intake % bw/day	Feed Ef.	Cond. I.
Diet A	0	0	1,85	2,485	0,95	1,61	1,12	1,54
Diet B	0	0,2	1,85	2,475	0,95	1,58	1,11	1,55
Diet C	0	0,4	1,8	2,395	0,95	1,61	1,08	1,52
Diet D	18,75	0	1,75	2,335	0,95	1,49	1,14	1,51
Diet E	18,75	0,2	1,75	2,325	0,95	1,56	1,09	1,53
Diet F	18,75	0,4	1,65	2,25	0,95	1,50	1,09	1,53
Diet G	37,5	0	1,4	1,795	1,10	1,42	0,98	1,43
Diet H	37,5	0,2	1,55	2,075	1,00	1,41	1,09	1,51
Diet I	37,5	0,4	1,55	2,009	1,05	1,51	1,01	1,47

In fact, fish fed diets with 0 % maize gluten had the highest growth expressed in SGR, GF3 and weight gain, and also feed consumption and feed efficiency, although it was not significantly different (Duncan range test) from fish fed diets with 18.75 % maize gluten. The results on FCR and condition index were very similar between this two levels of maize gluten.

Maize gluten incorporated at a level of 37.5 % revealed a significant influence in growth comparing to all the other diets, showing the poorest performance. It was clear that this effect increased with the feeding period (Fig. 4). After 3 week feeding all diets showed similar results, but with time the differences between diets started to increased. The growth expressed in GF3, SGR or weight gain was directly related to the feed intake, and both parameters showed parallel behaviour.

In diets G, H, I, (37.5 % maize gluten) fish fed P supplemented diets (H, I), revealed slightly better results than diet G with no supplementation.

The fat content of these 3 diets was also lower than all the others and had some effect on the growth performance of this diets (Fig. 5).

The protein digestibility coefficient was lower for the diets containing 0 % maize gluten and increased with the level of inclusion of maize. The opposite was the case for the fat digestibility coefficient (Tab. XI).

The apparent digestibility coefficient (ADC) for phosphorus revealed to be highly correlated with the amount of P available in the diet, although the standard deviation between duplicates of some diets (B, F, H, I) was too high. However, the diets with lower levels of P were the ones who showed higher ADC.

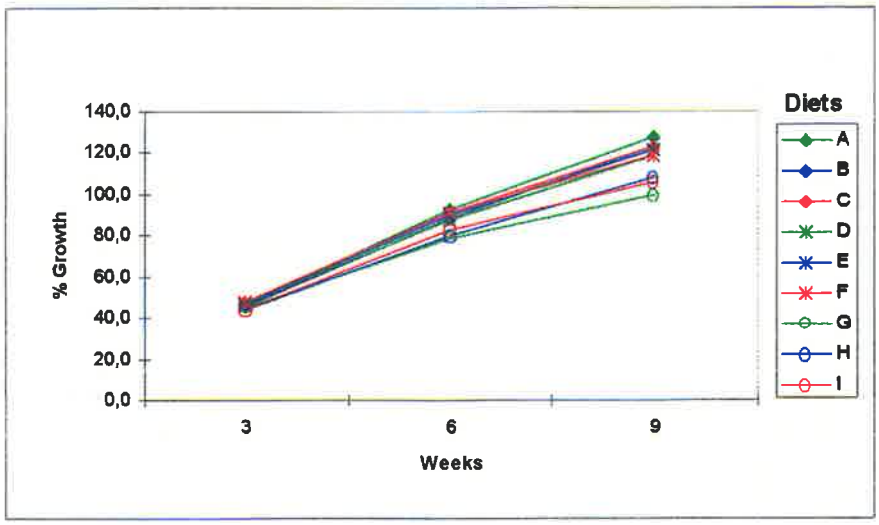

Figure 4 - Weight gain of the fish during the 9 weeks trial.

Table X - Results of the Duncan Multiple Range Test for the Maize gluten content and GF3

Dependent variable: GF3

Method:95% Duncan interval.
Table Ranges: 3.01 3.16
* denotes significantly different pairs. Vertical bars show homogeneous subsets.

group	cases	mean	37.	18.	0
37.5	6	1.95833333333		*	*
18.75	6	2.30333333333	*		
0	6	2.45166666667	*		

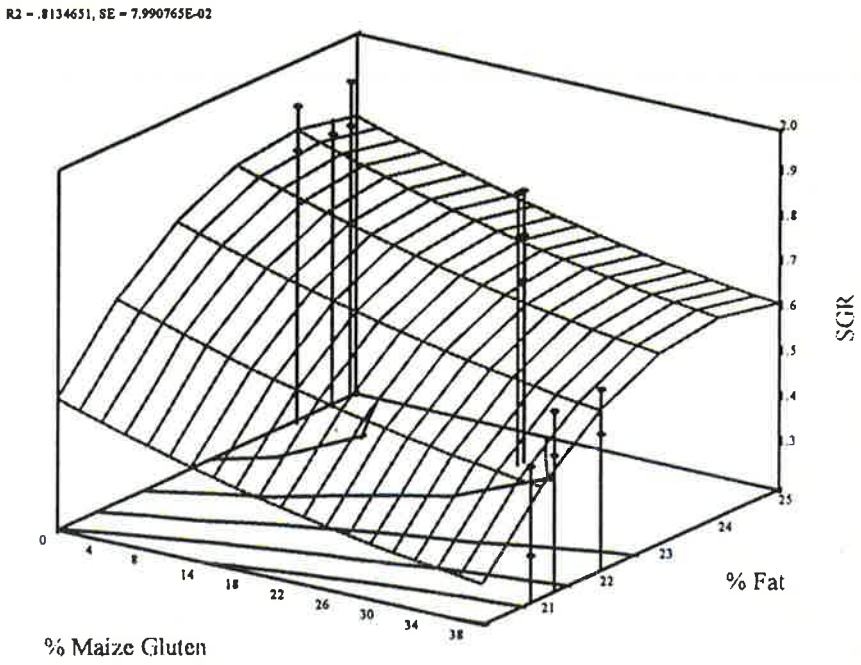

Figure 5 - Effect of maize gluten and fat content in the diet, on GF3.

Table XI - Digestibility results for the different experimental diets.

Diet ID.	M.G. % in Diet	P. Supl. %	Yttrium % DM	DC Protein	St. dev DC Protein	DC Fat	St. dev DC Fat	DC P	St. dev DC P.
Diet A	0	0	0,00935	91,6	0,5	95,6	0,1	32,6	0,8
Diet B	0	0,2	0,0091	91,6	0,5	95,2	0,4	28,7	2,4
Diet C	0	0,4	0,00927	91,7	0,1	95,7	0,6	27,5	0,1
Diet D	18,75	0	0,00968	92,7	0,4	95,2	0,3	36,1	0,9
Diet E	18,75	0,2	0,00934	92,9	0,1	95,3	0,4	37,0	0,5
Diet F	18,75	0,4	0,00949	92,3	0,1	94,7	0,2	30,7	2,2
Diet G	37,5	0	0,0102	93,7	0,1	94,3	0,8	48,6	0,5
Diet H	37,5	0,2	0,0101	94,1	0,0	93,4	1,1	44,0	5,5
Diet I	37,5	0,4	0,0103	93,3	0,8	92,6	0,2	37,5	2,0

3.3 - Body composition

As we can see from Tab. XII and Fig. 6, the results of the proximate body composition analyses from the fish fed the different diets revealed no significant differences. The levels of moisture in the whole body ranged from 63.5 to 66.8, and tended to increase in fish fed diets with higher percentage maize gluten. The opposite was seen for whole body fat content, where fish fed on this diets showed the lower percentage, with values ranging from 12.7 to 16.15 %. The results for whole body ash content were very similar, and were not correlated with the increasing ash contents of the diets fed to the fish. The values found for protein were also very similar ranging from 16.95 to 17.6 %.

However, the whole body analyses of the fish sample taken before the beginning of the trial showed a quite lower fat content compared with the fish fed the experimental diets, revealing a big increase in terms of fat during the trial period.

Table XII - Body composition of the fish fed the different tested diets.

Diet	% M.G.	P. Supl. %	Moisture % Ww	Ash % Ww	Protein % Ww	Fat % Ww
Diet A	0	0	64,5	1,9	17,1	15,65
Diet B	0	0,2	64,9	1,95	17,15	15,3
Diet C	0	0,4	64,35	2,4	17,5	15,6
Diet D	18,75	0	63,5	1,95	17,5	16,15
Diet E	18,75	0,2	65,1	2,15	17,5	14,95
Diet F	18,75	0,4	64,8	2,1	17,6	14,55
Diet G	37,5	0	66,8	2,1	17,5	12,7
Diet H	37,5	0,2	65,05	2,25	17,25	14,45
Diet I	37,5	0,4	65,95	2,2	16,95	14,1
Beginning			70.5	2.4	17	9.6

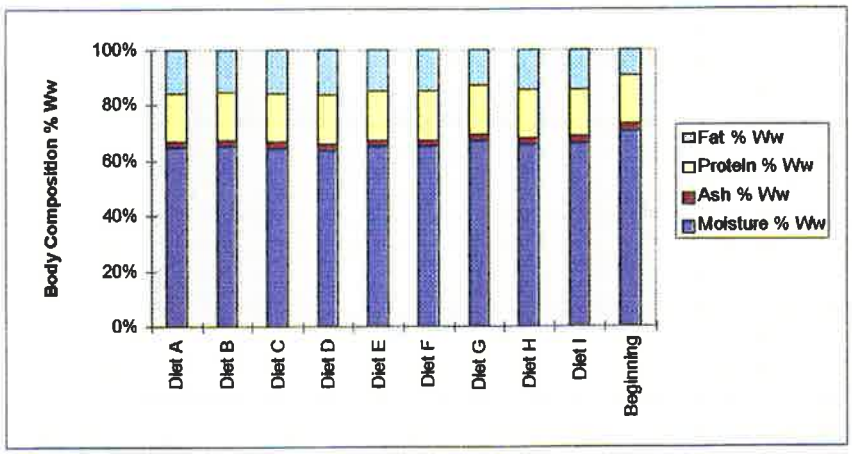

Figure 6 - Body composition of fish fed the different tested diets.

3.4 - Minerals

Mean mineral whole body compositions of the analysed fish are shown in Table XIII. The results obtained after the feeding experiment revealed that the Ca and P contents ranged from 3.72 to 4.75 and 3.32 to 4.3 mg/g respectively, with a Ca:P ratio close to 1 (1.05-1.18).

Regarding the other elements, Zinc and Manganese concentrations were very similar between diets, but showed a slight increase with increasing levels of maize gluten. The same was the case for Strontium and Sodium. Iron, copper and magnesium showed relatively constant levels in all diets.

The elemental composition of fish taken before the beginning of the dietary trial showed us higher concentration in all elements, specially for calcium and phosphorus.

Table XIII - Whole body mineral composition (mg/g wet weight).

Diet	Ca	P	Zn	Mg	K	Fe	Na	Cr	Cu	Mn	Sr	Y
Beginning	5,88	6,16	0,053	0,5	5,02	0,033	1,34	0,0003	0,0008	0,0016	0,017	0,0001
A	3,83	3,44	0,030	0,29	2,46	0,013	0,79	0,00020	0,0006	0,0012	0,010	0,00036
B	4,65	3,94	0,031	0,30	2,52	0,016	0,81	0,00023	0,0006	0,0013	0,012	0,00033
C	3,71	3,32	0,027	0,27	2,38	0,001	0,71	0,00018	0,0007	0,0012	0,010	0,00029
D	3,96	3,48	0,030	0,29	2,52	0,015	0,86	0,00024	0,0007	0,0013	0,011	0,00040
E	4,16	3,96	0,033	0,33	3,03	0,013	0,83	0,00021	0,0007	0,0013	0,013	0,00030
F	3,72	3,44	0,029	0,28	2,35	0,014	0,75	0,00019	0,0006	0,0013	0,012	0,00021
G	4,66	4,30	0,034	0,34	2,88	0,014	0,91	0,00020	0,0006	0,0012	0,015	0,00033
H	4,12	3,84	0,033	0,31	2,60	0,015	0,87	0,00020	0,0007	0,0015	0,014	0,00046
I	4,40	3,86	0,034	0,32	2,64	0,016	0,91	0,00019	0,0007	0,0013	0,014	0,00026

The ANOVA performed with the whole body mineral analyses of the 67 analysed fish (Tab. B, Annex 2), showed that the level of dietary phosphorus had no significant effect ($P>0.05$) on the phosphorus and calcium content of fish, but the level of maize gluten did. The results were similar between diets, although the variability in duplicates was high (fig. 7).

Nevertheless, and unexpectedly, the analyses revealed that diets with less available P were the ones that showed higher levels of body P (fig. 8), with the fish fed the diet with lower P content (diet G) showing the highest total body P content.

A negative correlation existed between the dietary phosphorus concentration supplied and the P digestibility coefficient (P DC) (fig. 9). That relationship was best described by the equation:

$Y = 68.58 - 2.0838(X)$, $r = 0.9335$

where Y is P DC coefficient and X is dietary phosphorus concentration.

A similar negative relationship existed for the amount of calcium in the diets and the P DC (fig. 10), best described by the equation:

$Y = 62.7579 - 0.0011(X)$, $r = 0.96165$

where Y is P DC and X is dietary Ca. Other potential mineral interactions were less clear.

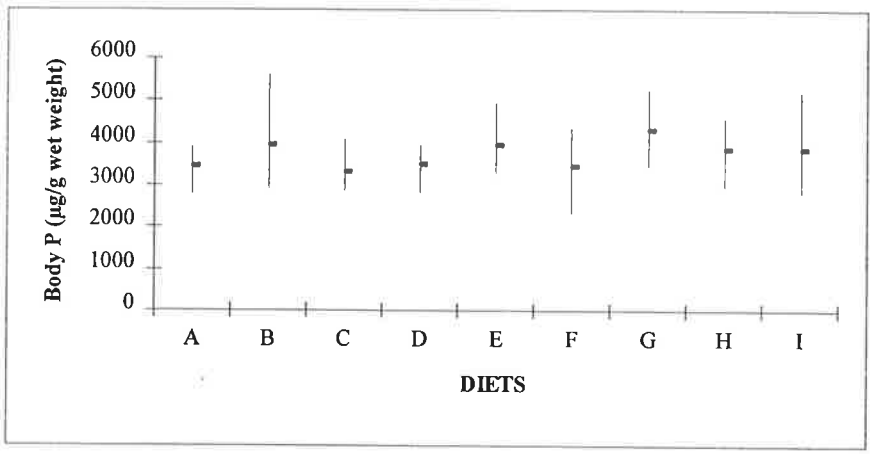

Figure 7 - Mean and variance of total body P content of fish fed the different diets.

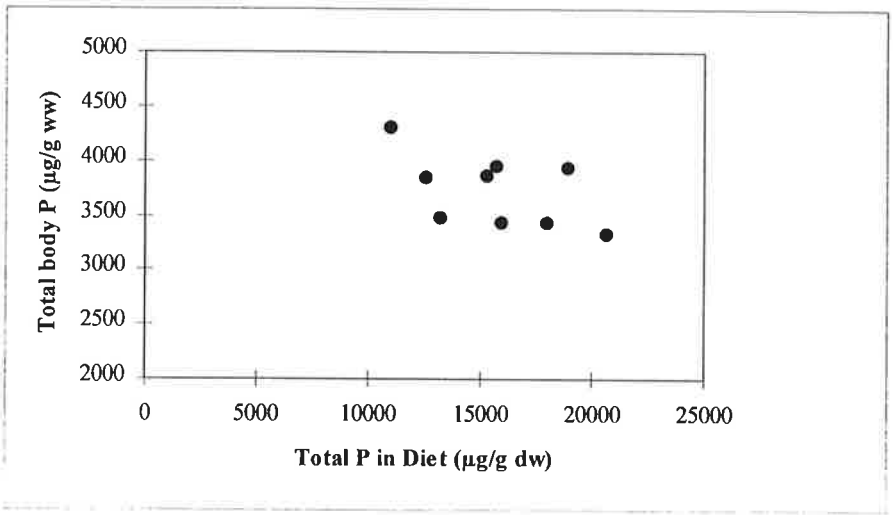

Figure 8 - Relation ship between total P and Body P.

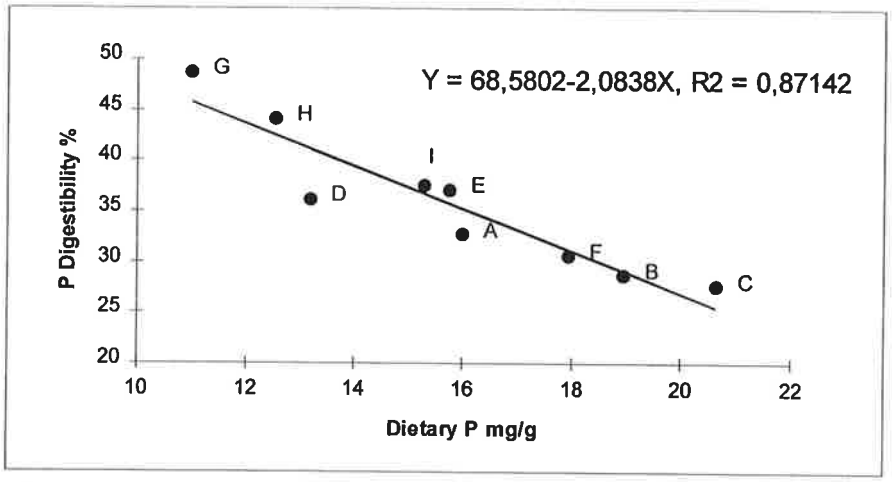

Figure 9 - Relation ship between available P in feed and total body P in fish.

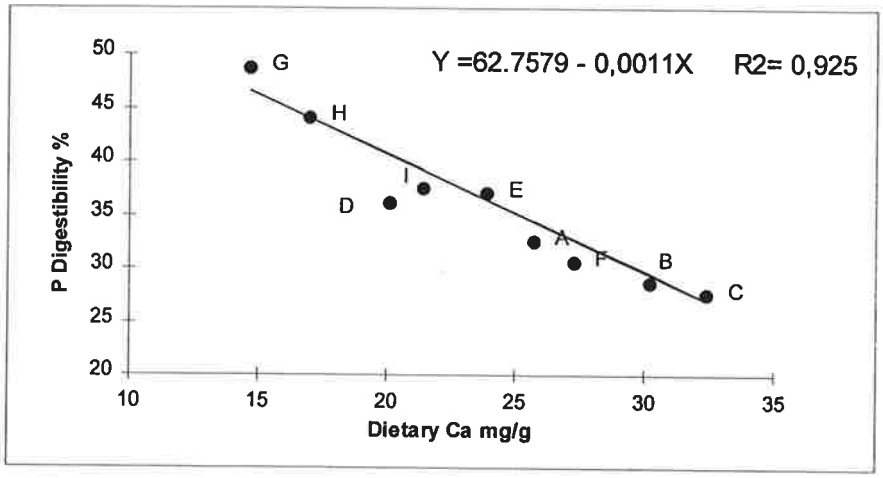

Figure 10 - Relationship between dietary Ca and P digestibility.

4 - DISCUSSION

We begin this chapter by discussing the differences found between the true diet composition and that expected from diet formulation, that in some degree affected the proposal of this experiment. In fact, the analyses performed on the different experimental diets after the trial, revealed some important differences from what we had proposed.

The most important one was the existence of higher values (± 0.4 %) in total P contents in all diets, that prevented us to see more clearly the influence of P supplementation, since almost every diet had more than 0.5 % available P, regarded by some author's to be sufficient to fulfil the P requirements of fish.

This fact was mainly related to an underestimation of the P content in the fish meal during diet formulation or to an over-supplementation. We assumed a P content of 2.1 %, but later analysis of the main raw materials showed a P content of 2.4 % for fish meal. Underestimation of P contents in the other raw materials, namely wheat, blood meal and the premix, may also have contributed to this P increment, since the content in maize gluten was lower (0.27 %) than predicted (0.5 %).

Thus, in order to correct the values of total and available P presented in the diets we should consider a P content of 2.4, 0.3 and 0.43 for fish meal, maize gluten and wheat respectively, that give us the levels presented in Tab. XIV.

Another relevant factor was the different levels of fat and protein contents existent in the diets. The diets were formulated in order to have equal levels in all diets, but the analysis showed that the diets with higher inclusion of maize gluten (G, H, I) had a fat content 2-3 % lower than proposed, and the values for protein were approximately 4 % higher than the other diets. This may indicate some difficulty in the incorporation of fat in diets containing high levels of maize gluten meal, probably due to the leakage of fat after the extrusion process. It also leads to the existence of some differences in terms of the digestible energy (DE) and digestible protein (DP) as well as in the relation DP/DE between the different diets, that could influence the growth performance.

The results after the nine weeks experiment revealed that P supplementation in fish meal based diets and in the maize gluten diets didn't affect growth performance of rainbow trout fed these diets.

This should be expected, since the content of available P in each diet was higher than the 0.4 %, regarded by Lall & Bishop (1977); Wiesmann *et al.* (1988) as the minimum level required for

maximum growth and feed utilisation efficiency in rainbow trout. On the other hand, Shearer & Hardy (1987) report that in fish fed diets containing different levels of P, ranging from 0.75-1.29 % dw (total P), average weights were similar among dietary treatments up to 18 weeks. After that, fish fed the diet with the lowest P content (total P - 0.75 % dw) began to show clinical signs of deficiency.

Table XIV - Corrected values for total chemical composition

CHEMCAL COMPOSITION

Diet code	A	B	C	D	E	F	G	H	I
Moisture	4,90	5,80	5,70	6,90	6,40	6,20	7,90	7,80	7,40
Protein	49,80	50,10	49,30	51,50	50,20	50,80	54,60	54,60	54,30
Fat	24,90	24,00	24,60	24,20	24,30	24,30	20,90	21,30	22,10
Ash	9,40	10,60	11,70	7,90	8,50	8,50	5,60	6,50	7,50
Carbohydrate (by difference)	11,00	9,50	8,70	9,50	10,60	10,20	11,00	9,80	8,70
Gross energy Kcal/Kg diet[a]	5,62	5,49	5,47	5,59	5,57	5,58	5,51	5,50	5,51
Available Phosphorus[b]	0,79	0,99	1,18	0,61	0,80	1,00	0,43	0,63	0,82
Total Phosphorus[c]	1,60	1,89	2,06	1,32	1,57	1,80	1,10	1,25	1,53

calculation was made based on the following parameters:

a- Based on 5.64 Kcal/g protein; 9.44 Kcal/g fat and 4.11 Kcal/g carbohydrate (NRC, 1993)

b -Fish meal - 2.4 % P (analysed) with 50% digestibility (LOVELL, 1989)

Maize gluten - 0.27 % P (analysed) - 25% digestibility (LOVELL, 1989)

Blood meal - 0.3% - 50 % digestibility (LOVELL, 1989)

CaP - 20% - 90 % digestibility (LOVELL, 1989)

Wheat - 0.43 % P - 50 % digestibility (LOVELL, 1989)

Premix - 8 % P - 90% digestibility (Cho & Cowey, 1991)

c - Analysed data

This suggested that some time is required to induce a phosphorus deficiency in rainbow trout. Therefore, the short period of the experiment, along with the adequate levels of dietary phosphorus, also contributed to the non-existence of clear differences among P treatments.

However, in diets with higher inclusion of maize gluten (G, H, I), diet G (0.43 % available P) had the poorest growth compared with the other diets, revealing that at some extent P supplementation improved the performance of these diets. This is in accordance with the study performed by Ketola & Richmond (1994) in which growth of rainbow trout was significantly improved by P supplementation in diets with levels up to 0.54 % non-phytin P. Little or further improvement in growth was observed with supplements beyond this level, as observed in our study.

Ketola & Richmond (1994) indicate that the minimum level of non-phytin phosphorus required for attaining maximum weight gain was between 0.34 and 0.54 of diet. The results of our study suggest that this level is between the 0.43% and 0.61 % in agreement to the levels 0.5-0.6 %

reported by (Ketola, 1975; Watanabe *et al.* 1980; Shearer, 1995), but are lower that the 0.7-0.8 proposed by Ogino & Takeda (1978).

The higher P requirement found could also be related to the high feed efficiency (gain/feed) observed in our study (1-1.1). Shearer (1995) reports that as feed efficiency increases, the requirement, when stated as a fraction of the diet, must also be increased if other factors affecting the requirement remain constant. A higher feed efficiency generally leads to a higher requirement.

Regarding the replacement of fish meal by maize gluten as main protein source, it was clear that the first level of inclusion (18.75 %) didn't affect growth performance, but a level of 37.5 % resulted in significantly reduced growth.

These results are in accordance with those of experiments performed by Groop *et al.* (1976), Alexis *et al.* (1985), Morales *et al.* (1995), Gomes *et al.* (1995), in which, diets containing levels of maize gluten up to 25 % dw showed similar results to the ones based on fish meal as main protein source. Gomes *et al.* (1995) also found that diets containing up to 38 % maize gluten (but with 100 % vegetable protein), caused significantly ($P<0.01$) lower growth performances than with diets containing less than 25 % maize gluten.

Data on the apparent digestibility coefficient (ADC) of the protein in maize gluten diets, indicated good digestive utilisation, and values were similar to those reported in the literature which report that protein digestibility of maize gluten meal is higher than or similar to that of fish meal (CHO *el al.*, 1982; HARDY, 1989; ANDERSON *et al.*, 1992; MORALES *et al.*, 1994).

Weight gain was directly related to feed intake. This relation would probably have been more pronounced with a different technique for collecting the uneaten food. In fact, we think that the methodology used for collection of not eaten food was not the most appropriate, since the water dropping from the outlet over the feed destroyed the pellets, leading to an overestimation of the feed intake. This effect was increased in tanks were the feed spoilage was higher, namely in fish fed diets G, H, I .

This could indicate that the depression of voluntary feed intake, along with the level of replacement of fish meal by maize gluten could cause a decrease in fish growth. This type of depression of voluntary feed intake has previously been observed by Gomes & Kaushik (1992, 1995). These authors suggest that some intrinsic characteristic of vegetable proteins may induce a significant reduction in feed intake and consequently in growth performance.

De la Higuera *et al.* (1988) also found a slight tendency toward a lower food intake with increasing substitution of fish meal by lupin seed meal, and explained this phenomenon through a progressively more difficult adaptation of fish to the organoleptic properties of such diets. Morales *et al.* (1994) attributed to a lower palatability of a casein diet, the significantly lower intake compared to the fish meal containing diets.

It should also be mentioned that because of the differences in fat and protein contents between diets, the levels of digestible energy (DE) and digestible protein (DP) were different between diets, a factor which could also influence the growth performance, as the relation DP/DE is one of the main factor controlling feed intake (Cho & Kaushik, 1985). From Fig. 4, we can see that in fact the growth performance of fish fed on diets with 37,5 % maize gluten was not only affected by the maize gluten content but also in some degree by the fat content in the diet.

In respect to the body proximate analyses, it is clear that have occurred a high increase in body fat occurred during the trial period, probably associated with the high level of fat in the feed and also to the high feeding level. Weatherley and Gill (1983b) also pointed out a decrease in water contents and increase in percentage lipid with increasing ration size and with increasing body weight.

Regarding the body composition of fish fed the different diets, our results are in accordance with those of Reinitz (1983) and Alexis *et al.* (1985) who reported that increasing the proportion of dietary fat resulted in an increased proportion of fat in fish carcasses and a decreased percent of moisture while the proportion of dietary protein did not appear to affect body composition significantly. This means that the observed associations among percent fat, protein, and moisture in fish carcasses and individual weight indicate that the additional energy stored as fat by fish simply replaces body water content and does not adversely affect the deposition of protein.

Therefore the observed increase in percent fat and decrease in percent moisture in the carcasses of fish fed the higher fat diets was most likely due to the increased proportion of dietary fat.

It was also clear from the body proximate analysis that in this case, the type of food, i.e. the substitution of fish meal by maize gluten, didn't influence significantly the body composition. These findings agree with the published results of Smith *et al.* (1988) who found no significant differences in body composition and taste acceptability from fish raised on diets high on plant protein, from those fed predominantly animal protein diets. However, this is not in accordance with

the study of Papoutsoglou *et al.* (1978), that found that the nutritional value and the quality of flesh in trout is mainly affected by the type of food.

Analysis of whole body mineral levels at the end of the feeding trial showed that except for Ca and P, dietary treatment did not affected levels of essential elements in fish. Hardy *et al.* (1984) found similar results, including Ca and P.

Regarding the elemental body composition, comparison of concentrations observed in our study and to those reported for trout by Shearer (1984) and for salmon Shearer *et al.* (1994) over their life cycle, revealed some similarity. Concentrations of Fe, K, Mn, Mg and Cr observed in our study agree with those reported by these authors. The Zn and Sr values reported for trout in fish of same size appear low when compared to our results, specially with levels in fish before the trial. However our results are similar compared with those of Hardy *et al.* (1984).

Like in the study performed by Porn-Ngam *et al.* (1993) the levels of body K, Na, Mn, and Cu were generally not affected by differences in dietary levels of Ca or P.

The Ca and P contents seem to be very low comparing to these and other authors. Lovell (1989) reports that the percentage of calcium in the whole, fresh (wet) body of finfish ranges from 0,5 to 1 % with a ratio of calcium to phosphorus of 0,7 to 1,6, and Shearer (1984) found values of 0.47-0.49 for phosphorus and 0.40-0.6 for Ca. Hardy *et al.* (1984) reports values of 4.4-6.7 for calcium and 3.8-4.7 for phosphorus.

The present results are difficult to explain comparing to the other authors. A possible source of error could be in the preparation of the samples (grinding and homogeneity) for mineral analyses. However, the results obtained for the other minerals were normal according to the same authors.

The mineral contents of the fish before the trial revealed higher levels of, Fe, K, Na, P (6 mg/g) and Ca (5.8 mg/g), leading us believe that some reduction on these elements may have occurred. The decline of P and Ca could be explained by the fact that muscle tissue grows faster than bone (main source of P and Ca) as fish grow, causing a decrease in larger fish comparing to small ones (Shearer pers. com.)

Regarding the other minerals, Weatherly and Gill (1983a,b) indicated that during post-juvenile growth, organs tend to grow at a slower rate than the carcass. The outcome of this negative allometry is the declining whole body concentration of some elements.

Hardy *et al.* (1991) reports that the body levels of P in fish reflect the amount of phosphorus fed during the course of the feeding period, with the level of body P decreasing with the level of P in the diet.

On the present experiment this was not the case. However, considering that the available P used by Hardy *et al.* (1991) didn't fulfil the requirement for the fish (higher availability 0,5 %) and in our study this requirements were satisfied (lower availability 0,43 %), it could be postulated that the fish don't absorb more phosphorus than is sufficient to satisfy requirement. In fact Hardy *et al.* (1991) also suggest that there is no dose-dependent relationship between apparent phosphorus retention and dietary phosphorus levels.

The negative correlation found between the dietary phosphorus concentration supplied and the P digestibility coefficient (P DC) come in support of this theory, revealing that rainbow trout might only use P equivalent to requirement and excrete excess amounts, as previously reported by Satoh *et al.* (1992). A similar negative correlation was found by Riche & Brown (1996), and Heinen *et al.* (1993) also found a lower phosphorus retention rate and greater phosphorus losses with increasing phosphorus contents in diet.

Nevertheless, Wisemann *et al.* (1988) and Ketola & Richmond (1994) report that at a level of 0,4 % available P, total body phosphorus content was reduced and that the P requirement of rainbow trout for bone mineralization is higher than that for growth.

In our study, opposite of what was expected, the fish fed the diets with lower levels of phosphorus were the ones who showed higher body P content, with fish fed diet G (0.43 available P) showing the highest body P content.

The Anova test shows that the level of maize gluten meal had an effect on body P but CaP did not. Shearer (1984, 1995), found a close relationship between fish size and elemental composition in post-juvenile or adult fishes, with the body burden (total amount) of P decreasing at a rate equivalent to the increasing in body weight of the fish. Thus, these results could be explained by fact that fish fed diets with higher levels of maize gluten had a smaller growth performance.

If we also take in account that Lall (1991) showed that 80-88 % of total body P of trout is located in the bones, and Rottiers (1993) found a negative correlation between % bone weight (wet and dry) and fish weight, considering that those fish showed lower body weight and condition index, the apparently high P content could be a consequence of a relatively high bone weight percentage in the analysed samples.

One consequence of size-dependent elemental body composition is that the direct comparison of whole body compositions among different dietary treatment groups, where treatments produce differential growth, may yield misleading results. Thus, we believe that in this case, analyses of

individual tissues concentrations, namely flesh and bone, could be more useful indicators of the effect of the dietary treatment than whole body elemental analysis.

The negative relationship between P DC and dietary calcium observed in this study was similar to that between dietary calcium concentration supplied by fish meal and the apparent P availability data for rainbow trout (Riche & Brown, 1996). A study by Porn-Ngam *et al.* (1993) and Satoh *et al.* (1993) also revealed that elevation of Ca levels in the diets, decreased gradually the absorption of P.

Another important issue is the fact that Satoh et al. (1993) suggest that due to an unbalanced Ca/P ratio in mono or tricalcium phosphate, diets supplemented with these P sources might not be suitable for rainbow trout. This authors also reports that the bone in the fish meal had the same effect as tricalcium phosphate. Riche & Brown (1996) suggest that skeletal tissue phosphorus (from the fish meal) hydrolysed in the acidic stomach may reform insoluble calcium phosphate compounds in the more alkaline environment of the intestine, difficult to hydrolyse, and resistant to digestion by rainbow trout.

Porn-Ngam *et al.* (1993) and Satoh *et al.* (1993) demonstrate that dietary P at 2 or 3 times higher that the P requirement might lower the growth of rainbow trout. However, these effects were weakened when Ca was included at the same level as P in diet, suggesting a optimum ratio of P to Ca of 1:1.

Thus, and looking at the body P contents, P DC and Ca:P ratios, we could say that maybe due to an unbalanced Ca:P ratio the fish fed fish meal and supplemented diets were not able to absorb greater amounts than those given by maize gluten and not supplemented diets. Nevertheless, this did not affected the growth performance of these diets since the P requirements were satisfied.

The correlation between phosphorus digestibility and dietary calcium may be useful, if verified in a more detailed manner, for the reduction of P output the environment, and an appropriate Ca:P ratio must studied and used in diet formulation.

5 - CONCLUSIONS

- P supplementation of the fish meal based diets was not required under the given conditions. The basal level of 1.6 % met all the requirements.

- A level of dietary available P of 0.43 % is not sufficient for maximum growth and feed efficiency. A level of 0.6% available P should be sufficient.

- Rainbow trout might only use P equivalent to requirement, and excrete excess amounts. Thus, excess amounts of P should not be added to rainbow trout diets.

- The negative correlation found between the dietary calcium concentration and the apparent P digestibility, revealed that the level of Ca in the diet is also of major importance for the reduction of the excreted phosphorus, due to in influence in P availability.

- Maize gluten meal was a good protein feedstuff at 19 % inclusion, but appeared sub-optimal at 38 %. This was partly but not completely corrected by P supplementation,.

- Body composition was not changed by the substitution of fish meal by maize gluten.

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ANNEX I

Table A- Individual growth performance of fish fed the different experimental diets.

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
135	893	18.61	0.2	145.9	299.8	1.26	1.53	1.28	153.90
135	872	18.62	0.2	183.9	527	1.85	2.6	1.61	343.10
135	886	18.63	0.2	170.9	531.9	1.99	2.73	1.54	361.00
135	895	18.64	0.2	141.5	380	1.73	2.18	1.45	238.50
135	881	18.65	0.2	212.9	694.1	2.07	3.09	1.78	481.20
135	871	18.66	0.2	183.8	354.2	1.15	1.49	1.37	170.40
135	900	18.67	0.2	173.9	422.6	1.56	2.06	1.45	248.70
135	898	18.68	0.2	185.5	610.4	2.09	2.98	1.78	424.90
135	869	18.69	0.2	151.5	353	1.48	1.86	1.26	201.50
135	894	18.70	0.2	208.4	538.3	1.66	2.36	1.54	329.90
135	883	18.71	0.2	151.8	423.3	1.80	2.33	1.45	271.50
135	868	18.72	0.2	128.6	435.5	2.14	2.71	1.53	306.90
135	887	18.73	0.2	169.6	473.7	1.80	2.42	1.67	304.10
135	896	18.74	0.2	175.8	611.6	2.19	3.09	1.73	435.80
135	882	18.75	0.2	207.2	545.8	1.70	2.41	1.63	338.60
135	889	18.75	0.2	175.2	430.1	1.58	2.09	1.31	254.90
135	885	18.75	0.2	158.1	366.7	1.48	1.87	1.52	208.60
135	873	18.76	0.2	172.6	583.9	2.14	2.99	1.78	411.30
135	880	18.77	0.2	194.2	565.6	1.88	2.65	1.53	371.40
135	897	18.78	0.2	229.5	433	1.11	1.54	1.26	203.50
135	892	18.79	0.2	102.7	320	1.99	2.31	1.54	217.30
135	A0600	18.80	0.2	171.4	570.9	2.11	2.93	1.51	399.50
135	A0457	18.81	0.2	197	448.8	1.44	1.97	1.52	251.80
135	AO590	18.82	0.2	184	544.5	1.90	2.65	1.46	360.50
135	A0596	18.83	0.2	178	554.8	1.99	2.77	1.53	376.80
135	A0461	18.84	0.2	158.6	333.4	1.30	1.63	1.31	174.80
135	A0468	18.85	0.2	179.7	579	2.05	2.88	1.69	399.30
135	899	18.86	0.2	173.2	332.7	1.15	1.45	1.57	159.50
135	879	18.87	0.2	138.5	403.8	1.88	2.37	1.42	265.30
135	888	18.88	0.2	188.9	583.9	1.98	2.80	1.78	395.00
136	814	37.5	0	188.2	346	1.07	1.38	1.21	157.80
136	863	37.5	0	253.3	467.6	1.08	1.54	1.57	214.30
136	810	37.5	0	229.6	592.3	1.66	2.43	1.53	362.70
136	854	37.5	0	181.7	420.5	1.47	1.96	1.31	238.80
136	866	37.5	0	285.4	587.1	1.27	1.92	1.43	301.70
136	801	37.5	0	136	366.8	1.74	2.16	1.64	230.80
136	851	37.5	0	172.8	567.4	2.09	2.90	1.76	394.60
136	A0455	37.5	0	153.3	442.2	1.86	2.43	1.41	288.90
136	807	37.5	0	189.6	296.8	0.79	0.99	1.32	107.20
136	867	37.5	0	126	217	0.95	1.07	1.29	91.00
136	862	37.5	0	184.7	365.9	1.20	1.56	1.43	181.20
136	864	37.5	0	133	361.6	1.75	2.16	1.34	228.60
136	865	37.5	0	152.8	426.3	1.80	2.33	1.50	273.50
136	812	37.5	0	168	420.9	1.61	2.12	1.51	252.90
136	857	37.5	0	197.6	442.1	1.41	1.92	1.44	244.50
136	813	37.5	0	194.8	462.5	1.52	2.07	1.68	267.70
136	809	37.5	0	167.4	418.5	1.61	2.11	1.52	251.10
136	811	37.5	0	161.1	321.1	1.21	1.51	1.39	160.00
136	808	37.5	0	96	328.6	2.16	2.49	1.53	232.60
136	802	37.5	0	148.8	309	1.28	1.56	1.32	160.20
136	852	37.5	0	102.6	297.8	1.87	2.14	1.56	195.20
136	855	37.5	0	259	549.7	1.32	1.95	1.56	290.70

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
136	858	37.5	0	230.8	391.8	0.93	1.27	1.54	161.00
136	803	37.5	0	196.6	443.2	1.43	1.94	1.52	246.60
136	860	37.5	0	221.6	398.5	1.03	1.40	1.38	176.90
136	856	37.5	0	194.5	372.9	1.14	1.50	1.35	178.40
136	806	37.5	0	208	482.5	1.48	2.05	1.42	274.50
136	A0473	37.5	0	168	379	1.43	1.84	1.43	211.00
136	A0460	37.5	0	138.5	539.8	2.39	3.18	1.65	401.30
137	831	37.5	0.2	210.9	590.1	1.81	2.61	1.56	379.20
137	815	37.5	0.2	153	509.3	2.11	2.82	1.83	356.30
137	822	37.5	0.2	168.8	387.4	1.46	1.89	1.33	218.60
137	816	37.5	0.2	175.2	389.2	1.40	1.83	1.44	214.00
137	839	37.5	0.2	196.1	386.7	1.19	1.58	1.30	190.60
137	829	37.5	0.2	201.1	488.1	1.56	2.16	1.59	287.00
137	834	37.5	0.2	232.4	694.6	1.92	2.90	1.77	462.20
137	835	37.5	0.2	161.7	314.3	1.17	1.45	1.29	152.60
137	832	37.5	0.2	237.6	457.3	1.15	1.62	1.48	219.70
137	823	37.5	0.2	219.5	426	1.16	1.60	1.43	206.50
137	843	37.5	0.2	232.6	525.7	1.43	2.06	1.40	293.10
137	826	37.5	0.2	231.2	590.5	1.65	2.41	1.72	359.30
137	818	37.5	0.2	183.1	383.1	1.30	1.70	1.45	200.00
137	828	37.5	0.2	212.2	502.2	1.51	2.12	1.44	290.00
137	820	37.5	0.2	237.8	543.1	1.45	2.10	1.58	305.30
137	830	37.5	0.2	205.7	472.5	1.46	2.02	1.44	266.80
137	817	37.5	0.2	174	395	1.44	1.88	1.33	221.00
137	841	37.5	0.2	216.8	480.8	1.40	1.96	1.35	264.00
137	838	37.5	0.2	126.2	229.8	1.05	1.19	1.66	103.60
137	837	37.5	0.2	208.1	585.7	1.82	2.61	1.63	377.60
137	840	37.5	0.2	118.7	307.2	1.67	1.96	1.45	188.50
137	825	37.5	0.2	225.6	399.4	1.00	1.37	1.38	173.80
137	836	37.5	0.2	170.5	558.7	2.08	2.88	1.58	388.20
137	844	37.5	0.2	174	385.3	1.39	1.81	1.43	211.30
137	821	37.5	0.2	159.9	374.8	1.49	1.91	1.40	214.90
137	842	37.5	0.2	160.5	440.9	1.77	2.33	1.38	280.40
137	824	37.5	0.2	268.5	723.8	1.74	2.71	1.75	455.30
137	833	37.5	0.2	156.2	554.5	2.22	3.03	1.72	398.30
137	827	37.5	0.2	187	413.6	1.39	1.85	1.29	226.60
137	A0562	37.5	0.2	234.2	742.9	2.03	3.10	1.75	508.70
138	848	37.5	0.4	184.5	419.5	1.44	1.92	1.38	235.00
138	A0951	37.5	0.4	245.5	583.1	1.52	2.24	1.47	337.60
138	A0970	37.5	0.4	218.2	597	1.77	2.57	1.49	378.80
138	850	37.5	0.4	273.1	463.1	0.93	1.34	1.40	190.00
138	AO963	37.5	0.4	148.1	570	2.36	3.21	1.63	421.90
138	846	37.5	0.4	157.8	453	1.85	2.44	1.51	295.20
138	A0961	37.5	0.4	158.6	428.8	1.74	2.28	1.47	270.20
138	A0968	37.5	0.4	217.9	519.8	1.53	2.17	1.45	301.90
138	A0952	37.5	0.4	172.9	360.3	1.29	1.65	1.49	187.40
138	A0957	37.5	0.4	218.6	563	1.66	2.39	1.41	344.40
138	847	37.5	0.4	160.6	282.8	0.99	1.21	1.20	122.20
138	A0962	37.5	0.4	219	383	0.98	1.32	1.40	164.00
138	A0960	37.5	0.4	170.2	408.2	1.53	2.01	1.59	238.00
138	A0972	37.5	0.4	220.4	514	1.49	2.11	1.48	293.60
138	A0958	37.5	0.4	113	285.7	1.63	1.88	1.81	172.70
138	A0964	37.5	0.4	155	355.2	1.45	1.83	1.37	200.20

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
138	A0583	37.5	0.4	155.2	445.5	1.85	2.42	1.59	290.30
138	A0465	37.5	0.4	158.1	439.2	1.79	2.35	1.47	281.10
138	A0595	37.5	0.4	125.7	222.9	1.00	1.13	1.20	97.20
138	A0959	37.5	0.4	182.8	410.9	1.42	1.88	1.49	228.10
138	A0971	37.5	0.4	178.2	373.8	1.30	1.69	1.36	195.60
138	A0974	37.5	0.4	192.5	433.2	1.42	1.92	1.45	240.70
139	A0989	0	0.4	185.8	616.6	2.10	3.00	1.57	430.80
139	A0987	0	0.4	208.8	563.8	1.74	2.49	1.57	355.00
139	A0901	0	0.4	185.6	397.5	1.34	1.76	1.63	211.90
139	A1000	0	0.4	224.5	809	2.25	3.47	1.79	584.50
139	A0993	0	0.4	169.3	408.4	1.54	2.02	1.44	239.10
139	A0996	0	0.4	209.5	767.4	2.28	3.44	1.60	557.90
139	A0994	0	0.4	170.3	491.6	1.86	2.51	1.59	321.30
139	A0997	0	0.4	194.6	612.8	2.01	2.89	1.56	418.20
139	A0977	0	0.4	202.7	527.4	1.68	2.36	1.33	324.70
139	A0995	0	0.4	145.8	597.2	2.47	3.38	1.68	451.40
139	A0998	0	0.4	239.6	655.1	1.76	2.65	1.62	415.50
139	A0902	0	0.4	241.7	715.3	1.90	2.91	1.59	473.60
139	A0979	0	0.4	173.5	461.8	1.72	2.30	1.41	288.30
139	A0985	0	0.4	179	475.3	1.71	2.32	1.51	296.30
139	A0984	0	0.4	233.3	659.1	1.82	2.73	1.47	425.80
139	A0990	0	0.4	194.7	512.8	1.70	2.36	1.47	318.10
139	A0976	0	0.4	204.4	440.6	1.35	1.84	1.41	236.20
139	A0982	0	0.4	171.4	400	1.49	1.94	1.47	228.60
139	A0983	0	0.4	157.1	392.7	1.61	2.06	1.47	235.60
139	A0904	0	0.4	191.3	365.6	1.14	1.49	1.38	174.30
139	A0978	0	0.4	128.1	300	1.49	1.77	1.43	171.90
139	A0975	0	0.4	170.8	377.6	1.39	1.80	1.55	206.80
139	A0988	0	0.4	167	387.2	1.48	1.91	1.48	220.20
139	A0992	0	0.4	181.5	457.6	1.62	2.19	1.40	276.10
139	A0980	0	0.4	204	540	1.71	2.42	1.60	336.00
139	A0903	0	0.4	160.8	572	2.23	3.06	1.48	411.20
139	A0991	0	0.4	129.9	526	2.45	3.22	1.75	396.10
139	A0466	0	0.4	189.4	416.9	1.38	1.85	1.47	227.50
140	A0594	0	0	160.8	480.8	1.92	2.56	1.44	320.00
140	A0917	0	0	167.5	561	2.12	2.93	1.60	393.50
140	A0932	0	0	178.5	754.9	2.53	3.72	1.70	576.40
140	A0911	0	0	124.5	277.2	1.40	1.63	1.46	152.70
140	A0908	0	0	235	432.4	1.07	1.49	1.49	197.40
140	A0919	0	0	239.2	774.3	2.06	3.18	1.94	535.10
140	A0925	0	0	179.2	443	1.59	2.13	1.49	263.80
140	A0920	0	0	148.3	302.1	1.25	1.52	1.38	153.80
140	A0934	0	0	142	435.6	1.97	2.53	1.45	293.60
140	A0589	0	0	123.1	575.7	2.71	3.58	1.56	452.60
140	A0913	0	0	229.5	489.6	1.33	1.88	1.40	260.10
140	A0921	0	0	112.3	389	2.18	2.65	1.66	276.70
140	A0909	0	0	263	771	1.89	2.96	1.64	508.00
140	A0905	0	0	240.8	562.9	1.49	2.18	1.52	322.10
140	A0914	0	0	175.2	518.5	1.90	2.61	1.60	343.30
140	A0930	0	0	164.8	363.3	1.39	1.77	1.43	198.50
140	A0927	0	0	193.4	421.8	1.37	1.84	1.68	228.40
140	A0928	0	0	142.3	366.9	1.66	2.07	1.65	224.60
140	A0922	0	0	113.4	369.3	2.07	2.50	1.53	255.90

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
140	A0490	0	0	156.1	390	1.61	2.06	1.44	233.90
140	A0489	0	0	117.6	435.5	2.30	2.87	1.46	317.90
140	AO584	0	0	139.2	371.4	1.72	2.15	1.48	232.20
140	A0487	0	0	115.4	315	1.76	2.07	1.23	199.60
163	A0177	18.75	0.2	124.3	236.5	1.13	1.28	1.24	112.20
163	A0181	18.75	0.2	166.7	362.4	1.36	1.74	1.28	195.70
163	A0179	18.75	0.2	181.9	454	1.60	2.16	1.66	272.10
163	A0160	18.75	0.2	182.7	466.4	1.64	2.23	2.15	283.70
163	A0173	18.75	0.2	125.8	345.3	1.77	2.15	1.37	219.50
163	A0190	18.75	0.2	148	379	1.65	2.08	1.39	231.00
163	A0187	18.75	0.2	167.4	406.9	1.56	2.03	1.63	239.50
163	A0171	18.75	0.2	216.4	676.3	2.00	2.97	1.66	459.90
163	A0166	18.75	0.2	171	373.8	1.37	1.77	1.36	202.80
163	A0172	18.75	0.2	229.3	609.1	1.71	2.52	1.51	379.80
163	A0182	18.75	0.2	182.4	556.9	1.96	2.74	1.48	374.50
163	A0183	18.75	0.2	174.6	456.2	1.68	2.26	1.53	281.60
163	A0164	18.75	0.2	153.3	489.7	2.04	2.71	1.45	336.40
163	A0188	18.75	0.2	190.4	493.4	1.67	2.30	1.62	303.00
163	A0485	18.75	0.2	143	328.5	1.46	1.79	1.28	185.50
163	A0454	18.75	0.2	176.5	542.3	1.97	2.72	1.74	365.80
163	A0588	18.75	0.2	177.2	377.7	1.33	1.73	1.34	200.50
163	A0585	18.75	0.2	103.7	348.1	2.12	2.50	1.64	244.40
163	A0493	18.75	0.2	106.3	308.1	1.87	2.16	1.33	201.80
163	A0467	18.75	0.2	192	614.8	2.04	2.93	1.70	422.80
163	A0482	18.75	0.2	183	572.5	2.00	2.81	1.64	389.50
163	A0498	18.75	0.2	177.4	522.7	1.90	2.61	1.44	345.30
163	A0500	18.75	0.2	159.7	466.5	1.88	2.49	1.45	306.80
163	A0499	18.75	0.2	234.4	673	1.85	2.78	1.67	438.60
163	A0495	18.75	0.2	197.3	434.2	1.38	1.87	1.38	236.90
164	A0867	0	0.4	155.4	330	1.32	1.64	1.27	174.60
164	A0882	0	0.4	184.7	441.2	1.53	2.05	1.23	256.50
164	A0950	0	0.4	165.2	461.6	1.80	2.40	1.46	296.40
164	A0944	0	0.4	180.7	560.4	1.99	2.77	1.53	379.70
164	A0949	0	0.4	160.4	323.4	1.23	1.53	1.43	163.00
164	A0884	0	0.4	213.1	578.1	1.75	2.52	1.62	365.00
164	A0941	0	0.4	176	354.4	1.23	1.58	1.41	178.40
164	A0874	0	0.4	126.6	271.6	1.34	1.56	1.46	145.00
164	A0881	0	0.4	185.9	405.8	1.37	1.82	1.61	219.90
164	962	0	0.4	139.1	437.3	2.01	2.58	1.40	298.20
164	A0597	0	0.4	153.3	465.5	1.95	2.57	1.52	312.20
164	A0475	0	0.4	189.3	617.9	2.08	2.97	1.50	428.60
164	A0479	0	0.4	129.4	357.9	1.78	2.19	1.55	228.50
164	A0586	0	0.4	125.8	249.3	1.20	1.37	1.31	123.50
164	A0477	0	0.4	132.8	585.1	2.60	3.49	1.53	452.30
164	A0464	0	0.4	146.5	444.8	1.95	2.53	1.42	298.30
164	A0476	0	0.4	164	423.3	1.66	2.18	1.43	259.30
164	A0470	0	0.4	166.7	496.3	1.91	2.58	1.53	329.60
164	A0587	0	0.4	228.4	463.5	1.24	1.74	1.38	235.10
164	A0481	0	0.4	180	373.8	1.28	1.67	1.74	193.80
164	A0496	0	0.4	134.1	506.1	2.33	3.05	1.62	372.00
164	A0453	0	0.4	130.9	488	2.31	2.99	1.49	357.10
164	A0488	0	0.4	145	524.9	2.26	3.01	1.73	379.90
164	A0591	0	0.4	171.3	533	1.99	2.73	1.54	361.70

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
164	A0599	0	0.4	139	344.6	1.59	1.96	1.40	205.60
164	A0459	0	0.4	151.2	424.3	1.81	2.34	1.57	273.10
165	754	18.75	0	192.7	495.9	1.66	2.29	1.38	303.20
165	A0892	18.75	0	107.1	429.8	2.44	2.99	1.53	322.70
165	A0894	18.75	0	159.2	402.7	1.63	2.10	1.49	243.50
165	A0893	18.75	0	189.3	436.8	1.47	1.98	1.55	247.50
165	A0888	18.75	0	164.1	500.8	1.96	2.64	1.42	336.70
165	755	18.75	0	232.4	703.4	1.94	2.94	1.47	471.00
165	A0896	18.75	0	191.7	581.2	1.95	2.76	1.59	389.50
165	A0889	18.75	0	231.1	513.8	1.40	2.00	1.42	282.70
165	751	18.75	0	118.5	254.6	1.34	1.53	1.45	136.10
165	767	18.75	0	206.8	465.1	1.42	1.96	1.61	258.30
165	759	18.75	0	216.8	514.9	1.52	2.15	1.59	298.10
165	758	18.75	0	191.3	499.7	1.68	2.33	1.47	308.40
165	764	18.75	0	204	534.8	1.69	2.39	1.68	330.80
165	760	18.75	0	147.3	390.9	1.71	2.17	1.45	243.60
165	A0895	18.75	0	214.4	415.8	1.16	1.58	1.37	201.40
165	A0890	18.75	0	152.8	443.6	1.87	2.44	1.55	290.80
165	757	18.75	0	226.4	712.5	2.01	3.04	1.78	486.10
165	A0891	18.75	0	160.4	502.9	2.00	2.70	1.77	342.50
165	761	18.75	0	230.7	708.1	1.97	2.98	1.62	477.40
165	1000	18.75	0	256.7	576	1.42	2.10	1.50	319.30
165	A0593	18.75	0	174.9	433	1.59	2.11	1.30	258.10
166	797	37.5	0.2	172.3	315.2	1.06	1.33	1.24	142.90
166	792	37.5	0.2	142.2	398.7	1.81	2.29	1.70	256.50
166	771	37.5	0.2	245.7	510.9	1.28	1.85	1.43	265.20
166	798	37.5	0.2	197.2	421.1	1.33	1.79	1.31	223.90
166	770	37.5	0.2	184	369.5	1.22	1.59	1.30	185.50
166	796	37.5	0.2	172.7	408.7	1.51	1.98	1.47	236.00
166	782	37.5	0.2	164	490.6	1.92	2.58	1.57	326.60
166	777	37.5	0.2	160.9	381.1	1.51	1.94	1.28	220.20
166	779	37.5	0.2	198.5	408.6	1.27	1.70	1.37	210.10
166	870	37.5	0.2	224.3	397.7	1.00	1.37	1.53	173.40
166	A0915	37.5	0.2	213.4	521	1.57	2.22	1.67	307.60
166	787	37.5	0.2	234.4	450.1	1.14	1.60	1.45	215.70
166	768	37.5	0.2	178	522.5	1.89	2.60	1.69	344.50
166	783	37.5	0.2	215	560.1	1.68	2.41	1.66	345.10
166	781	37.5	0.2	179	414.5	1.47	1.95	1.52	235.50
166	772	37.5	0.2	153	423.1	1.78	2.31	1.63	270.10
166	776	37.5	0.2	176	334	1.12	1.43	1.30	158.00
166	A0956	37.5	0.2	191.6	573	1.92	2.72	1.57	381.40
166	884	37.5	0.2	144.7	392.3	1.75	2.22	1.51	247.60
166	A0875	37.5	0.2	170.8	396.4	1.48	1.92	1.53	225.60
166	788	37.5	0.2	141.8	379.3	1.73	2.17	1.73	237.50
166	784	37.5	0.2	130.9	317	1.55	1.86	1.63	186.10
166	780	37.5	0.2	194.8	629	2.06	2.97	1.60	434.20
167	A0084	0	0.2	141.9	353.8	1.60	1.99	1.35	211.90
167	994	0	0.2	102	311	1.96	2.25	1.33	209.00
167	988	0	0.2	174.7	620.6	2.22	3.15	1.65	445.90
167	A0088	0	0.2	211.4	585.4	1.79	2.58	1.64	374.00
167	993	0	0.2	161.6	612.2	2.34	3.26	1.78	450.60
167	A0095	0	0.2	162.2	364.1	1.42	1.81	1.45	201.90
167	A0091	0	0.2	149.4	318.8	1.33	1.63	1.52	169.40

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
167	999	0	0.2	156.5	441	1.82	2.38	1.51	284.50
167	996	0	0.2	229.7	447.3	1.17	1.63	1.47	217.60
167	998	0	0.2	180.8	416.6	1.46	1.94	1.62	235.80
167	984	0	0.2	126	439.2	2.19	2.77	1.38	313.20
167	995	0	0.2	140.4	262.7	1.10	1.29	1.20	122.30
167	985	0	0.2	175.3	344.9	1.19	1.52	1.75	169.60
167	983	0	0.2	191.9	571.3	1.91	2.71	1.59	379.40
167	A0083	0	0.2	159.9	578.7	2.26	3.11	1.58	418.80
167	A0087	0	0.2	225.4	834	2.30	3.56	1.70	608.60
167	992	0	0.2	203.3	536.5	1.70	2.40	1.55	333.20
167	990	0	0.2	203.7	458	1.42	1.95	1.51	254.30
167	A0090	0	0.2	237.2	447.6	1.11	1.56	1.50	210.40
167	991	0	0.2	195.7	569.3	1.87	2.66	1.71	373.60
167	A0086	0	0.2	148.6	538.4	2.26	3.04	1.58	389.80
167	A0093	0	0.2	150.9	461.1	1.96	2.57	1.52	310.20
168	965	18.75	0.4	182.6	486	1.72	2.34	1.42	303.40
168	958	18.75	0.4	214.8	490	1.45	2.03	1.55	275.20
168	978	18.75	0.4	187.3	595	2.03	2.88	1.51	407.70
168	969	18.75	0.4	178.9	490.2	1.77	2.41	1.61	311.30
168	967	18.75	0.4	169.4	382.4	1.43	1.85	1.42	213.00
168	982	18.75	0.4	135.5	391.4	1.86	2.33	1.42	255.90
168	951	18.75	0.4	119.1	267.1	1.42	1.63	1.23	148.00
168	959	18.75	0.4	220.4	451.5	1.26	1.75	1.62	231.10
168	968	18.75	0.4	166.9	416.5	1.60	2.10	1.50	249.60
168	979	18.75	0.4	164.8	403.2	1.57	2.04	1.67	238.40
168	972	18.75	0.4	175	469.9	1.73	2.33	1.55	294.90
168	970	18.75	0.4	147.8	441.1	1.92	2.49	1.45	293.30
168	952	18.75	0.4	175.3	493.5	1.82	2.47	1.53	318.20
168	975	18.75	0.4	170.7	390	1.45	1.88	1.28	219.30
168	953	18.75	0.4	194.1	425.8	1.38	1.86	1.46	231.70
168	980	18.75	0.4	149.3	469	2.01	2.64	1.57	319.70
168	954	18.75	0.4	127.2	365.6	1.85	2.27	1.55	238.40
168	971	18.75	0.4	115.2	411.6	2.23	2.75	1.69	296.40
168	977	18.75	0.4	121.5	304.5	1.61	1.90	1.42	183.00
168	957	18.75	0.4	192.2	430.7	1.42	1.91	1.52	238.50
168	964	18.75	0.4	173	455.1	1.70	2.27	1.47	282.10
168	956	18.75	0.4	241.2	516.3	1.34	1.92	1.42	275.10
168	961	18.75	0.4	240.2	633.6	1.70	2.54	1.75	393.40
168	960	18.75	0.4	192.6	516.6	1.73	2.41	1.48	324.00
168	966	18.75	0.4	175.8	602.5	2.16	3.04	1.72	426.70
168	955	18.75	0.4	192.2	467.1	1.56	2.13	1.47	274.90
168	974	18.75	0.4	151.2	430.8	1.84	2.38	1.52	279.60
168	973	18.75	0.4	98.1	202.1	1.27	1.34	1.36	104.00
168	976	18.75	0.4	167.4	393.1	1.50	1.94	1.47	225.70
168	963	18.75	0.4	137.3	366.4	1.72	2.14	1.50	229.10
169	A0105	37.5	0.4	150.8	315.3	1.29	1.59	1.75	164.50
169	A0102	37.5	0.4	244.7	489	1.21	1.74	1.52	244.30
169	A0121	37.5	0.4	176	399.1	1.44	1.88	1.62	223.10
169	A0106	37.5	0.4	196.4	411	1.30	1.74	1.45	214.60
169	A0883	37.5	0.4	203.4	430.5	1.32	1.79	1.45	227.10
169	A0128	37.5	0.4	111	217.9	1.18	1.30	1.48	106.90
169	A0114	37.5	0.4	190.8	386.6	1.24	1.64	1.36	195.80
169	A0109	37.5	0.4	128.4	413.6	2.05	2.58	1.61	285.20

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
169	A0124	37.5	0.4	175	476.3	1.76	2.37	1.54	301.30
169	A0103	37.5	0.4	155.3	378.1	1.56	1.99	1.21	222.80
169	A0120	37.5	0.4	154	328.5	1.33	1.65	1.39	174.50
169	A0107	37.5	0.4	129.9	328.5	1.63	1.96	1.35	198.60
169	A0110	37.5	0.4	122.3	344.3	1.82	2.19	1.41	222.00
169	A0108	37.5	0.4	169.9	440.7	1.67	2.22	1.44	270.80
169	A0168	37.5	0.4	100.8	244.2	1.55	1.71	1.53	143.40
169	A0112	37.5	0.4	185.4	438.6	1.51	2.03	1.53	253.20
169	A0111	37.5	0.4	207.1	315.3	0.74	0.95	1.36	108.20
169	A0119	37.5	0.4	144.6	310	1.34	1.63	1.41	165.40
169	A0104	37.5	0.4	117.5	478.5	2.46	3.13	1.61	361.00
169	A0101	37.5	0.4	171	413	1.55	2.03	1.47	242.00
169	A0117	37.5	0.4	140.2	483.2	2.17	2.84	1.50	343.00
169	A0126	37.5	0.4	233	417	1.02	1.41	1.47	184.00
169	A0097	37.5	0.4	220	463.6	1.31	1.82	1.56	243.60
169	A0122	37.5	0.4	254.6	594	1.49	2.21	1.70	339.40
169	A0099	37.5	0.4	130.6	262.9	1.23	1.43	1.32	132.30
169	A0113	37.5	0.4	144.4	505.2	2.20	2.91	1.44	360.80
169	A0100	37.5	0.4	168.2	414	1.58	2.07	1.44	245.80
169	A0127	37.5	0.4	112.4	369	2.09	2.51	1.72	256.60
169	A0123	37.5	0.4	168.6	582.6	2.18	3.03	1.54	414.00
169	A0918	37.5	0.4	131.3	354.2	1.74	2.13	1.35	222.90
170	A0147	18.75	0.4	221.3	492.4	1.40	1.98	1.58	271.10
170	A0131	18.75	0.4	195	721.9	2.30	3.40	1.66	526.90
170	A0152	18.75	0.4	176.3	388	1.38	1.81	1.71	211.70
170	A0140	18.75	0.4	229.1	583.6	1.64	2.40	1.42	354.50
170	A0154	18.75	0.4	146.2	429.5	1.89	2.44	2.04	283.30
170	A0150	18.75	0.4	184	545.7	1.91	2.66	1.56	361.70
170	A0148	18.75	0.4	141.6	450.9	2.03	2.63	1.67	309.30
170	A0146	18.75	0.4	193.6	448.8	1.48	2.00	1.58	255.20
170	A0155	18.75	0.4	144	411	1.84	2.35	1.52	267.00
170	789	18.75	0.4	175.8	393.5	1.41	1.85	1.40	217.70
170	A0132	18.75	0.4	252.4	730.6	1.86	2.88	1.76	478.20
170	A0141	18.75	0.4	241.5	522.9	1.36	1.96	1.46	281.40
170	A0133	18.75	0.4	225	661.7	1.89	2.82	1.63	436.70
170	A0134	18.75	0.4	177.6	410	1.47	1.94	1.47	232.40
170	A0159	18.75	0.4	198.2	540	1.76	2.48	1.40	341.80
170	A0135	18.75	0.4	203.9	503	1.58	2.21	1.70	299.10
170	A0139	18.75	0.4	187.4	503.6	1.73	2.39	1.54	316.20
170	A0142	18.75	0.4	240	630.1	1.69	2.52	1.43	390.10
170	A0158	18.75	0.4	193.6	762.3	2.40	3.59	1.72	568.70
170	A0144	18.75	0.4	121.5	305.5	1.62	1.91	1.52	184.00
170	A0129	18.75	0.4	191.2	532.7	1.80	2.51	1.31	341.50
170	A0151	18.75	0.4	149.7	439.9	1.89	2.46	1.43	290.20
170	A0156	18.75	0.4	192.2	364.7	1.12	1.47	1.39	172.50
170	A0145	18.75	0.4	251	577	1.46	2.16	1.51	326.00
171	A0227	37.5	0	199.2	387.1	1.17	1.55	1.45	187.90
171	A0237	37.5	0	170.7	322.3	1.12	1.40	1.32	151.60
171	A0250	37.5	0	145.4	306.2	1.31	1.59	1.26	160.80
171	A0249	37.5	0	200.3	281.5	0.60	0.75	1.23	81.20
171	A0230	37.5	0	153.6	398.6	1.67	2.15	1.62	245.00
171	A0246	37.5	0	102.4	204.1	1.21	1.29	1.31	101.70
171	A0244	37.5	0	182.1	407	1.41	1.87	1.48	224.90

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
171	A0223	37.5	0	113.6	248.7	1.37	1.55	1.38	135.10
171	A0245	37.5	0	169.4	382.7	1.43	1.85	1.42	213.30
171	A0242	37.5	0	223.9	457.8	1.25	1.75	1.46	233.90
171	A0243	37.5	0	218	529	1.56	2.21	1.53	311.00
171	A0236	37.5	0	178.6	368.1	1.27	1.64	1.36	189.50
171	A0221	37.5	0	221.5	408.2	1.07	1.46	1.37	186.70
171	A0229	37.5	0	158.2	319.8	1.23	1.53	1.38	161.60
171	A0222	37.5	0	187.2	390.5	1.29	1.70	1.55	203.30
171	A0228	37.5	0	238	570.8	1.53	2.25	1.47	332.80
171	A0463	37.5	0	255.4	387.7	0.73	1.01	1.30	132.30
171	A0238	37.5	0	186.1	443.3	1.52	2.05	1.52	257.20
171	A0226	37.5	0	137.3	314.4	1.45	1.76	1.29	177.10
171	A0224	37.5	0	180.7	361.8	1.22	1.58	1.41	181.10
171	A0225	37.5	0	211.9	447	1.31	1.80	1.49	235.10
171	A0232	37.5	0	171.7	409.6	1.53	2.00	1.58	237.90
172	A0351	0	0	188.1	482.4	1.65	2.26	1.47	294.30
172	A0193	0	0	213.7	579.4	1.75	2.52	1.58	365.70
172	A0202	0	0	206.2	620.5	1.93	2.81	1.51	414.30
172	A0200	0	0	197.8	538.8	1.76	2.47	1.51	341.00
172	A0207	0	0	94.6	193	1.25	1.31	1.40	98.40
172	A0210	0	0	200	373.5	1.10	1.45	1.53	173.50
172	A0212	0	0	159.2	551.3	2.18	2.98	1.68	392.10
172	A0217	0	0	141.1	373	1.71	2.13	1.38	231.90
172	A0209	0	0	240.6	665	1.78	2.69	1.56	424.40
172	A0215	0	0	104.5	390.5	2.31	2.78	1.88	286.00
172	A0206	0	0	156.3	457.7	1.88	2.48	1.51	301.40
172	A0208	0	0	95.7	315	2.09	2.39	1.65	219.30
172	A0216	0	0	176.5	493.2	1.80	2.45	1.45	316.70
172	A0211	0	0	196.2	439	1.41	1.92	1.28	242.80
172	A0195	0	0	257.3	796.1	1.98	3.11	1.64	538.80
172	A0213	0	0	177.8	579.8	2.07	2.91	1.50	402.00
172	A0203	0	0	161	513.7	2.04	2.75	1.60	352.70
172	A0201	0	0	176.1	432.5	1.58	2.10	1.48	256.40
172	A0204	0	0	168	543	2.06	2.83	1.79	375.00
172	A0220	0	0	122.8	405.2	2.09	2.60	1.39	282.40
172	A0197	0	0	170	393.3	1.47	1.91	1.39	223.30
172	A0196	0	0	161.4	380	1.50	1.93	1.71	218.60
172	A0205	0	0	158.4	545.6	2.17	2.96	1.56	387.20
172	A0198	0	0	168.3	403.2	1.53	2.00	1.44	234.90
172	A0352	0	0	166.5	378.9	1.44	1.86	1.34	212.40
172	A0191	0	0	132.3	355	1.73	2.13	1.47	222.70
173	A0453	0	0.2	185.1	633.3	2.16	3.09	1.52	448.20
173	A0337	0	0.2	173.2	376	1.36	1.76	1.44	202.80
173	A0336	0	0.2	159.3	384	1.54	1.98	1.42	224.70
173	A0330	0	0.2	197.4	540.5	1.77	2.49	1.40	343.10
173	A0338	0	0.2	158.8	448.6	1.82	2.40	1.31	289.80
173	A0341	0	0.2	273.4	823.6	1.93	3.09	1.50	550.20
173	A0324	0	0.2	197.9	533.1	1.74	2.44	1.52	335.20
173	A0332	0	0.2	177	483.6	1.76	2.39	1.52	306.60
173	A0333	0	0.2	175	686.2	2.40	3.45	1.66	511.20
173	A0342	0	0.2	147.3	385.8	1.69	2.14	1.76	238.50
173	A0322	0	0.2	148.3	500	2.13	2.83	1.60	351.70
173	A0346	0	0.2	220.3	707.8	2.05	3.07	1.62	487.50

Tank	Fish ID	% M.G.	P Sup.	In. Weight	F. Weight	SGR	GF3	C. Factor	Weight gain
173	0A327	0	0.2	140.8	547.8	2.38	3.19	1.52	407.00
173	A0343	0	0.2	156.3	621.9	2.42	3.37	1.90	465.60
173	A0349	0	0.2	153.7	473.9	1.98	2.61	1.64	320.20
173	A0347	0	0.2	181	624.1	2.17	3.09	1.66	443.10
173	A0340	0	0.2	206.4	462.3	1.41	1.95	1.37	255.90
173	A0344	0	0.2	191.4	371.3	1.16	1.52	1.38	179.90
173	A0348	0	0.2	181.3	520.7	1.85	2.55	1.70	339.40
173	A0331	0	0.2	155.7	569.7	2.28	3.12	1.69	414.00
173	A0323	0	0.2	212.6	638	1.93	2.83	1.71	425.40
174	A0383	18.75	0	170.4	371.4	1.37	1.76	1.40	201.00
174	A0386	18.75	0	202.8	428.6	1.31	1.78	1.42	225.80
174	A0374	18.75	0	118.1	325	1.78	2.11	1.37	206.90
174	A0387	18.75	0	194.7	606.4	1.99	2.86	1.46	411.70
174	A0400	18.75	0	146.8	341.1	1.48	1.83	1.40	194.30
174	A0398	18.75	0	229.5	516.9	1.42	2.04	1.51	287.40
174	A0395	18.75	0	150.5	351	1.49	1.86	1.31	200.50
174	A0376	18.75	0	206.4	632.3	1.96	2.86	1.59	425.90
174	A0392	18.75	0	105.4	397.4	2.33	2.81	1.44	292.00
174	A0394	18.75	0	161.5	572	2.22	3.06	1.88	410.50
174	A0397	18.75	0	104.2	309.6	1.91	2.20	1.34	205.40
174	A0393	18.75	0	222	458.7	1.27	1.77	1.54	236.70
174	A0389	18.75	0	127.8	326.6	1.65	1.98	1.30	198.80
174	A0375	18.75	0	180	408	1.44	1.90	1.44	228.00
174	A0399	18.75	0	191.9	609.8	2.03	2.90	1.71	417.90
174	A0391	18.75	0	198	466	1.50	2.06	1.58	268.00
174	A0384	18.75	0	150.1	300.5	1.22	1.48	1.37	150.40
174	A0377	18.75	0	187.3	718.3	2.36	3.46	1.78	531.00
174	A0372	18.75	0	185.2	477.9	1.66	2.27	1.49	292.70
174	A0396	18.75	0	130.4	484	2.30	2.98	1.66	353.60
174	A0381	18.75	0	118.3	388.1	2.08	2.55	1.24	269.80
174	A0388	18.75	0	179.2	503.3	1.81	2.48	1.51	324.10
174	A0355	18.75	0	159.6	530.2	2.11	2.86	1.48	370.60

ANNEX II

Table B - Individual mineral composition of the sampled fish, fed the experimental diets (µg/g dry weight).

Fish ID	% M.G.	P Sup. %	P	Ca	Zn	K	Fe	Mg	Mn	Na	Sr
896	18.75	0.2	3350	3783	32	2379	13.8	276	1.2	729	11.4
882	18.75	0.2	3343	3381	24.6	3822	12.6	300	1.1	670	8.7
897	18.75	0.2	4648	4538	31	3256	15.2	379	1.6	984	14.1
892	18.75	0.2	3638	3982	28.3	2764	13.4	310	1.3	811	13.5
863	37.5	0	4427	4525	31.5	2984	14.2	358	0.9	919	15.2
857	37.5	0	4537	4721	30.8	3212	14.2	377	1.1	961	14.9
811	37.5	0	4097	4340	32.4	3005	14.4	338	1.4	828	13.9
858	37.5	0	3614	4105	31.4	2721	15.7	314	1.8	852	10.6
839	37.5	0.2	3447	3992	34.2	2497	15.5	295	0.9	869	10.6
834	37.5	0.2	2960	3363	25.9	2154	12.8	254	1.8	792	9.5
825	37.5	0.2	4358	4452	38.3	2849	16.5	350	1.5	897	13.7
836	37.5	0.2	3905	4034	27.6	2450	13.6	383	1.1	847	16
850	37.5	0.4	3713	4214	34.1	2693	15.1	325	1.2	942	13.3
846	37.5	0.4	3773	4204	32.8	2624	15.3	315	1.6	855	14.1
A0952	37.5	0.4	3248	3279	27.8	2648	22.7	387	1.8	843	8.2
A0959	37.5	0.4	3110	3683	32.6	2125	13.5	264	1.3	740	10.3
A0994	0	0.4	3120	3613	23.7	2264	10.1	261	1.2	658	9.4
A0902	0	0.4	3272	3489	23.9	2009	11.6	249	1.3	668	11.8
A0980	0	0.4	3264	3641	31.2	2458	12.9	234	1.1	822	9.4
A0903	0	0.4	3739	4053	30.7	2496	13.1	297	1.9	820	1.5
A0932	0	0	3080	3579	22.1	2140	10.7	252	0.9	642	9.9
A0925	0	0	3705	4125	34.7	2785	14.2	324	1.4	925	11.4
A0922	0	0	3898	4279	41	2793	14.1	335	1.4	964	11.4
A0490	0	0	3849	4078	34.5	3152	12.5	356	1.1	880	10.4
A0177	18.75	0.2	4526	4657	43.4	3333	12.5	375	1.1	862	16.8
A0190	18.75	0.2	3964	4415	36.8	2990	13.2	343	1.4	872	12.5
A0166	18.75	0.2	4916	4889	42	3493	13.1	399	1.5	1004	19.6
A0467	18.75	0.2	3266	3614	25.7	2238	10.7	270	1.3	700	11.7
A0949	0	0.4	3128	3671	27.9	2413	10.9	272	1	627	8.9
A0477	0	0.4	3116	3527	25.8	2276	11.8	269	1.4	703	9.6
A0587	0	0.4	2087	3368	25.5	2229	9.9	244	1	562	7.9
A0481	0	0.4	4066	4296	29.4	2878	11.5	323	1	789	13.2
755	18.75	0	3873	4349	34.5	2617	13.2	316	1.2	976	14.2
1000	18.75	0	3448	3904	29.5	2602	12.6	292	0.8	721	10.7
797	37.5	0.2	4010	4415	40.3	2823	15.7	338	1	991	14.5
988	0	0.2	2897	3341	24.6	2056	11.4	241	0.9	663	7.7
A0095	0	0.2	3771	4091	31.4	2353	17.1	299	1.2	828	11.5
984	0	0.2	3916	4324	37.3	2740	30.5	310	2.6	887	12.3
985	0	0.2	4596	6277	33.4	2496	17.2	337	1	927	15.6
969	18.75	0.4	4338	4611	39	2606	14.8	326	1.6	984	16.3
968	18.75	0.4	4315	4511	32.2	2587	13.7	339	1.5	924	15.1
980	18.75	0.4	3672	3926	32.3	2360	11.6	283	1.8	729	13.1
960	18.75	0.4	2894	2590	27.9	2613	14.7	277	1	868	6.6
A0103	37.5	0.4	5197	7147	43.4	2946	16.2	379	1.7	1087	22.1
A0119	37.5	0.4	5019	5161	41.2	3331	16.8	398	1.5	1072	18.3
A0104	37.5	0.4	2829	3154	25.6	2099	12.4	243	0.9	711	11.1
A0100	37.5	0.4	3993	4340	36.9	2677	13.3	330	1.4	1006	12.8
A0131	18.75	0.4	2348	2783	20.1	1618	9.6	188	1.4	530	8
A0155	18.75	0.4	4270	5195	33.4	2959	28.3	348	1.3	752	16.7
A0132	18.75	0.4	3275	3565	26.6	2200	10.8	252	1.2	679	11.2
A0158	18.75	0.4	2372	2569	21.7	1896	9.7	211	0.6	517	7.1
A0237	37.5	0	4373	6131	37.6	2374	14.5	327	1.8	925	19

Fish ID	% M.G.	P Sup. %	P	Ca	Zn	K	Fe	Mg	Mn	Na	Sr
A0223	37.5	0	4007	4251	38.8	2732	12.9	324	1.2	836	14.4
A0228	37.5	0	3447	3944	35.7	2453	13.1	295	1.2	828	12.5
A0463	37.5	0	5243	5164	37.3	3590	16.1	425	1.3	1152	16.5
A0209	0	0	3287	3807	25.6	2207	9.8	265	1	724	11.4
A0215	0	0	2705	2938	28.2	2693	11.4	239	1.2	737	8.2
A0213	0	0	2965	3270	23.6	2291	11.3	260	1	687	9.8
A0205	0	0	3246	3725	26.9	2275	18.8	270	1.2	730	11.2
A0333	0	0.2	3099	3376	27.1	2498	12.6	266	0.9	678	10.3
A0346	0	0.2	3233	3632	26.4	2520	12.2	271	1.4	749	8.8
0A327	0	0.2	3605	3987	29.3	2414	11.2	282	1.7	760	11.5
A0344	0	0.2	5613	3201	36.8	3054	12.1	386	1.5	975	20
A0376	18.75	0	2841	3214	25	2110	13.6	243	1.1	708	8.6
A0393	18.75	0	3952	4441	38.1	2852	17.7	330	1.2	1002	12.2
A0399	18.75	0	3033	3207	26	2446	15.1	277	1.3	733	9.3
A0355	18.75	0	3375	3538	30	2776	22	308	1.9	812	9.6