Impact of the Quality of First Food on the Digestive Enzymes and Development of the Anterior Intestine, Liver and Pancreas of Genetically Male Nile Tilapia (GMT), Oreochromis niloticus L.

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INTRODUCTION

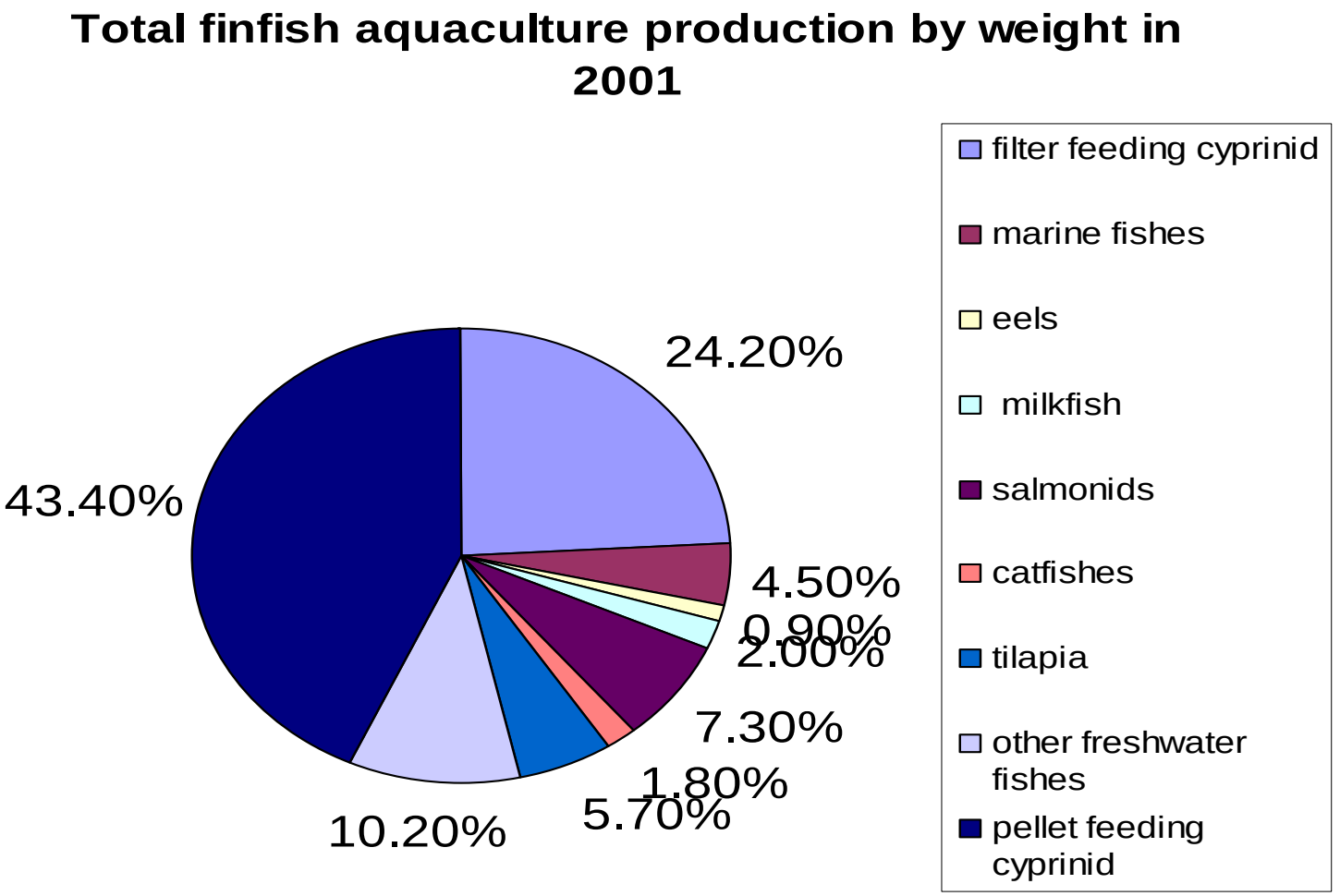
Aquaculture is the fastest animal production sector in the world

It has been dedicated in finding and answering the continuous demands for quality “aqua” foods for human consumptions

Malnutrition is the no.1 cause of deaths

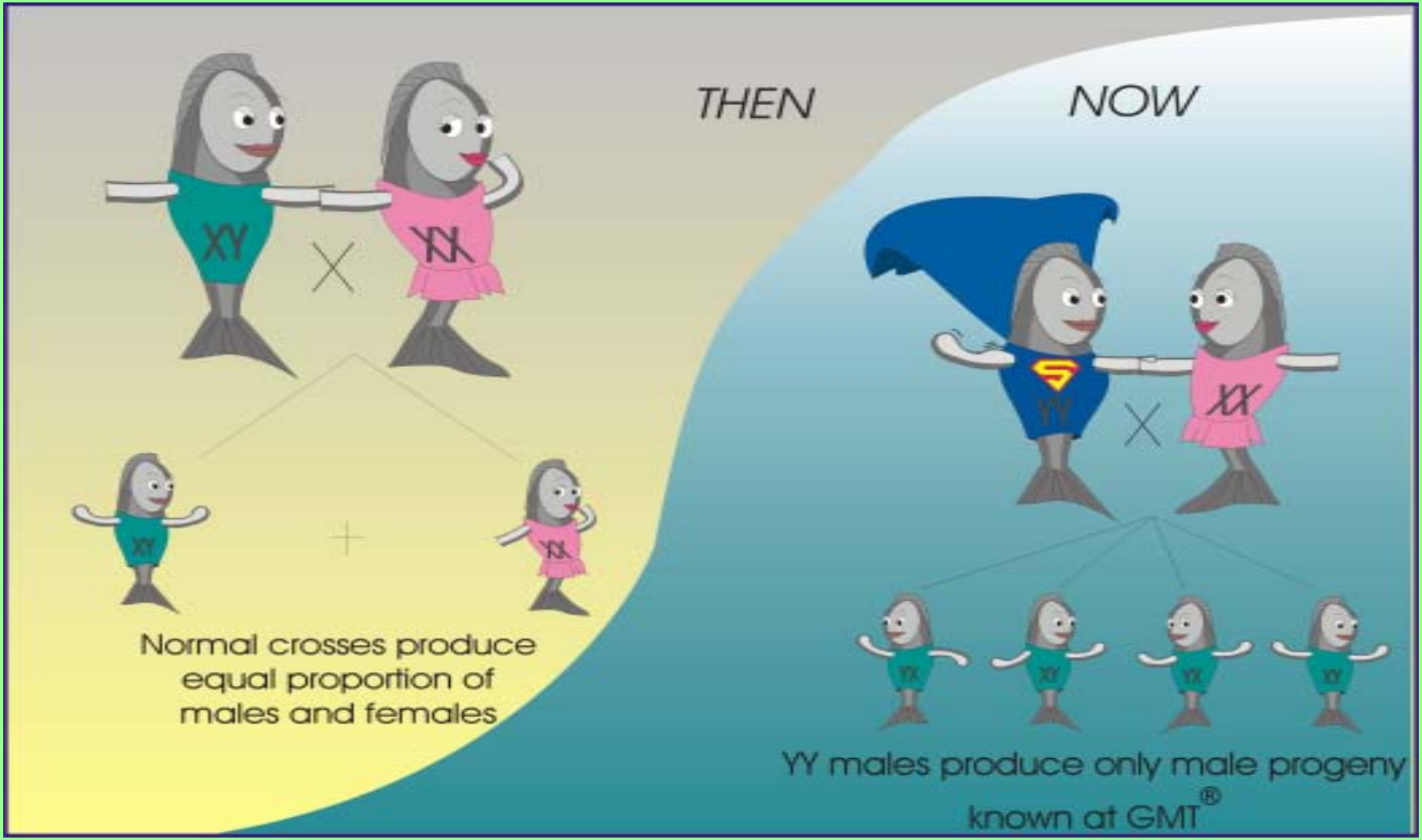
Tilapias are emerging as one of the important cultured food fish

Tilapia Production



Source : FAOSTAT 2003

GMT Production



Tilapia Nutrition

Protein is an important constituent of the fish diet. It is an essential nutrient needed for maintenance, growth and reproduction.

The optimum dietary protein level for tilapia appears to be influenced by age and size of the fish and ranges from 28%-50% (Santiago and Lovell, 1988; El-Sayed and Teshima, 1992; Shiau, 2002)

Fish meal is used as the main conventional protein source in aquaculture feeds.

Tilapia Nutrition

Dietary lipids are the only source of essential fatty acids needed by fish for normal growth and development; they are important carriers and assist in the absorption of fat-soluble vitamins.

The optimal dietary lipid level for tilapia was quantified by Chou and Shiau (1996); 5% of dietary lipid appeared to be sufficient to meet the minimal requirement of the juvenile tilapia, but a level of 12% was needed for maximal growth.

Tilapia Nutrition

Carbohydrates are poorly utilized by fish and the main sources of energy in fish appear to be protein and lipids, in contrast to mammals in which carbohydrates and lipids are more important (Ogunji and Wirth, 2000).

Cereal grain products are generally used as carbohydrate source in feed formulation.

Tilapia Nutrition

Vitamins likely to be missing in commercial tilapia rations containing oilseed meals, animal by products, and grains are: vitamins C, A, D, niacin, panthothenic acid riboflavin, and possibly vitamins E and K (Popman and Lovshin, 1994).

Because of the possible consequences of vitamin deficiency, vitamin premixes are usually added to fish feeds.

Minerals are needed by fish for osmoregulation, tissue formation and various metabolic processes.

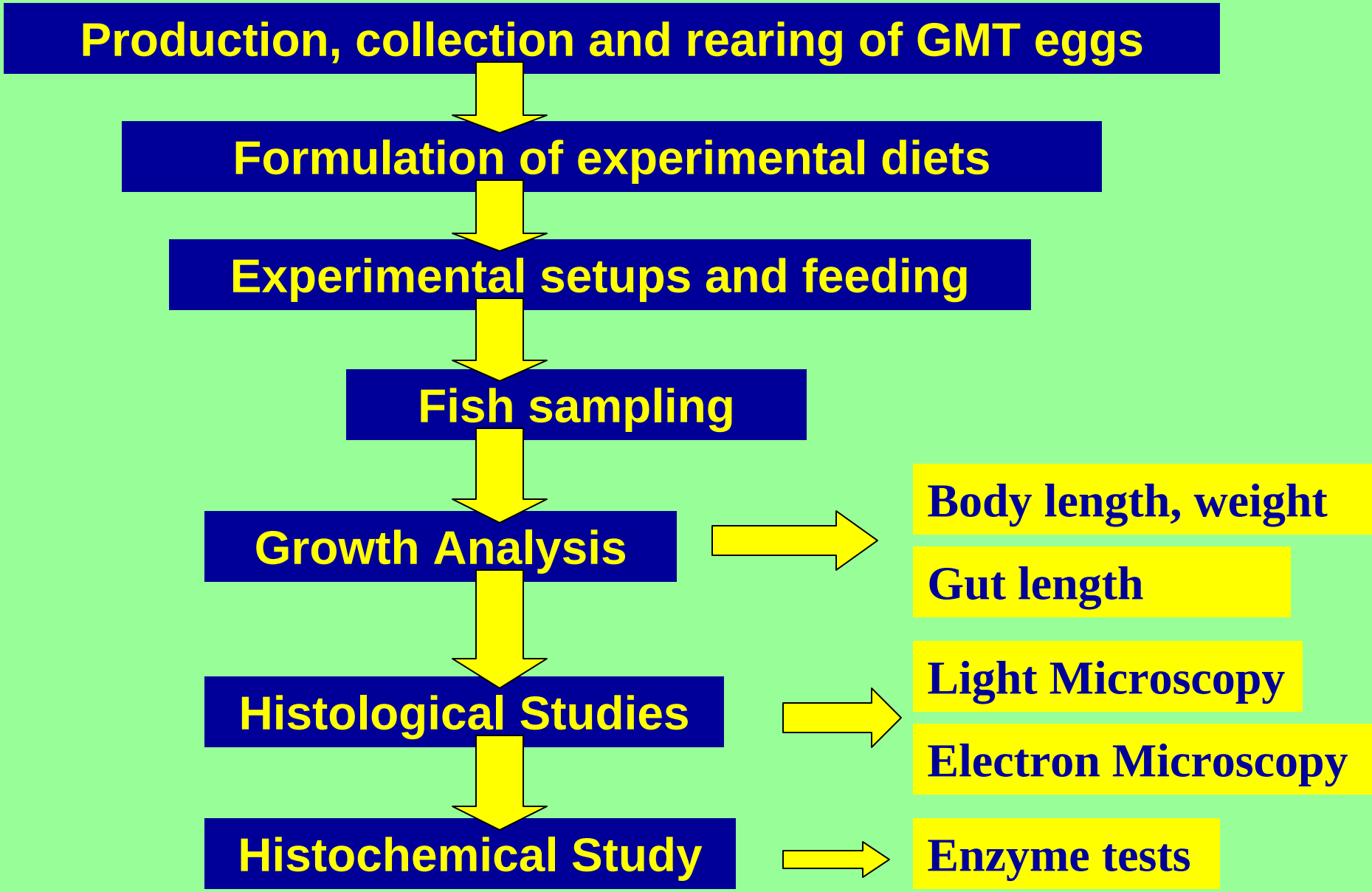
OBJECTIVES

This study was undertaken to:

- **present the development of the gut (primarily anterior intestine) and associated organs – liver and pancreas Nile tilapia fed with different first food diets through light, scanning and transmission electron microscopy.**

- **investigate the effects of the different first food diets on some enzymes – lipase, esterase, amylase and phosphatase in 150-day old Nile tilapia.**

MATERIALS AND METHODS



Production, collection and rearing of GMT eggs

Experimental Diets

DIET 1 – Plankton (*Moina*) only

DIET 2 – Fish meal + Rice bran

DIET 3 – Fry booster (Tateh)

DIET 4 – *Moina* + Fish meal + Rice bran

DIET 5 – *Moina* + Fry booster

First setup
(0-30 days post-hatch)

Second setup
(31-150 days post-hatch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Five different diets used in the study.

Treatments	Diets	
	Period I (day 0-30)	Period II (day 31-150)
T1	*Moina* (plankton)	fish meal
T2	fish meal + rice bran	fish meal
T3	fry booster	fish meal
T4	*Moina* + fish meal + rice bran	fish meal
T5	*Moina + fry booster*	fish meal

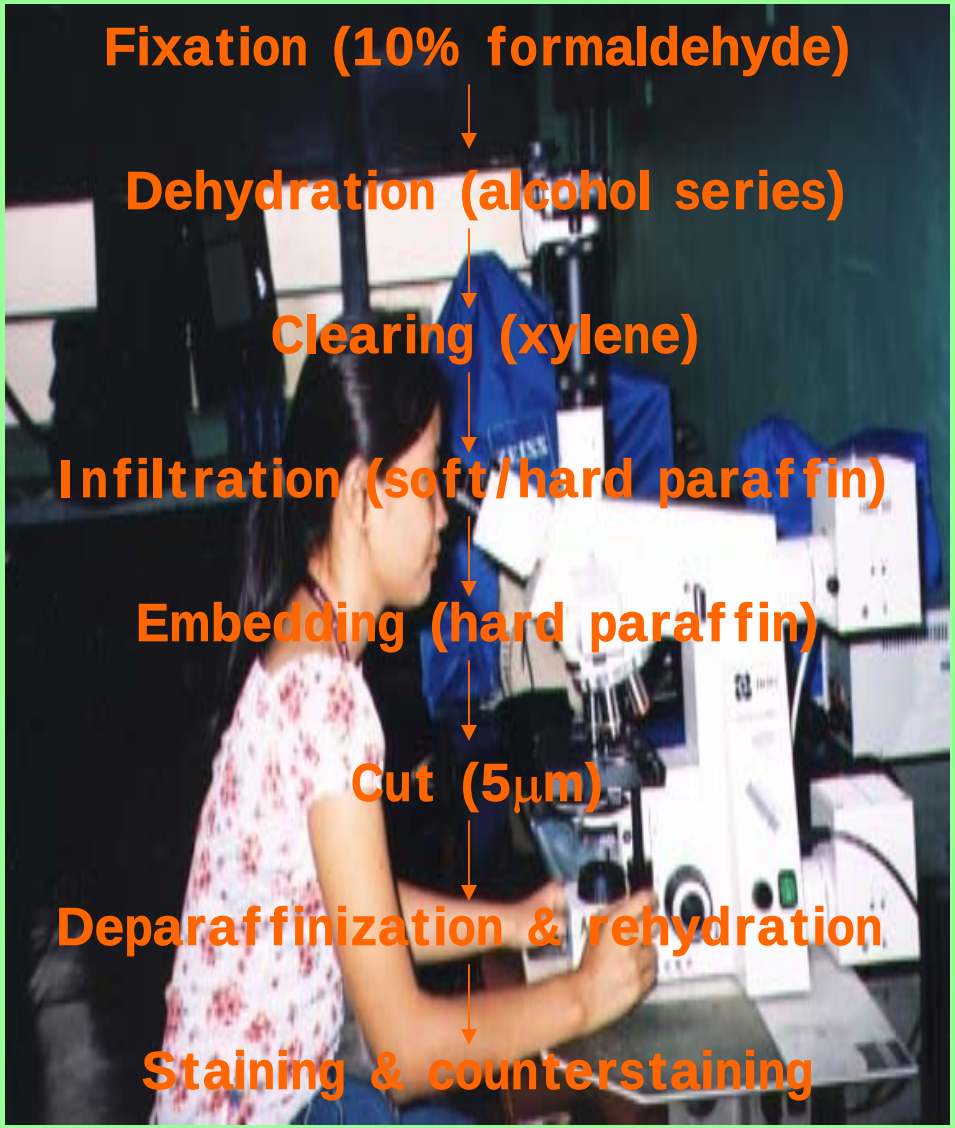
Fish Sampling

15 samples

- **per treatment (T1, T2, T3, T4, T5)**
- **per sampling date (10, 20, 30, 60, 90, 120, 150 dph)**

Histological Studies

Light Microscopy



Organ Histology

Anterior & Posterior Intestine

- muscularis, mucosal folds, goblet cells

Liver

- hepatocytes, HPV, lipid inclusions

Pancreas

- pancreatic cells, zymogen granules

Ultrastructure Studies

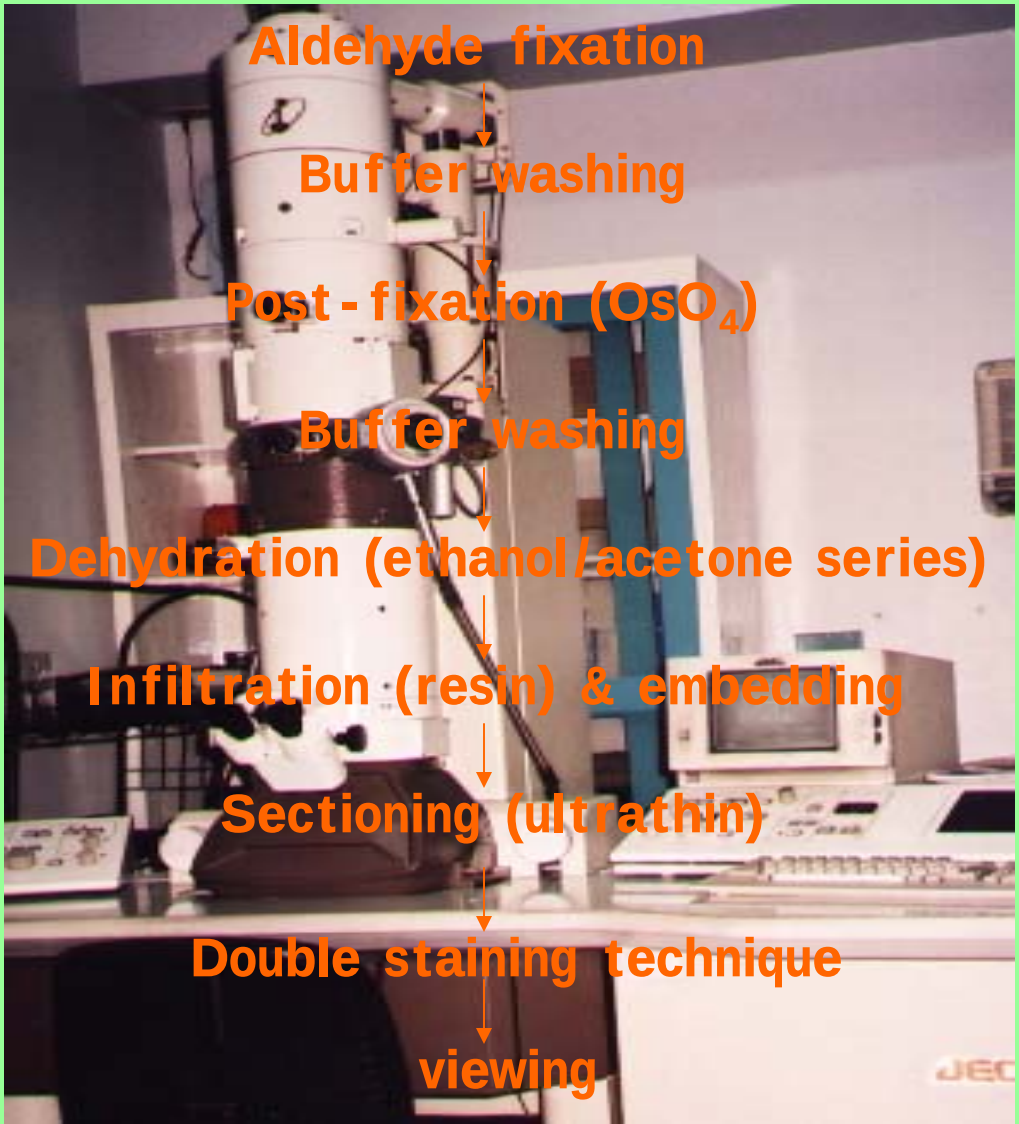
Scanning Electron Microscopy

Anterior Intestine

- **1 cm long, approximately most anterior part**
- **mucosal folds, microvilli**

Ultrastructure Studies

Transmission Electron Microscopy



Anterior Intestine

- **1 cm long, approximately most anterior part**
- **microvilli, goblet cell, mitochondria**

Enzyme Histochemistry

Cryostat cutting

Fresh samples of anterior intestine (1 cm long) and pancreas of 150-day old Nile tilapia were brought to National Kidney Institute for cryostat cutting

Enzyme tests were done at the Developmental Biology Thesis Room of Institute of Biology

Enzyme Tests

Azo-Coupling Technique for Alkaline Phosphatase

(Kiernan, 1990)

Incubation Medium

0.05M Tris buffer, pH 10.0 – 10ml

Sodium salt – 10mg

$MgCl_2$ – 10mg

Fast blue RR salt – 10mg

Result: colored purple to black

Mount cryostat sections on slides

↓

Wash sections

↓

Incubate (20mins)

↓

Transfer to H_2O (1min)

↓

Transfer to acetic acid (1min)

↓

Rinse in water

↓

Mount and cover

Enzyme Tests

Simultaneous Coupling Method for Non-specific Esterases

(After Gomori, 1952; Burstone 1962 in P.J. Stoward and A.G.E. Pearse, eds., 1980)

Incubation Medium

0.1M phosphate buffer, pH 7.4 – 20ml

α-naphthyl acetate – 0.25ml

Fast blue B – 50-100mg

Result: black

Mount cryostat sections on slides

↓

Air dry

↓

Incubate (1-15mins)

↓

Wash in running H_2O (2mins)

↓

Counterstain (4-6 mins)

↓

Wash (4-6 mins)

↓

Mount and cover

Enzyme Tests

Tween Method for Lipase

(After Gomori, 1945 in Kiernan, 1990)

Incubation Medium

0.5M Tris-HCl buffer, pH 7.4 – 5ml

10% $CaCl_2$ – 2ml

Tween 60 – 2ml

Distilled H_2O – 40ml

Mount cryostat sections on slides

↓

Air dry

↓

Incubate (3-12hrs)

↓

Wash in distilled H_2O

↓

Immerse in 1% lead nitrate (15mins)

↓

Wash in running H_2O (1-2mins)

↓

Immerse in 1% sodium sulphide (1-2mins)

↓

Wash and counterstain w/ eosin (5 min)

↓

Wash, mount and cover

Result: brownish-black

Enzyme Tests

Starch Film Method for α-Amylase

(Smith and Frommer, 1973 in P.J. Stoward and A.G.E. Pearse, eds., 1980)

Starch film

5% sol'n of starch in 0.02M borate- 0.01M NaOH buffer, pH 9.2, warm in water bath

Dip clean slides in the sol'n for 15 s, redip for 30s and air dry

Result: unstained

Mount cryostat sections on slides

↓

Air dry

↓

Fix in 50;10:50 (by vol) methanol, acetic acid and water (1h)

↓

Rinse in tap H_2O

↓

Immerse in 1% Lugol's iodine sol (1min)

↓

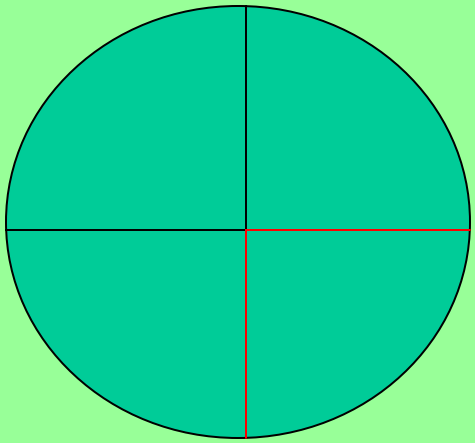
Rinse in H_2O

↓

Mount and cover

Enzyme Tests

Qualitative analysis (visual analysis) was done through the intensity of the color reactions under LPO. Subsequent color ranking scheme, quantitative analysis was employed by assigning numerical values representing the intensity of color reactions. Cells with color reaction were likewise counted by concentrating on the lower right quadrant of every section under HPO.



Statistical Analysis

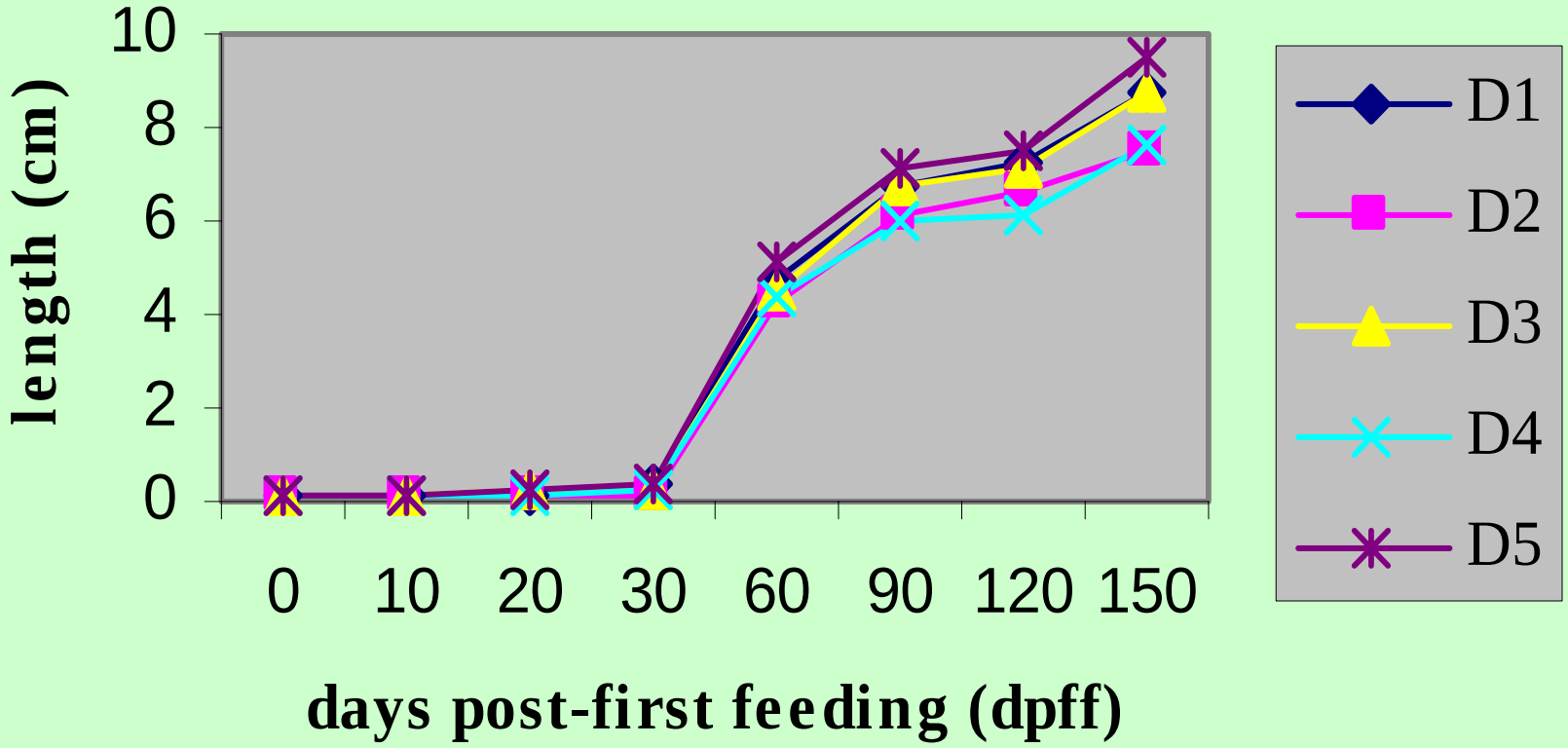
One-way ANOVA and DMRT using SAS package

- **total body length**
- **total body weight**
- **gut length**
- **anterior (muscularis, mucosal folds and goblet cells)**
- **liver (HPV)**

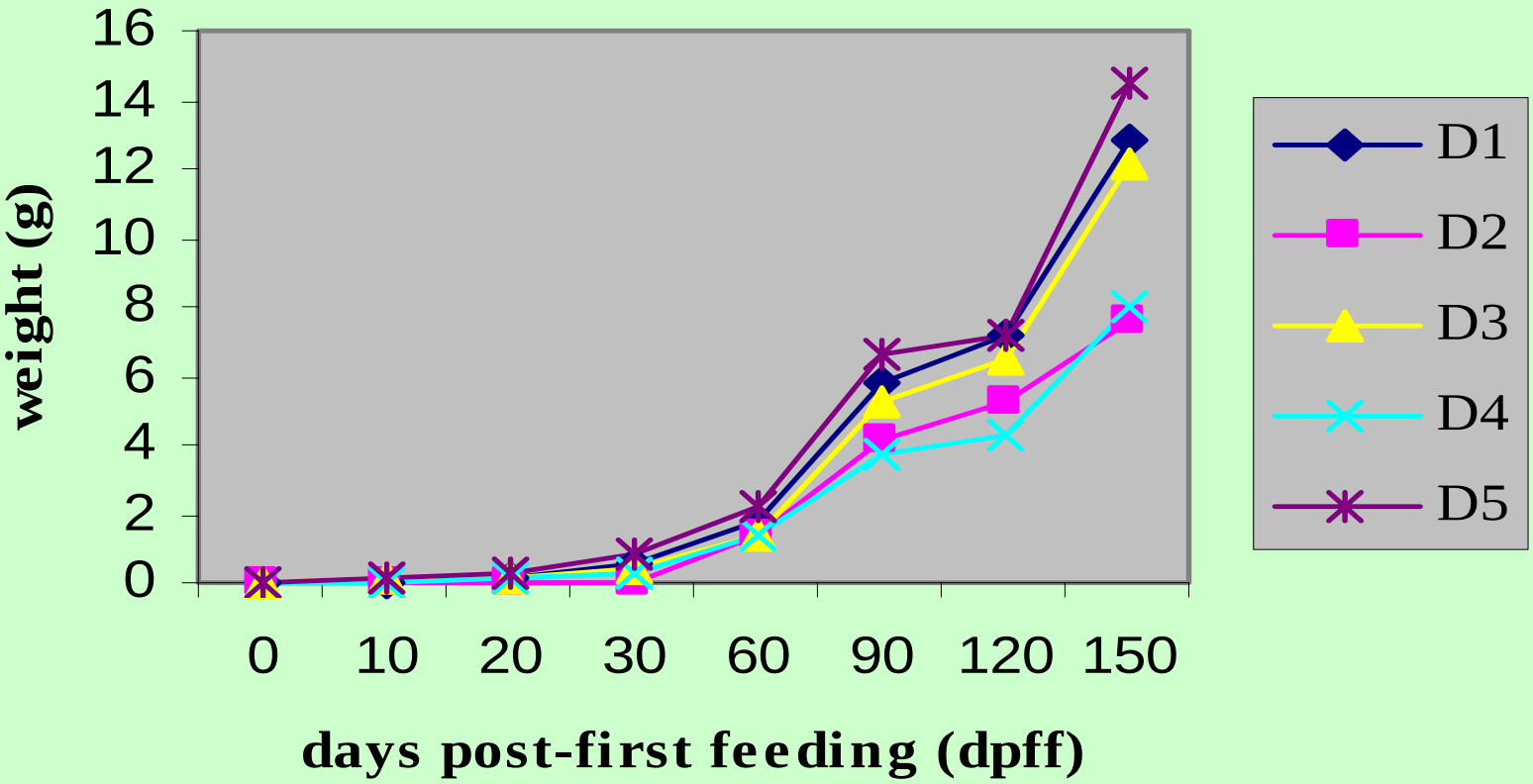
Proximate composition of the different ingredients used as experimental first food diets for Nile tilapia, *Oreochromis niloticus* L., for thirty days

Nutrients	*Moina*	Fish meal	Rice bran	Fry booster
Crude protein	50%	66.7%	11.64%	48.0% max
Crude lipid/fat	8.7%	10.5%	11.93%	12.0% min
Crude fiber	4-6%	1.4%	7.20%	5.0% max
Crude ash		20.8%	8.89%	16.0% max
Moisture				12.0% max

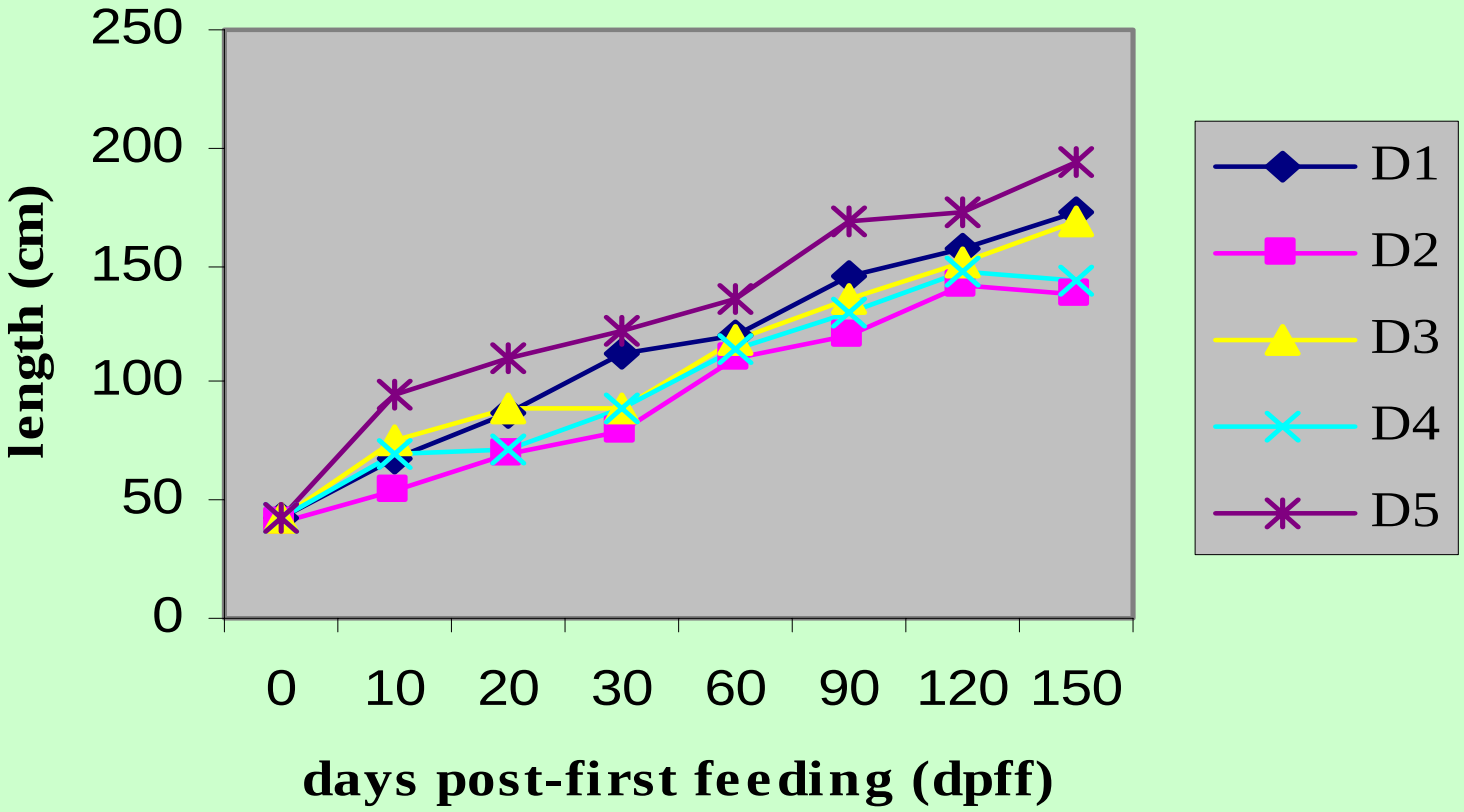
Total body length of developmental stages of *Oreochromis niloticus* L. (GMT) fed with different first food diets.



Total body weight of developmental stages of *Oreochromis niloticus* L. (GMT) fed with different first food diets.



Gut length of developmental stages of *Oreochromis niloticus* L. (GMT) fed with different first food diets.



Effects of the Different First Food Diets on the Body length and weight and Gut length

Al-Ogaily *et al.* (1996) reported a decrease of growth performance of both carp and tilapia when fed with pelleted diets containing high levels of different grains, which are high in carbohydrate.

Similar study conducted by Viola et al. (1988) concluded that inclusion of high fiber feed ingredients such as wheat bran at levels up to 60% caused impairment of growth (Swick, 2001).

The poor performance of fish fed with T2 diet may due to its higher crude fiber (7.20%) and low protein (11.64%) contents.

Effects of the Different First Food Diets on the Body length and weight and Gut length

Protein level of the diet is the most important consideration especially during the fry stage (0.5 – 10g). T2 (Fishmeal, 66.7%; Rice bran, 11.64%), T4 (*Moina*,50% ; Fishmeal, 66.7%; Rice bran, 11.64%) and T5 (Fry booster, 48%; Rice bran, 11.64%) diets have higher protein level.

In general, plant proteins are low in some essential and limiting (methionine, cystine and lysine) amino acids (Akiyama, 2001) and contain antinutritional components that have adverse effect on the growth performance.

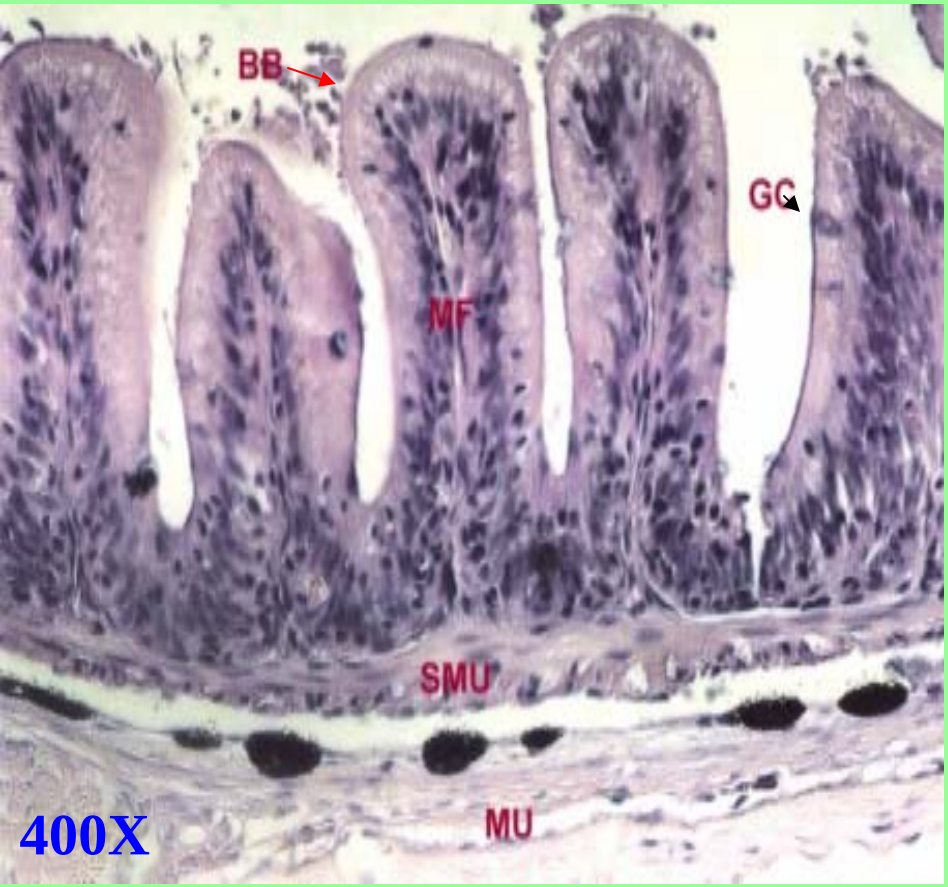
Effects of the Different First Food Diets on the Body length and weight and Gut length

Moreover, the good growth performance of the T1, T3 and T5 fish may due to the increased total fat concentration in the diets: T1 (*Moina*, 8.7%), T3 (Fry booster, 12.0%) and T5 (*Moina*, 8.7% and Fry booster, 12.0%).

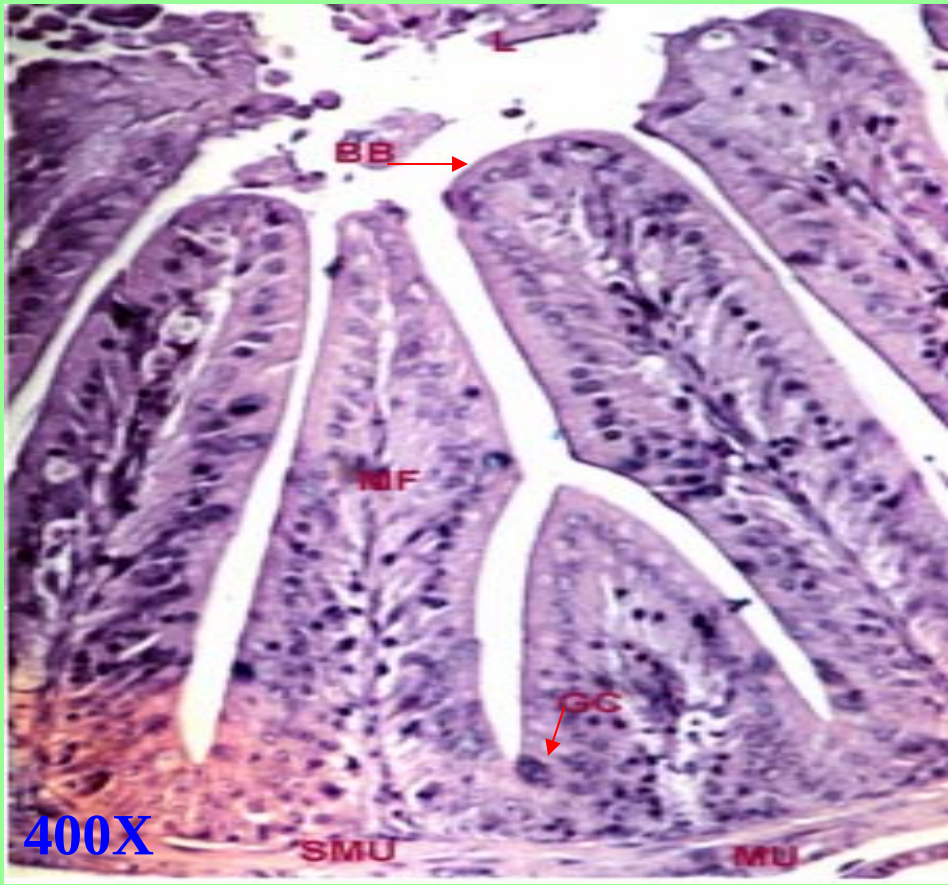
Ahlgren *et al.* (1999) found that increased total fat concentration in the diets seemed to have beneficial effects on both growth and survival of grayling raised in aquaculture systems. Lipids have protein-sparing effect.

Best growth performance of T5 fish was due to high nutrient content (high protein content – *Moina*, 50%; fry booster, 48% and high fat content - (*Moina*, 8.7% and Fry booster, 12.0%), good digestibility (low fiber content – *Moina*, 4-6%; fry booster, 5%) and palatability.

Anterior Intestine

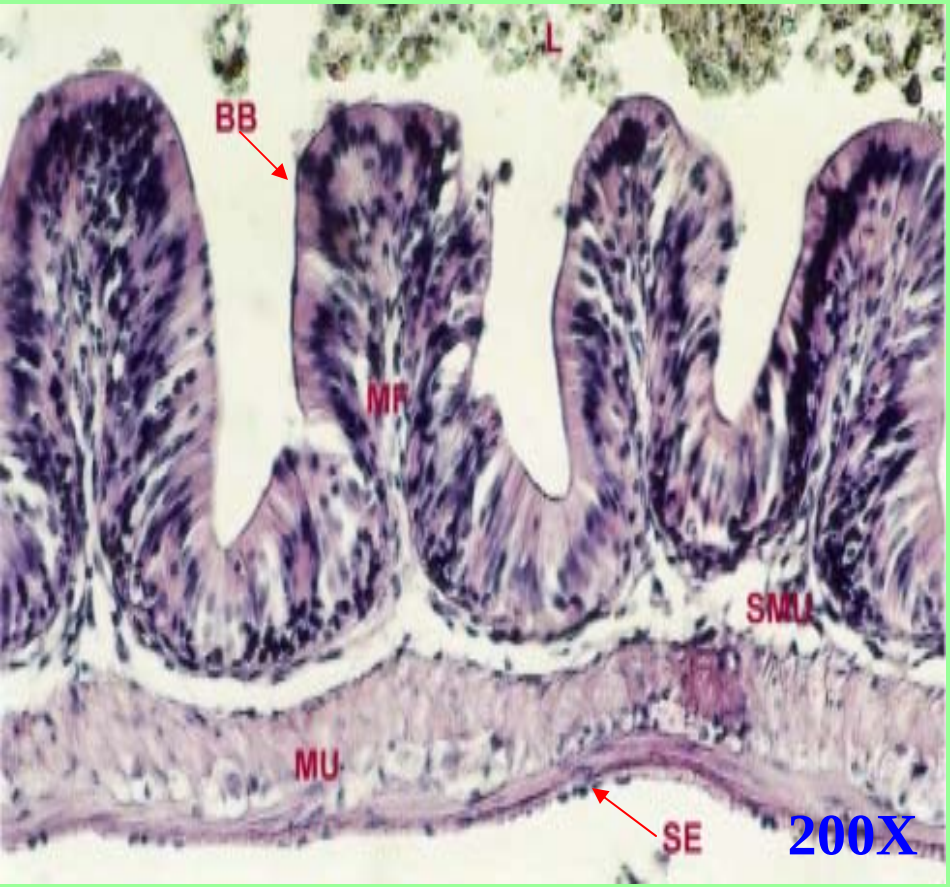


D2 – 30 dph

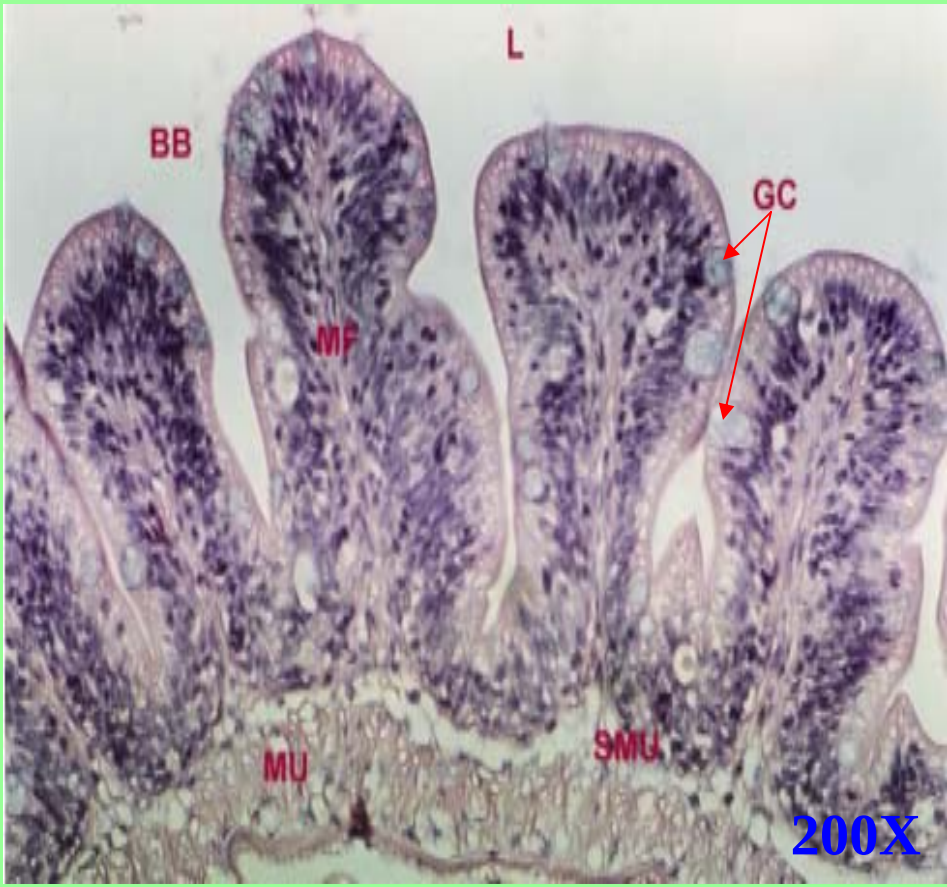


D5 – 30 dph

Anterior Intestine

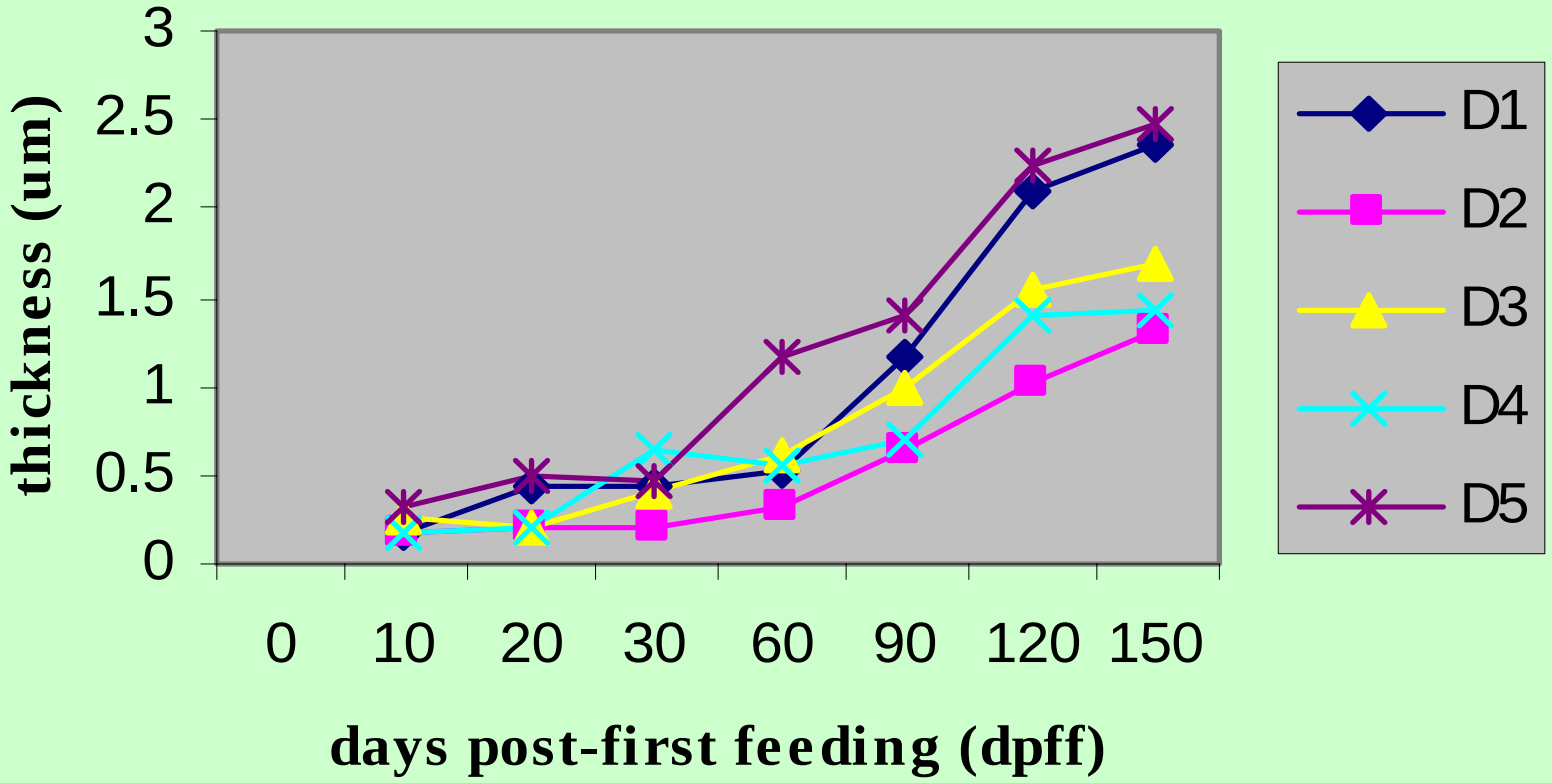


D2 – 150 dph

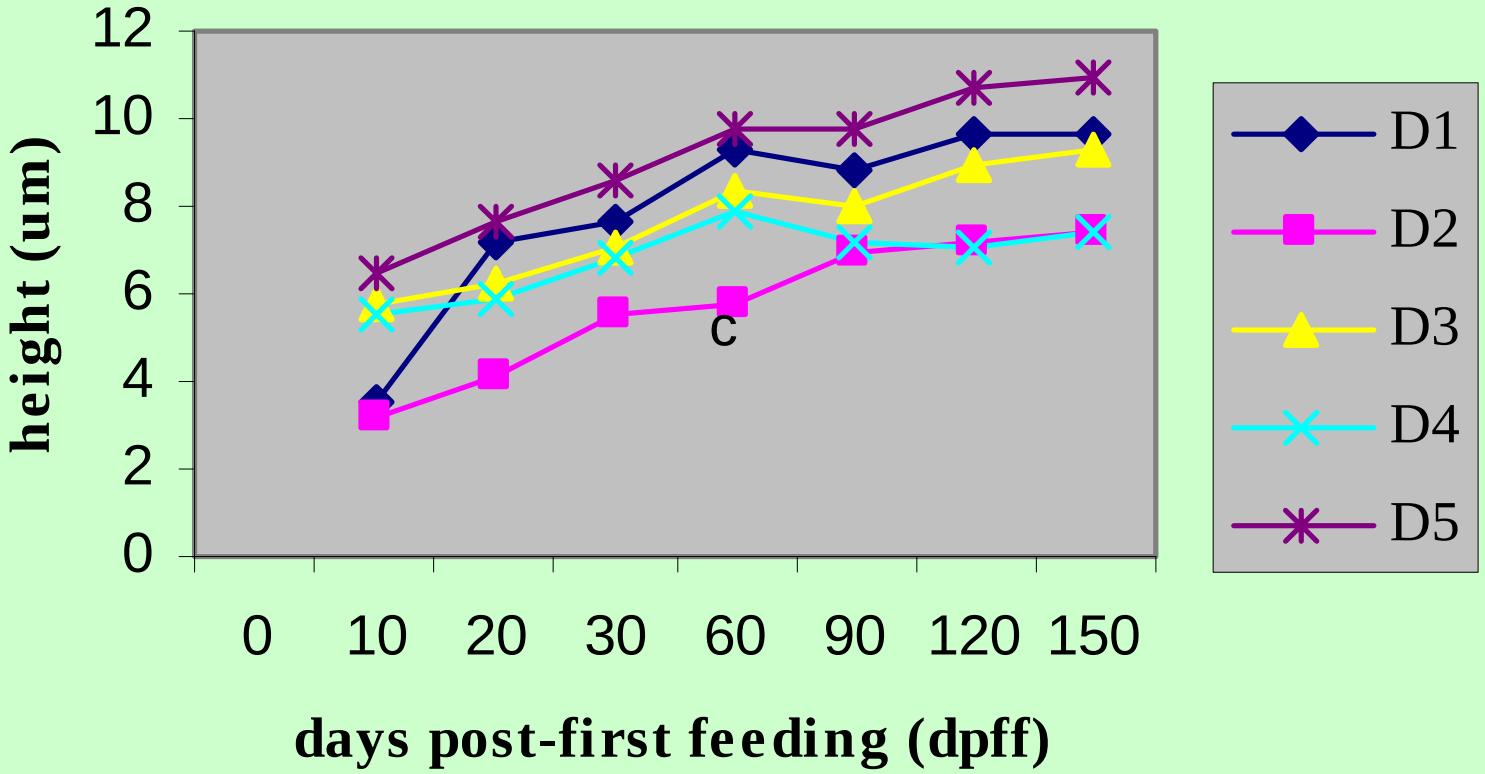


D5 – 150 dph

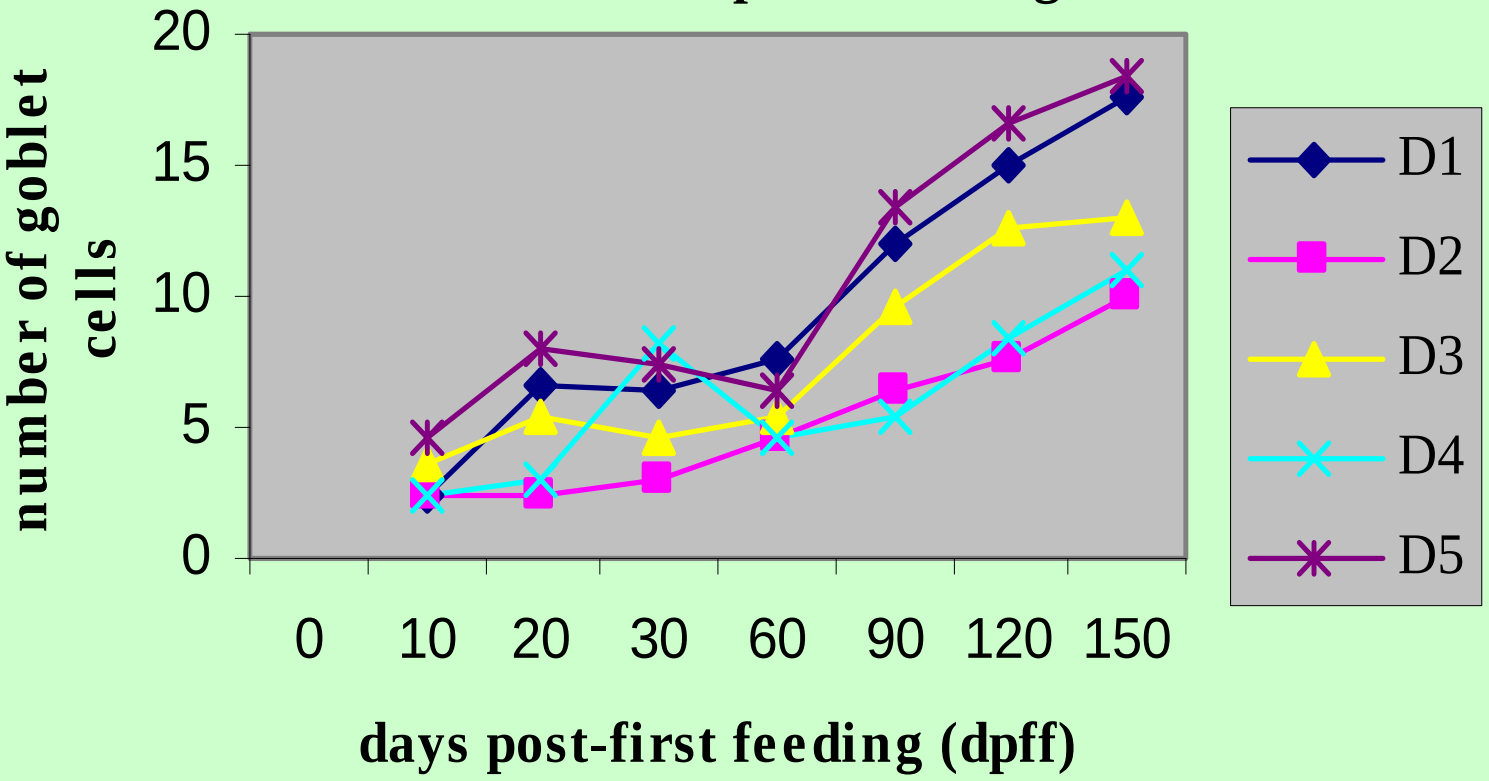
Thickness of anterior intestine muscularis of *Oreochromis niloticus* L. (GMT) in different developmental stages.



Height of anterior intestine mucosal folds of *Oreochromis niloticus* L. (GMT) in different development stages.

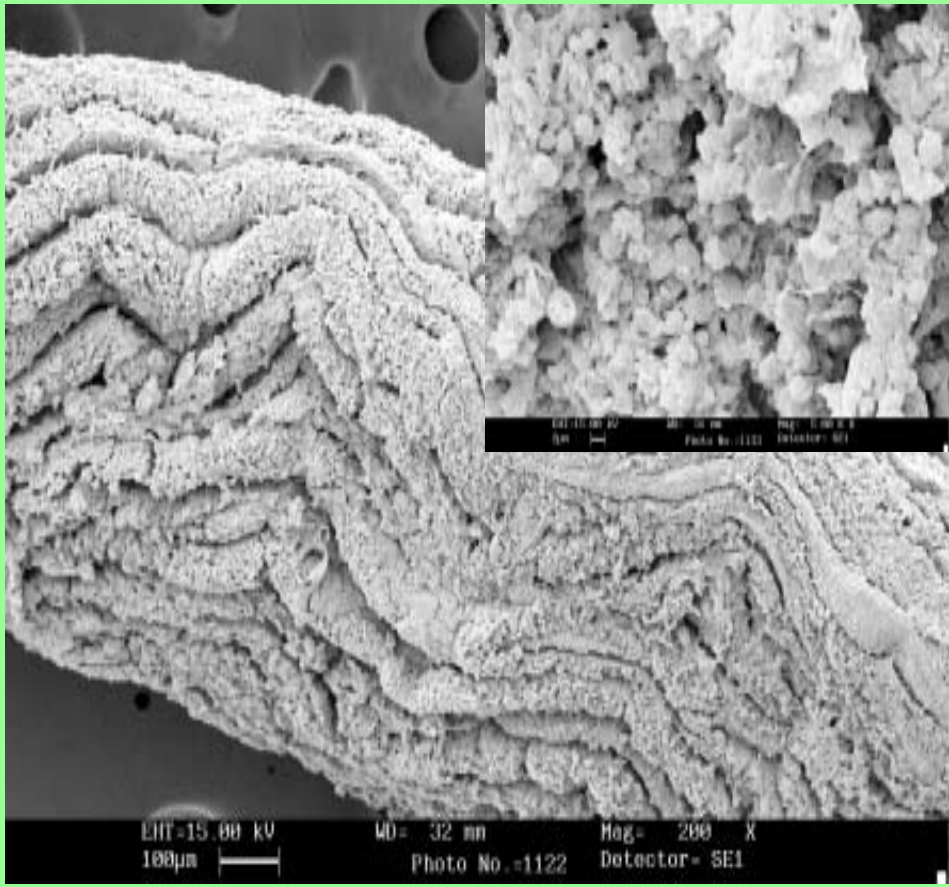


Number of goblet cells seen in the tallest section of a mucosal fold in the anterior intestine of *Oreochromis niloticus* L. (GMT) in different developmental stages.



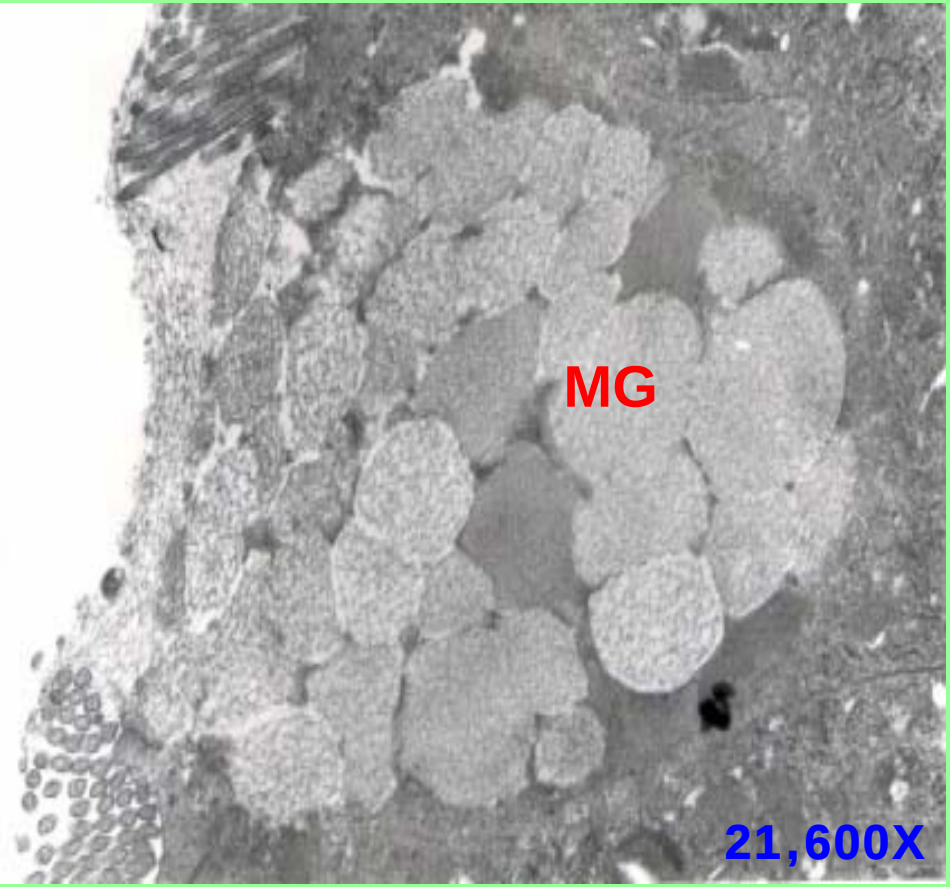
Anterior Intestine - SEM

D2 – 150 dph

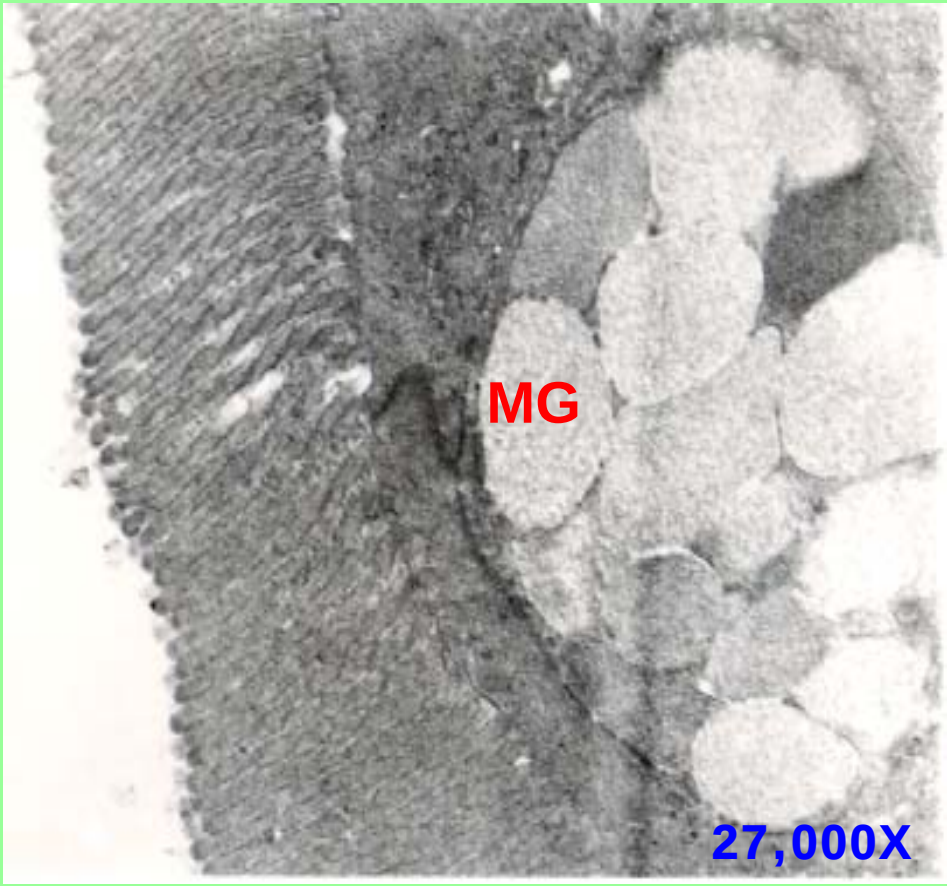


D5 – 150 dph

Anterior Intestine - TEM



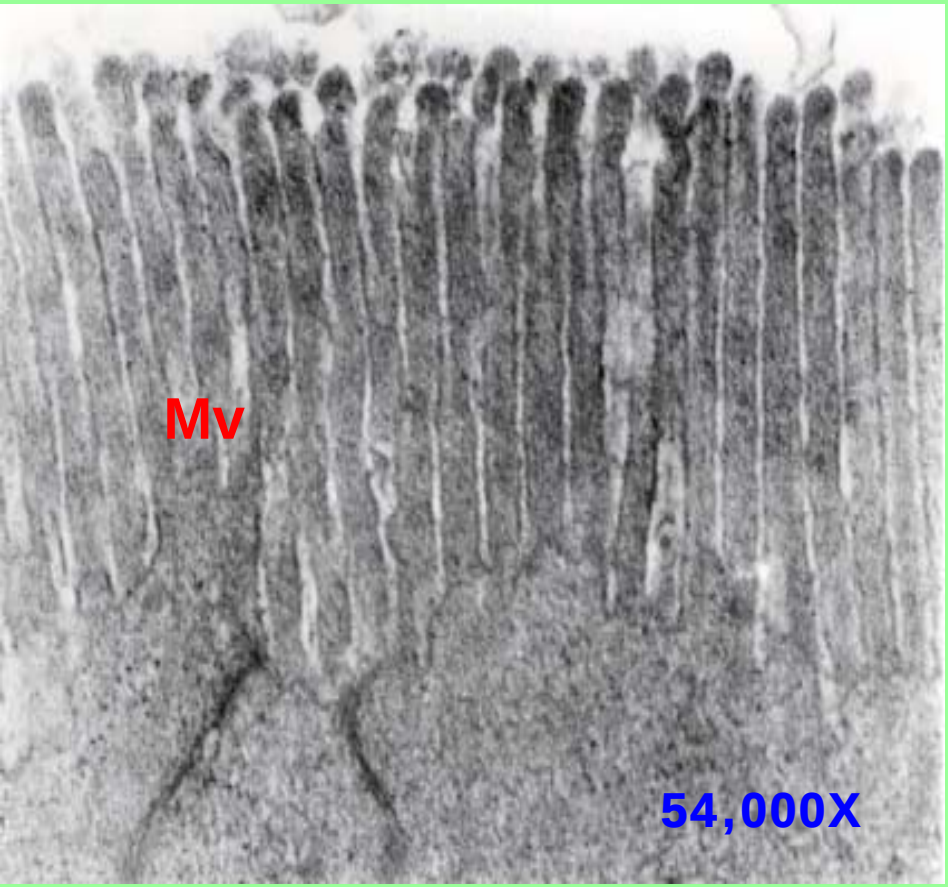
D2 – 150 dph



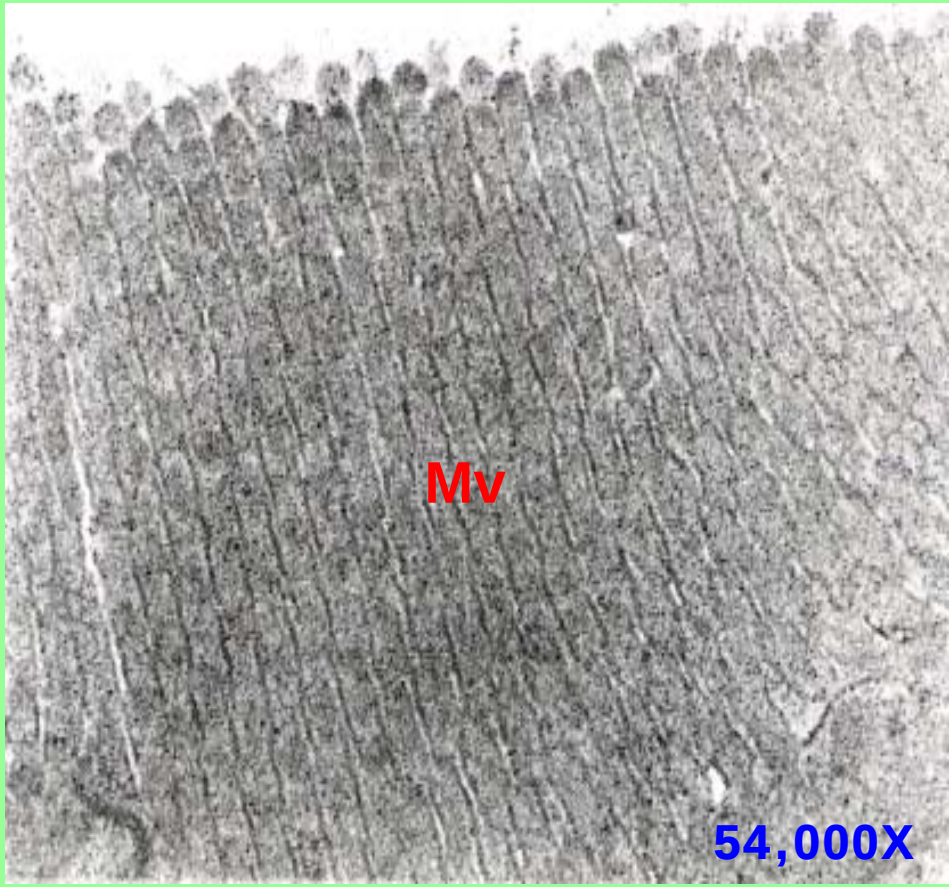
D5 – 150 dph

Goblet Cell

Anterior Intestine - TEM



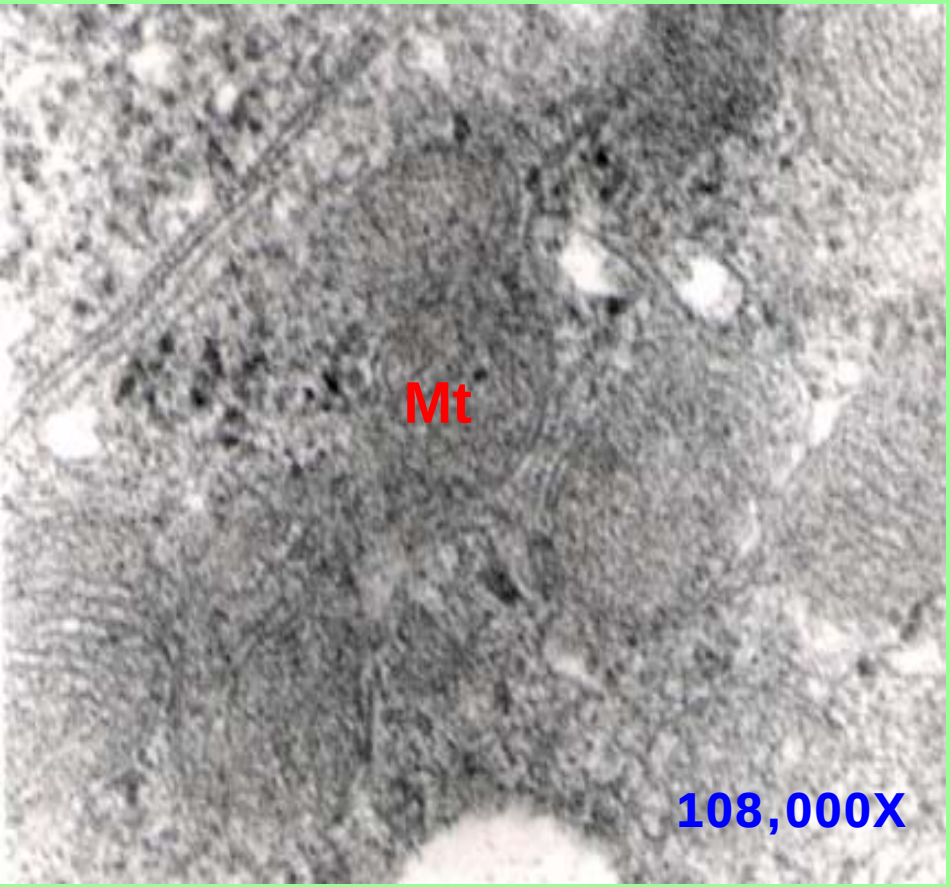
D2 – 150 dph



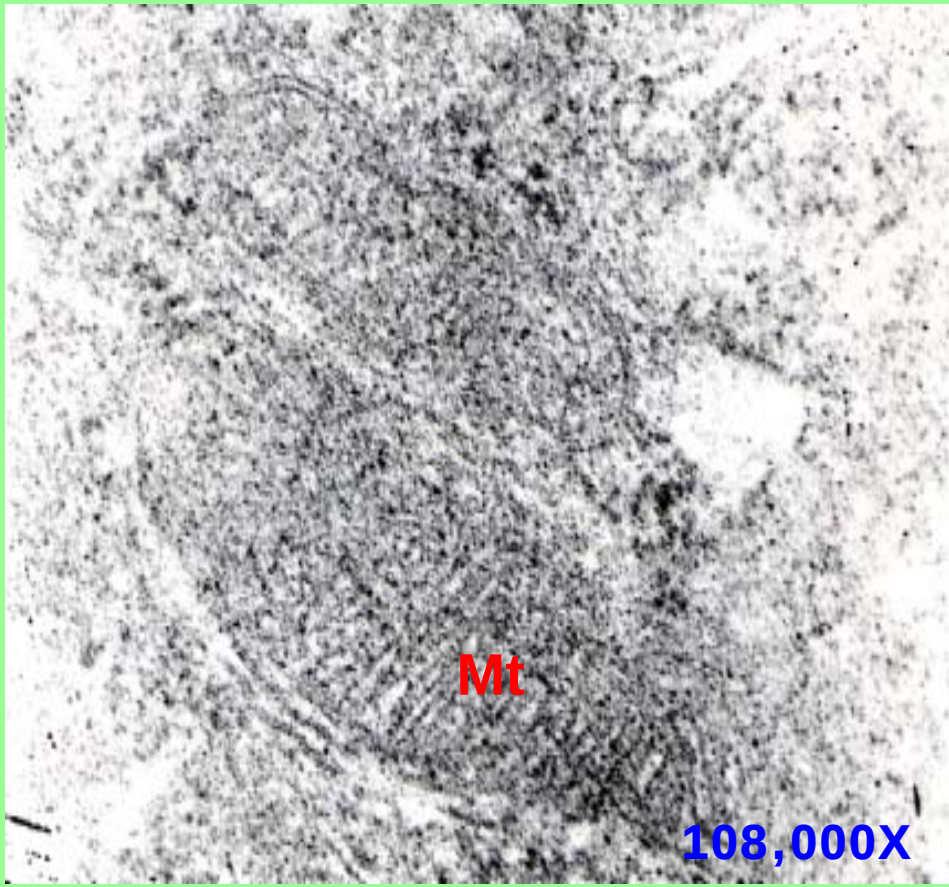
D5 – 150 dph

Microvilli

Anterior Intestine - TEM



D2 – 150 dph



D5 – 150 dph

Mitochondria

Effects of the Different First Food Diets on the Anterior Intestine

Histological changes include a reduction in the height and number of mucosal folds in winter flounders *Pseudopleoronectes americanus*, smaller and fewer mucous cells in rainbow trout *Oncorhynchus mykiss*, and a loose, fragile submucosa in the bluegill sunfish *Lepomis macrochinus* (Hall and Bellwood, 1995).

The mechanism involving these changes are well documented in mammals where it is believed that the decreased luminal concentration of nutrients, and lack of direct stimulation by food, is responsible, at least in part, for the atrophy of the intestinal mucosa (Hall and Bellwood, 1995).

Effects of the Different First Food Diets on the Anterior Intestine

The atrophy of the epithelium demonstrated in both stomach and intestine during starvation, is due to the use of the atrophied tissue for nourishment (Hall and Bellwood, 1995)

In fish fed with T2 diet, there was a reduced intake of food and less absorbed nutrients as shown in the marked decrease of growth performance. This may due to marginal level of nutrients of rice bran, poor digestibility and palatability.

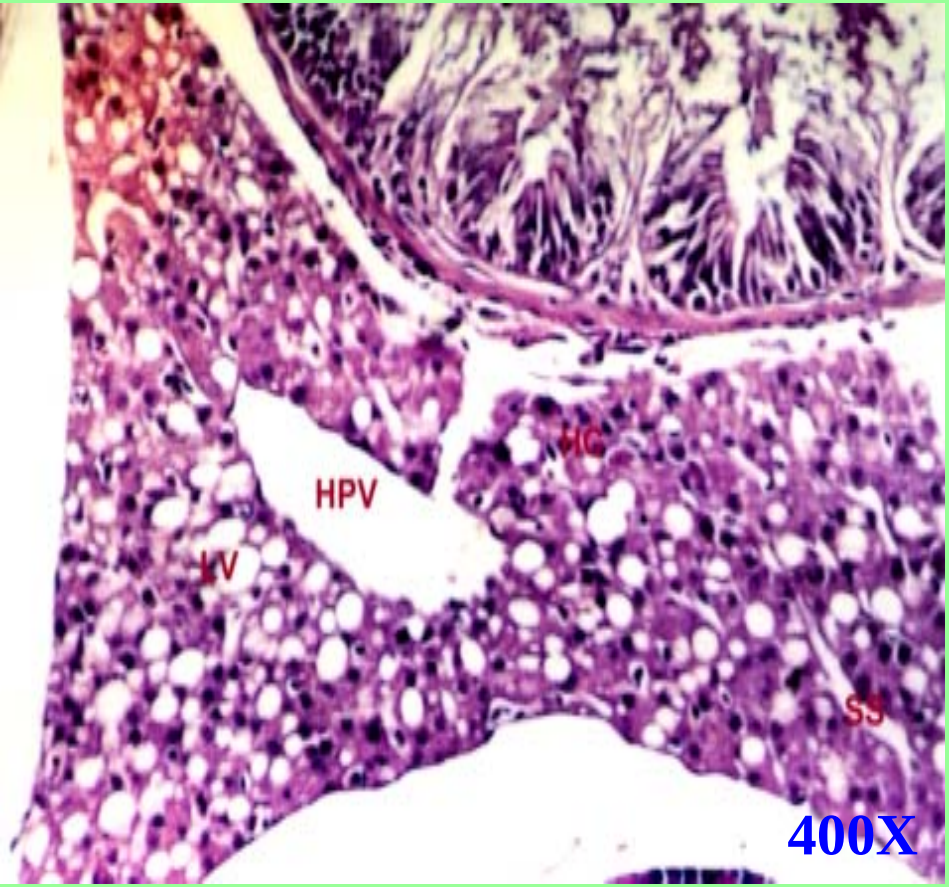
Possibly, decrease of food intake, poorer digestion and absorption affected the intestine by inhibiting maximal development of gut tissues.

Effects of the Different First Food Diets on the Anterior Intestine

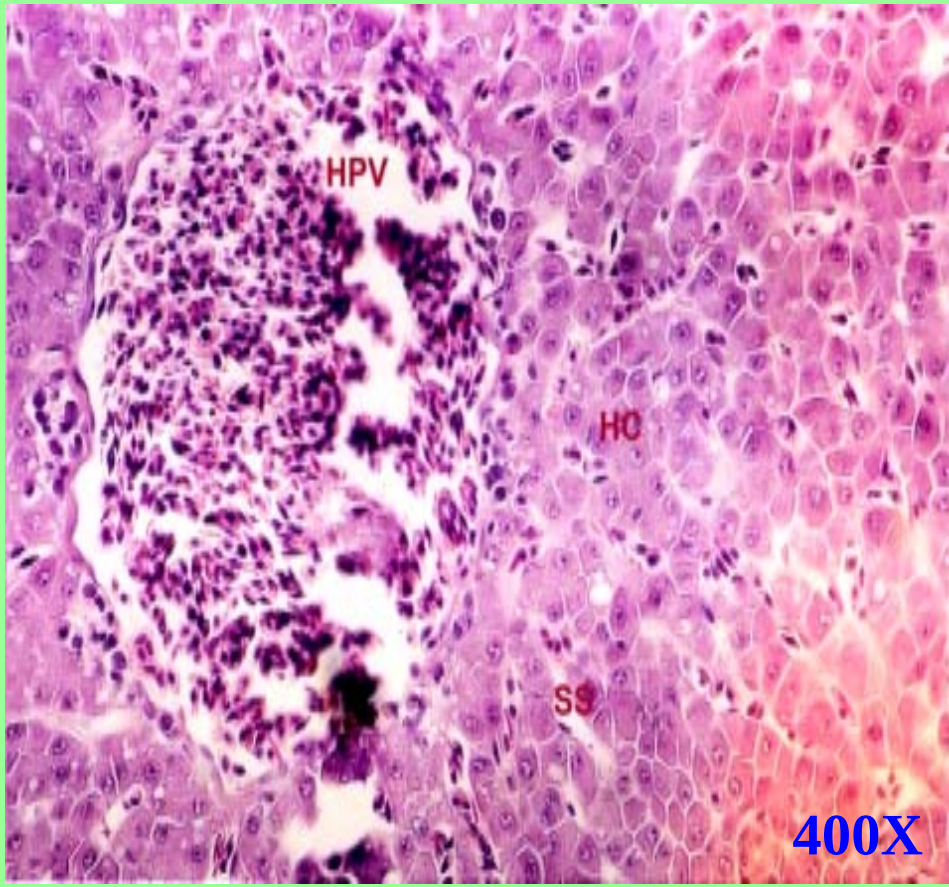
Goblet cells are responsible for the secretion of mucus coating the intestine. Secretion of mucus is elicited primarily by irritating stimuli rather than in response to hormones (Cross and Mercer, 1993).

Mucus serves an important role in mitigating shear stresses on the epithelium and contributes to barrier function in several ways (Cross and Mercer, 1993)

Liver

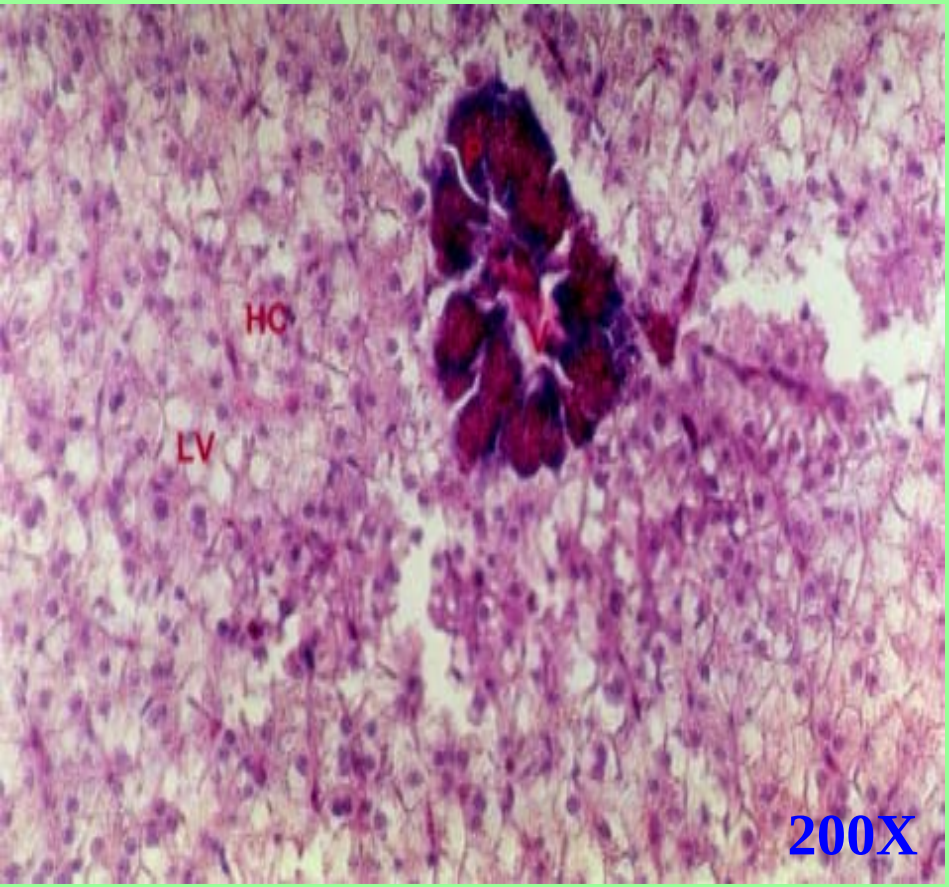


D2 – 30 dph

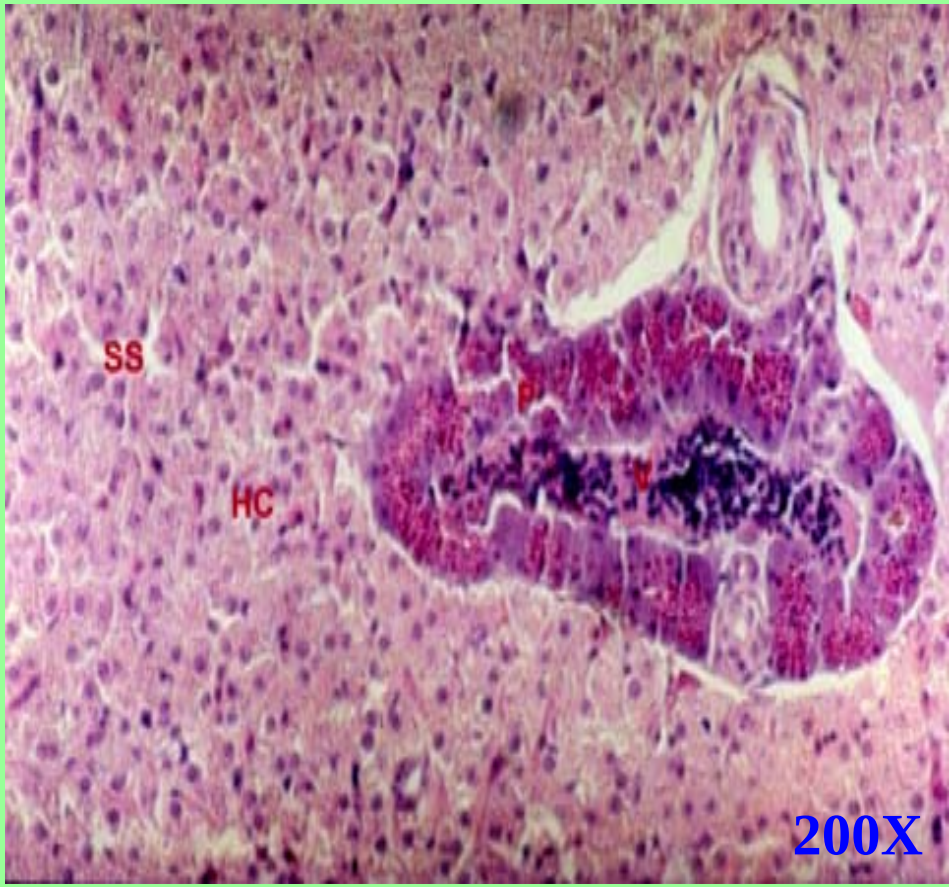


D5 – 30 dph

Liver

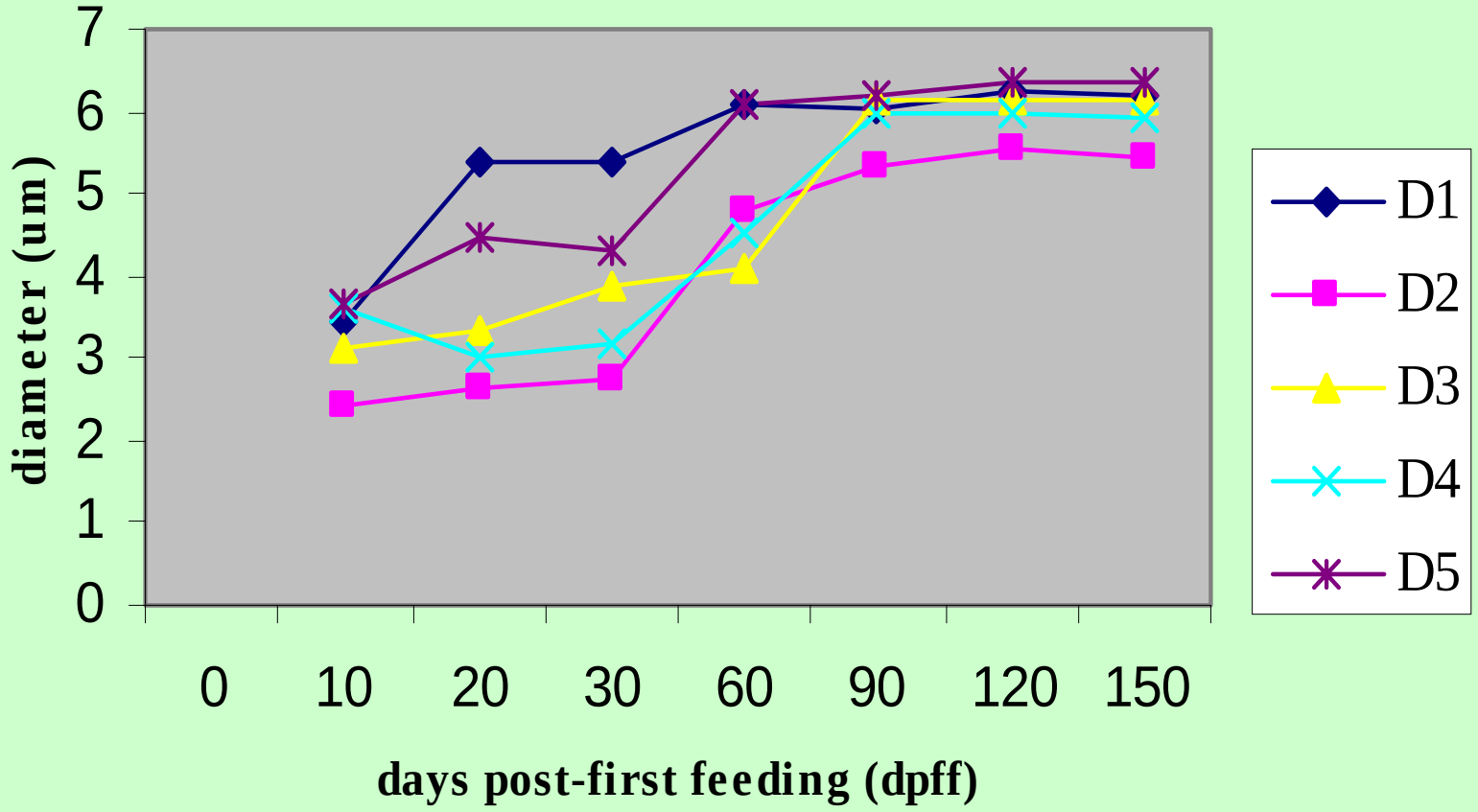


D2 – 150 dph



D5 – 150 dph

Diameter of hepatic portal vein of *Oreochromis niloticus* L. in different developmental stages fed with different first food diets.



Effects of the Different First Food Diets on the Liver

The hepatocytes contain glycogen and the amount of which in the liver cells depends upon the nutritional state of the animal. They are few in number in the normal liver but are dramatically increased after consumption of hepatotoxic substances (Junqueira *et al.*, 1995).

Fish hepatocytes are good indicator of dietary quality (Kugler and Pequignot, 1988).

Bigger hepatocytes of fish fed with T5 diet may be due to the increased glycogen inclusion.

Effects of the Different First Food Diets on the Liver

Lipid vacuolations were prominent and abundant in fish fed with T2 diet especially during the early developmental stages (10-30 dph).

Increased liver lipid deposits may indicate diet of insufficient vitamin content, carbohydrate-rich diet and high-unsaturated fatty acids (Kugler and Pequignot, 1988).

High lipid infiltration revealed in the liver of fish with T2 diet was probably due to the high carbohydrate and unsaturated fatty acids and low vitamin content of the rice bran used.

Effects of the Different First Food Diets on the Liver

Dietary protein deficiency may have contributed to the lipid accumulation in the liver.

Very low-density lipoproteins (VLDL) are the transport vehicle of triglycerides in the bloodstream and are synthesized from the triglycerides and apolipoproteins in the liver, and secreted as triglycerides-rich lipoprotein (Ogunji and Wirth, 2000).

Apolipoprotein deficiency results in impaired secretion of lipid from the liver, causing accumulation of lipids in the liver (Ogunji and Wirth, 2000).

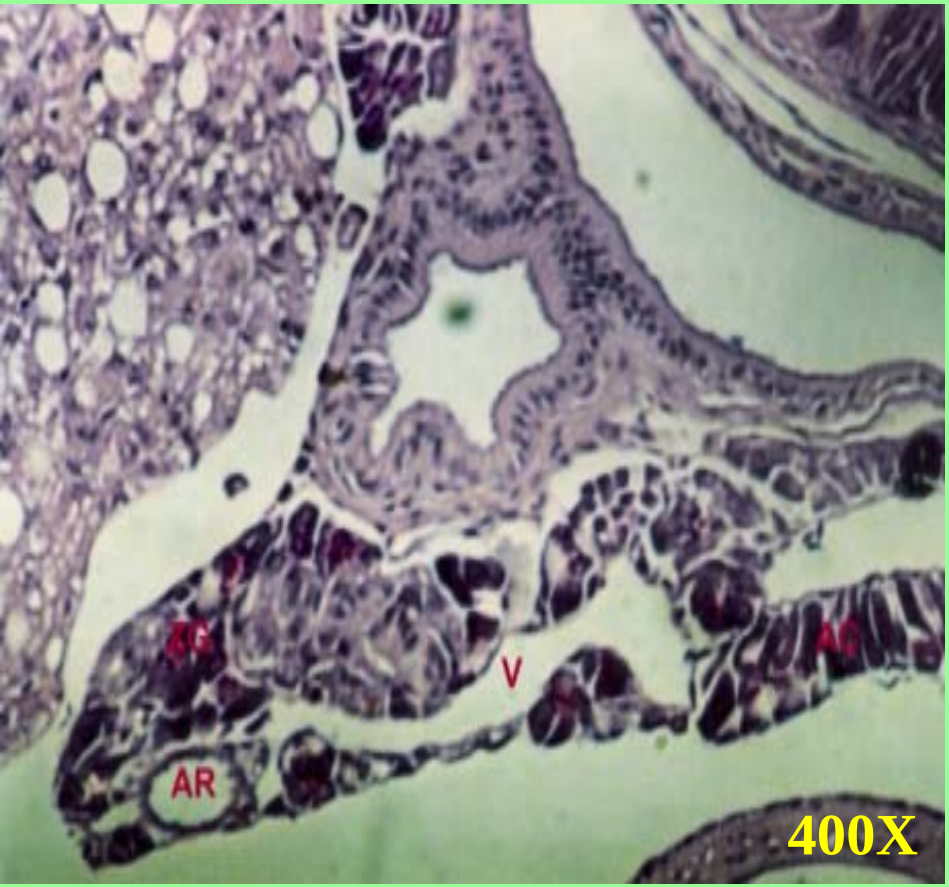
Effects of the Different First Food Diets on the Liver

Although fry booster contains fishmeal and rice bran, the incorporation of zooplankton (*Moina*) in the mixed food diet (T5) may have counteracted the disturbed lipid metabolism caused by feeding a diet rich in carbohydrate.

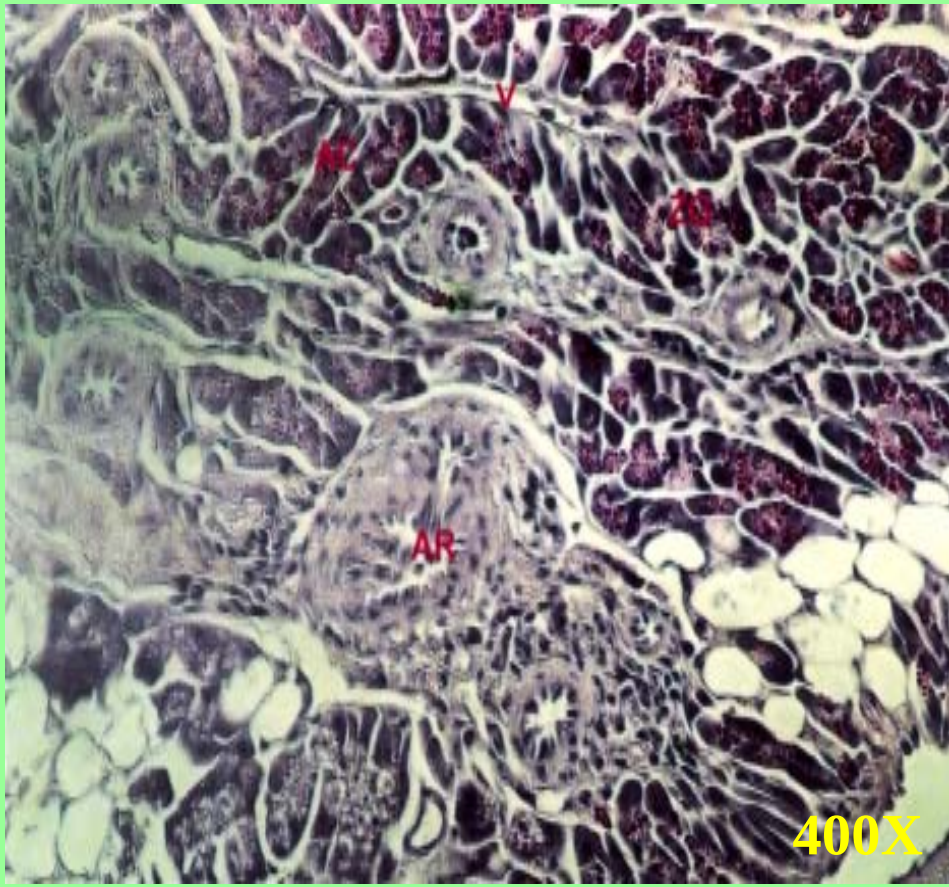
The major contribution of natural food organisms to the nutrition of commercially cultured fish may be from nutrients that are required in trace amounts such as vitamins, minerals, and essential fatty acids (Robinson, 2003)

Bigger hepatic portal vein diameter was revealed in fish with T5 diet suggesting more blood supply in the liver.

Pancreas

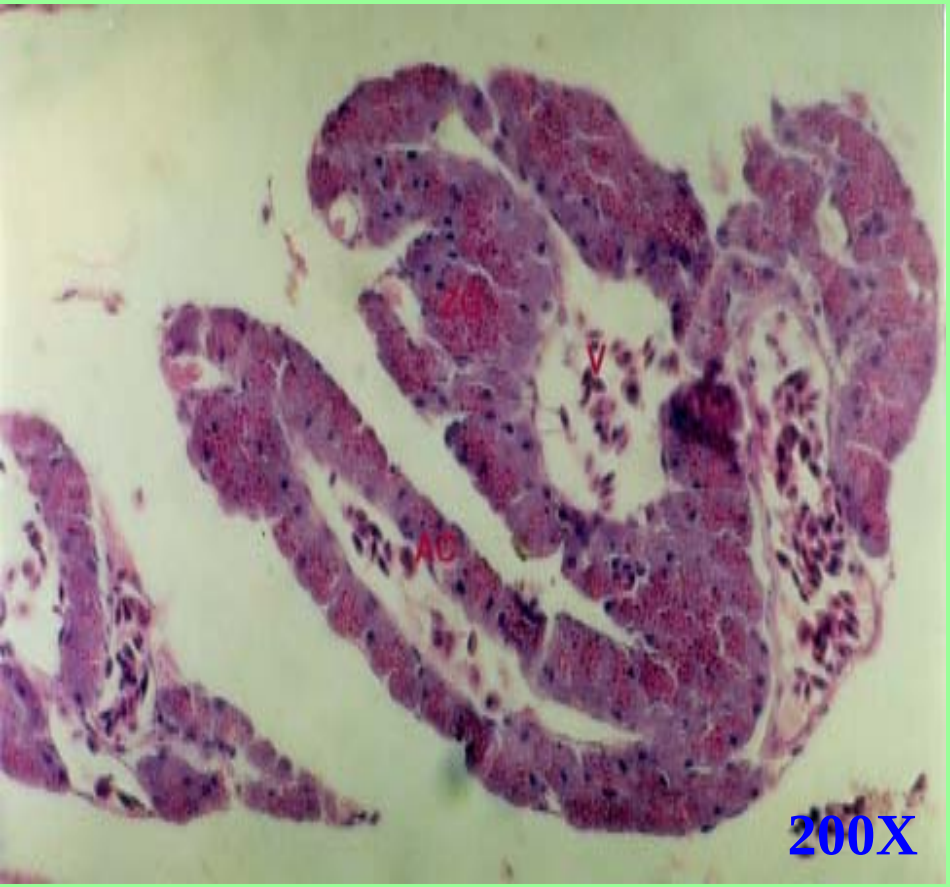


D2 – 30 dph

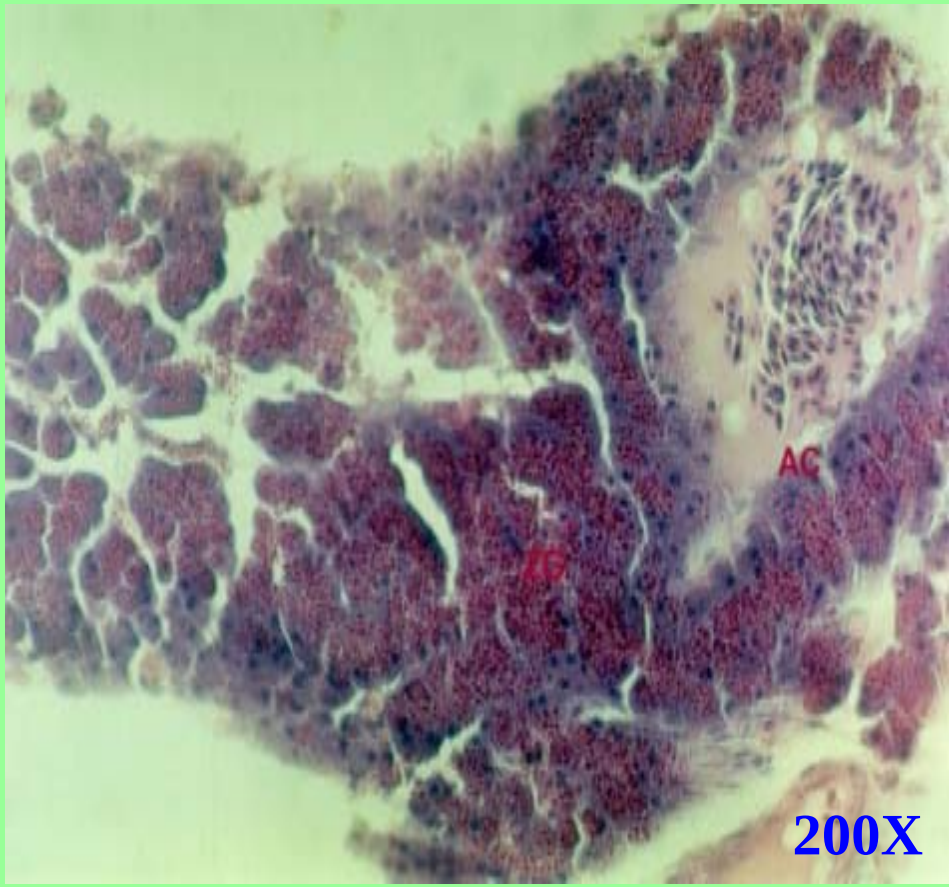


D5 – 30 dph

Pancreas



D2 – 150 dph



D5 – 150 dph

Effects of the Different First Food Diets on the Pancreas

Proenzymes, stored within zymogen granules, are inactive precursors of digestive enzymes that become active within the duodenum (Cross and Mercer, 1993), anterior intestine in Nile tilapia.

zymogen – an inactive protein that can be activated by specific hydrolysis of peptide bonds

Each zymogen granule appears to contain all the pancreatic enzymes; however, the concentration of individual enzymes varies between granules and is sensitive to changes in diet (Cross and Mercer, 1993).

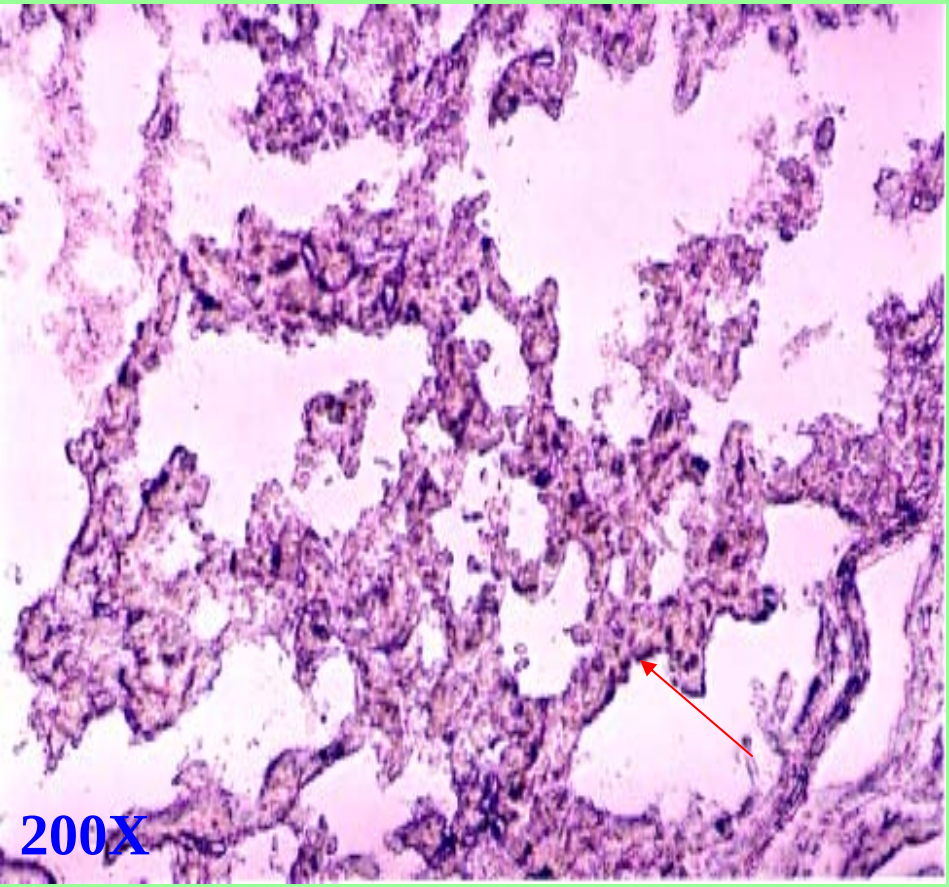
Effects of the Different First Food Diets on the Pancreas

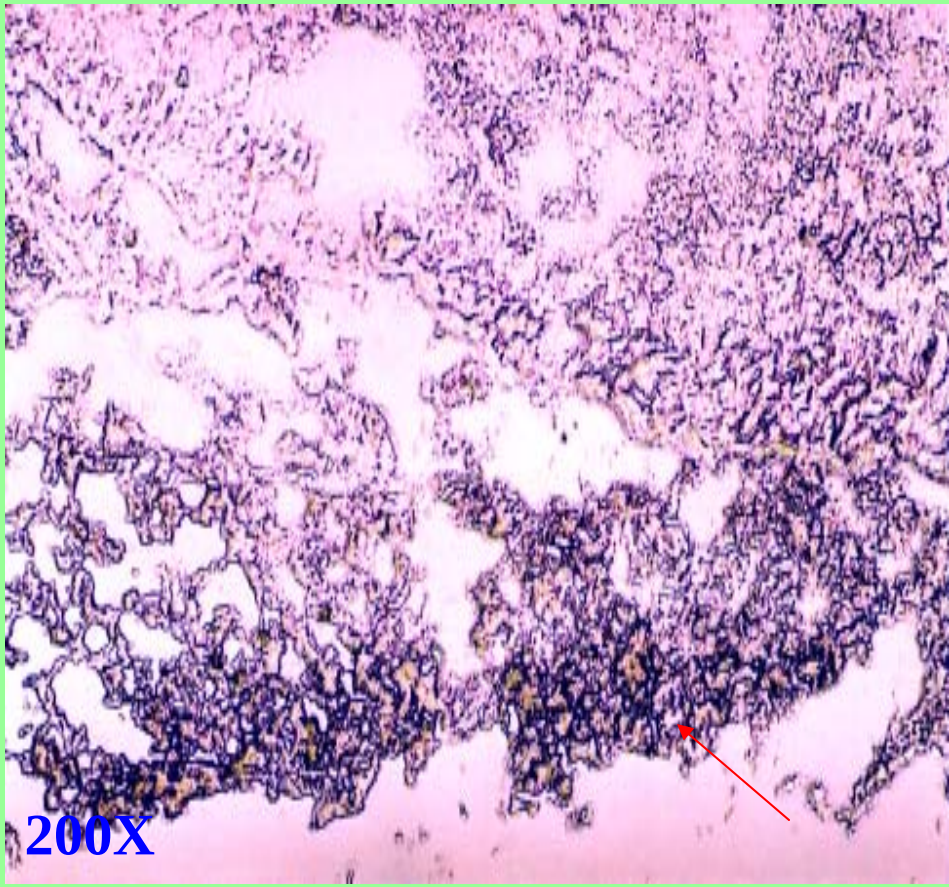
High-protein diets result in a high concentration of proteases, whereas high-carbohydrate and fat diets are reflected in high levels of amylase and lipase respectively (Fawcett, 1994).

In this study, abundance of these granules in acinar cells suggest active production and secretion of pancreatic enzymes like protease, lipase, amylase esterase and phosphates indispensable for the digestion of macromolecules – protein, carbohydrates, and lipids/fats.

T5 (fry booster plus *Moina*) fish had bigger acinar cells (3-7 μm). This may be due to the abundant zymogen granules in the pancreatic acinar cells.

Enzyme Histochemistry- Anterior Intestine



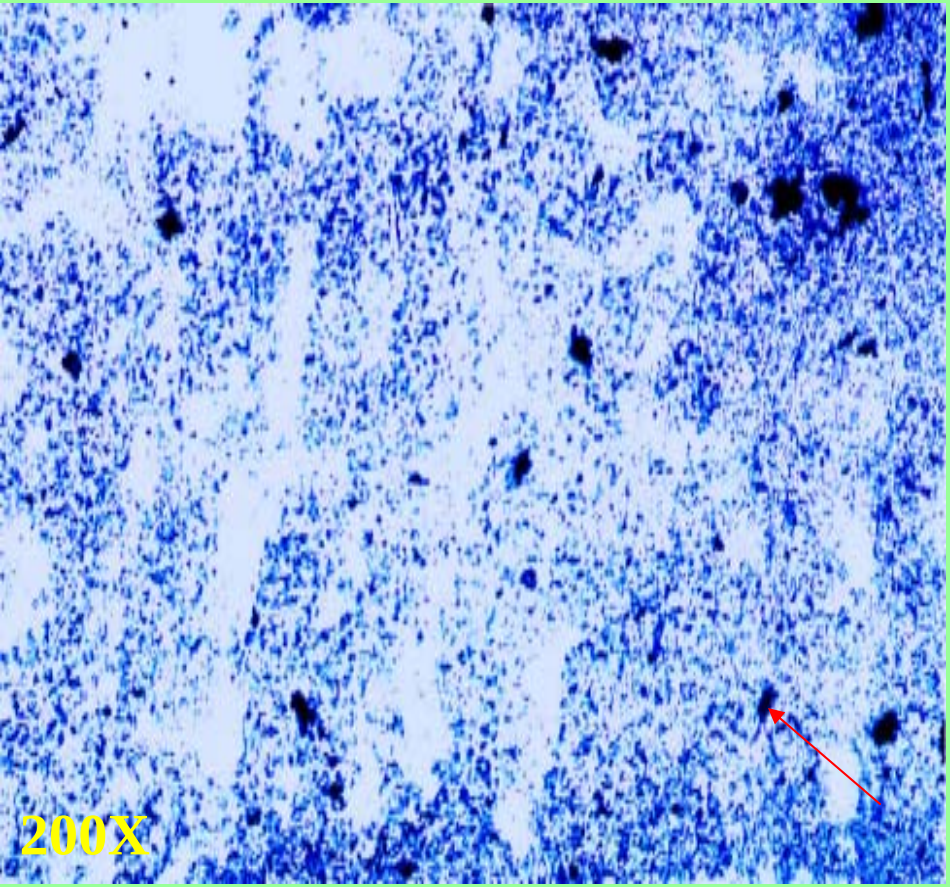


D2 – 150 dph

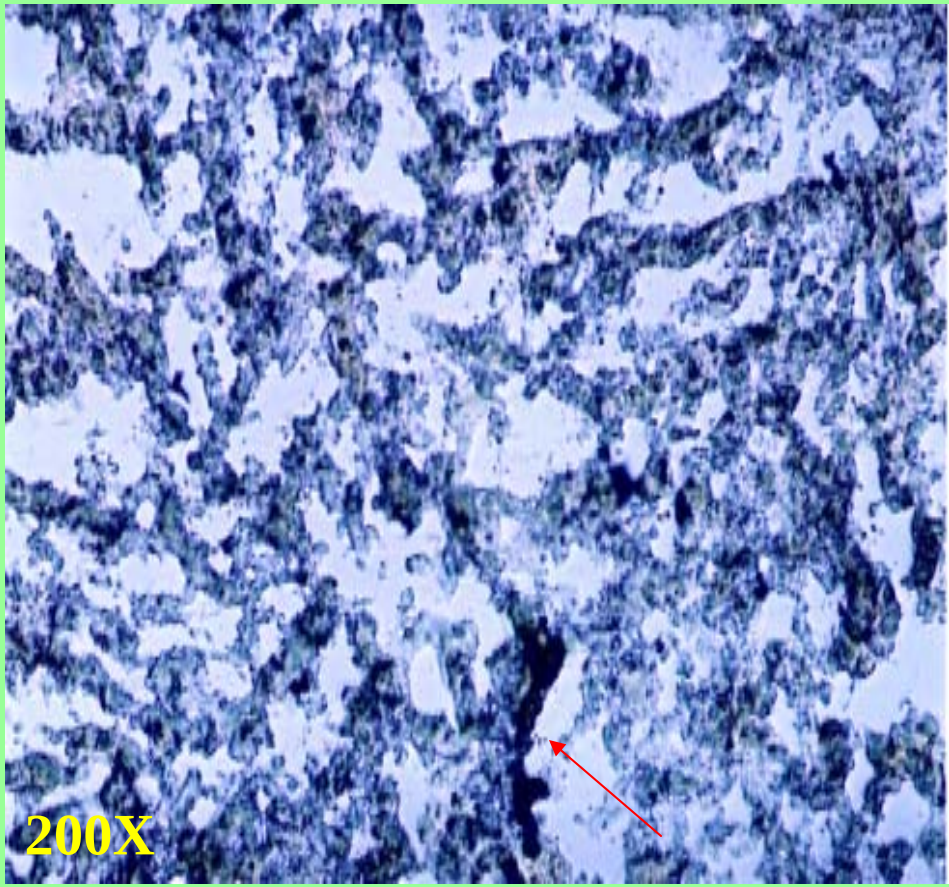
D5 – 150 dph

Alkaline Phosphatase

Enzyme Histochemistry- Anterior Intestine



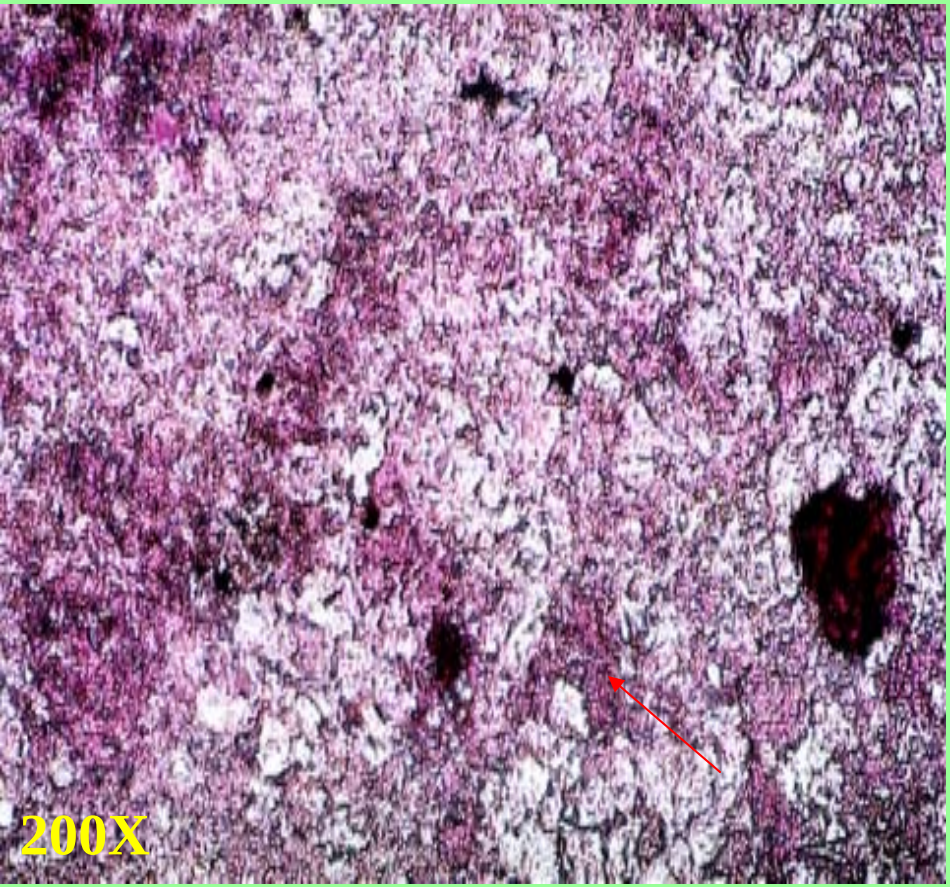
D2 – 150 dph



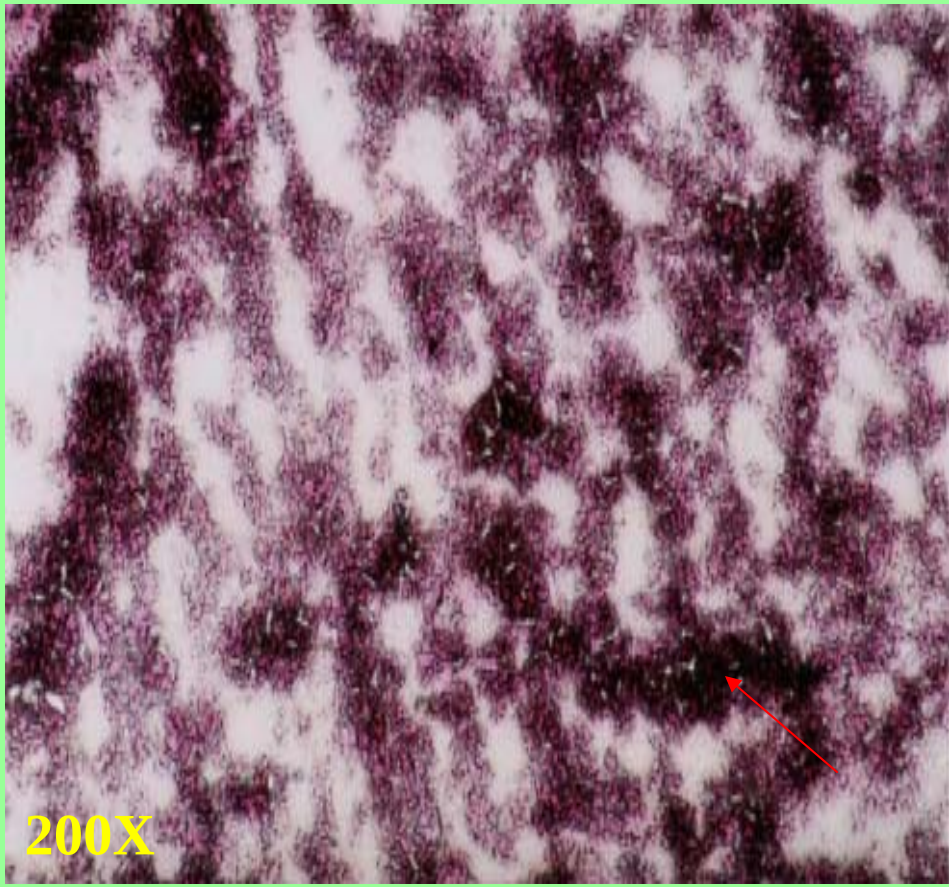
D5 – 150 dph

Esterase

Enzyme Histochemistry- Anterior Intestine



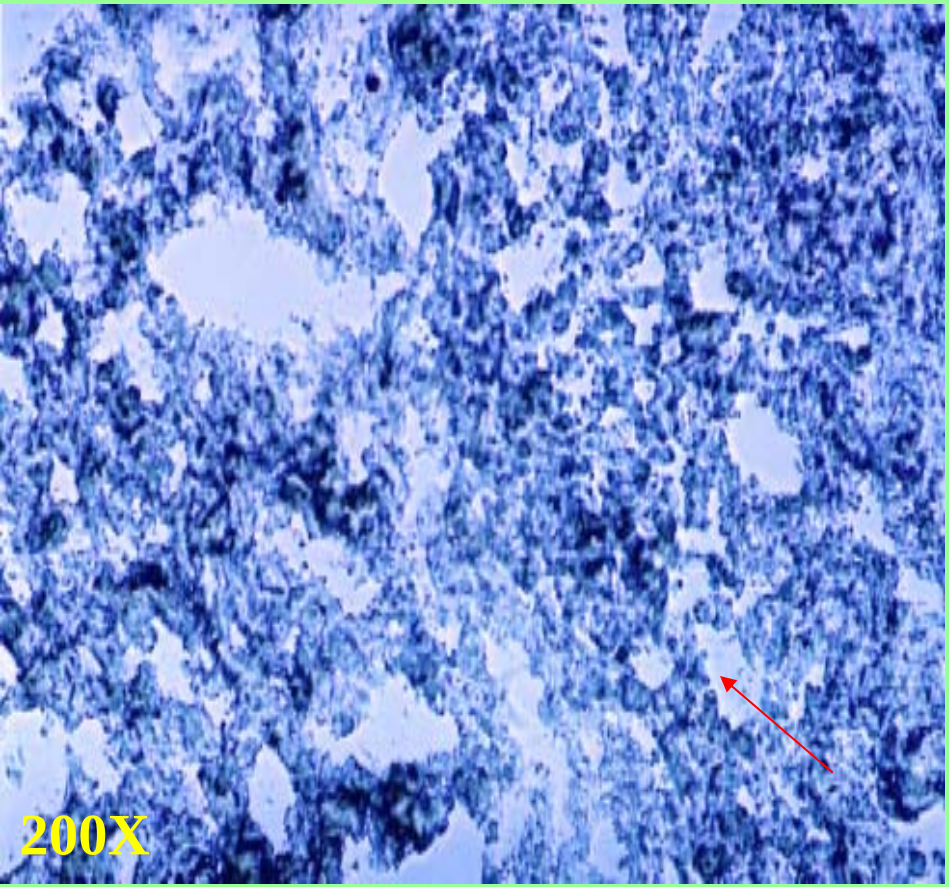
D2 – 150 dph

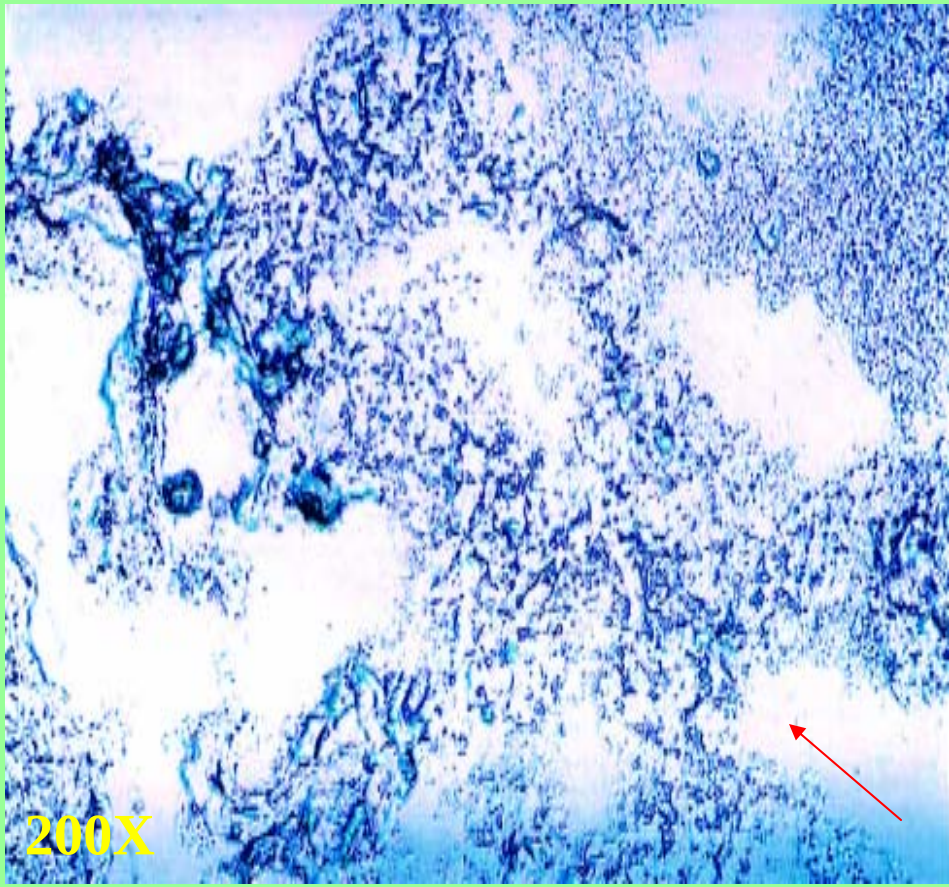


D5 – 150 dph

Lipase

Enzyme Histochemistry- Anterior Intestine



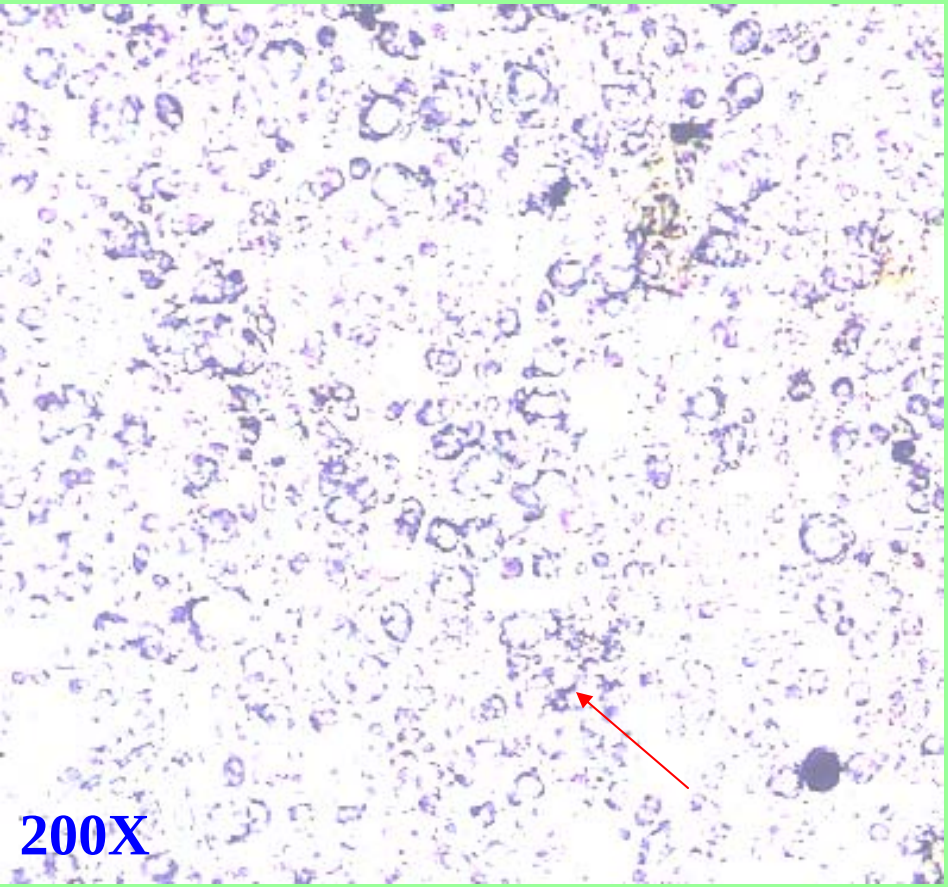


D2 – 150 dph

D5 – 150 dph

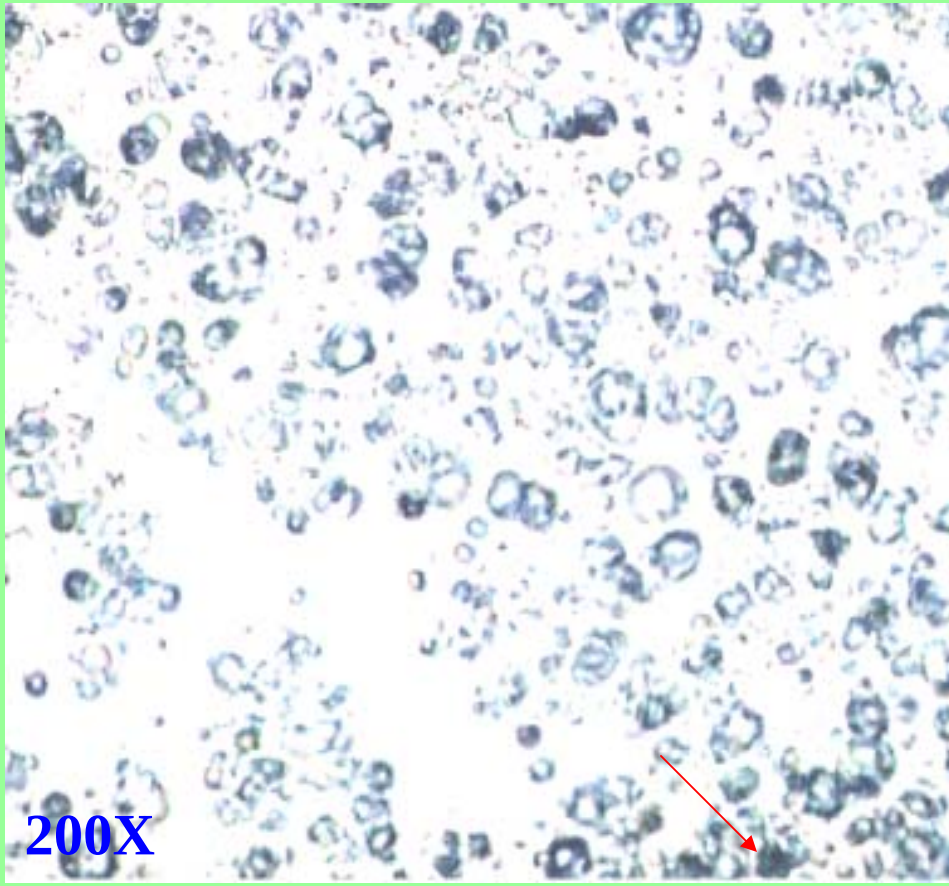
Amylase

Enzyme Histochemistry - Pancreas



D1 – 150 dph

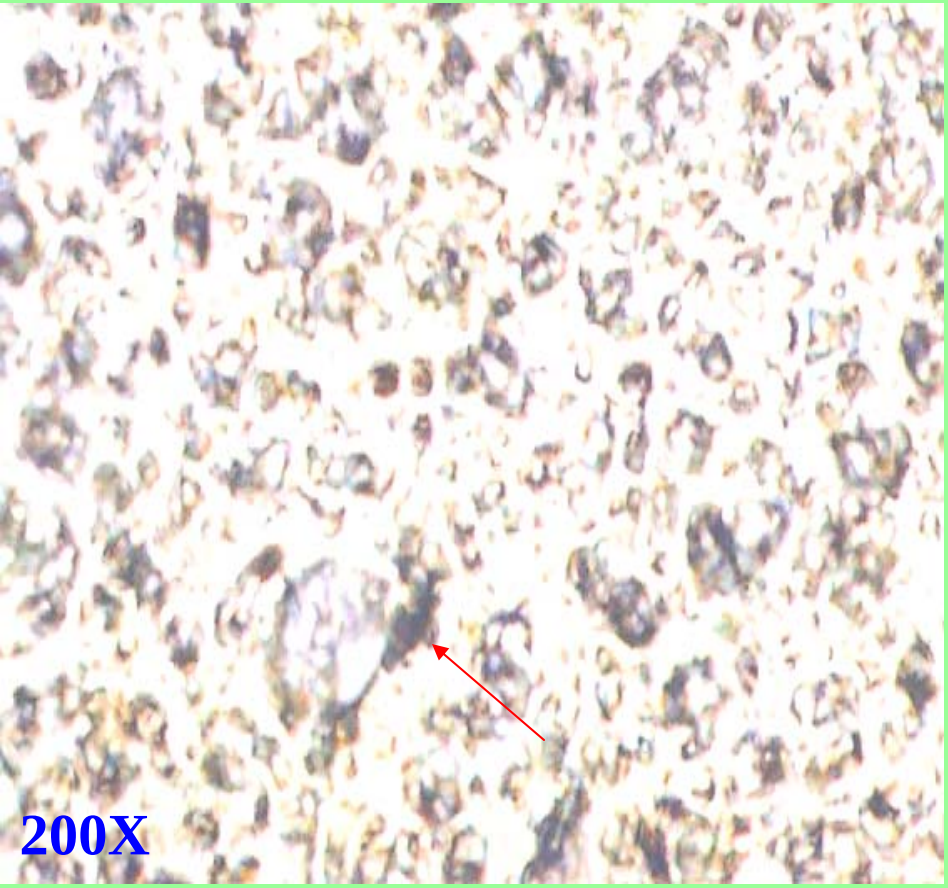
Alkaline Phosphatase



D2 – 150 dph

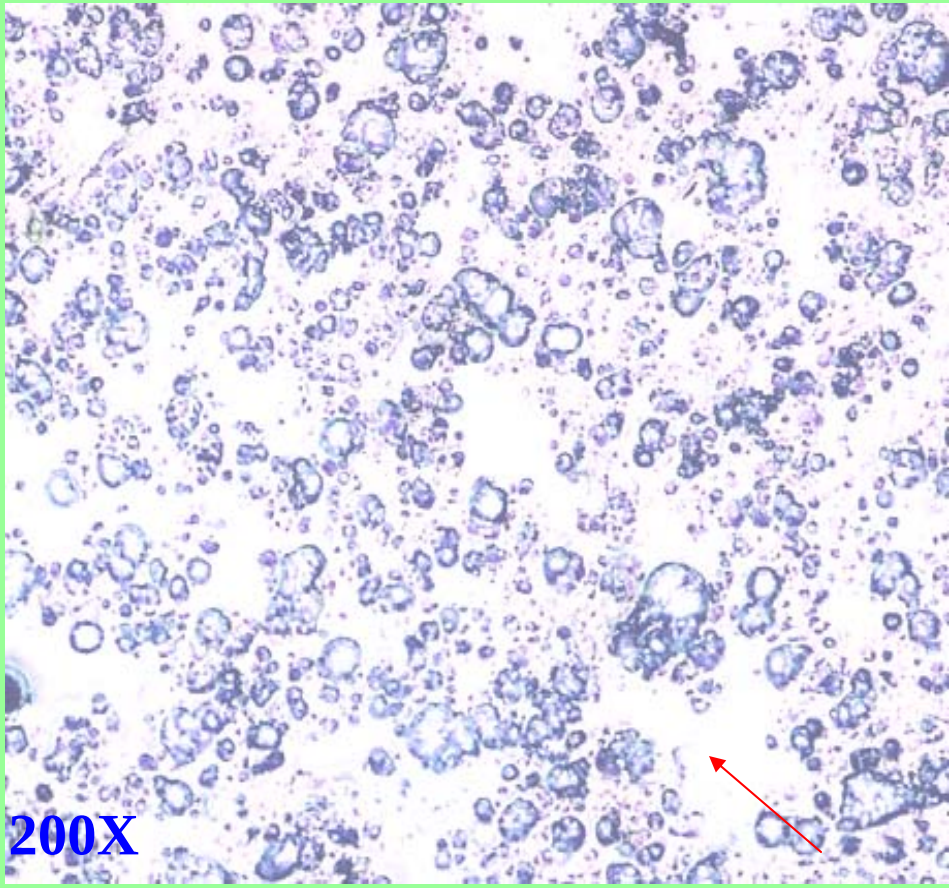
Esterase

Enzyme Histochemistry - Pancreas



D3 – 150 dph

Lipase



D5 – 150 dph

Amylase

Effects of the Different First Food Diets on the Some Enzymes

In freshwater teleosts, digestive enzyme activity is affected by feeding behaviour and biochemical composition of the food (Kumar and Chakrabarti, 1998).

More intense (> 300 cells stained) activity of alkaline phosphatase, non-specific esterase, lipase, and amylase were exhibited in fish with T5 diet while T2 (> 200 cells stained) diet showed weak enzymatic activity.

Aside from high nutrient content of the diet, increased food intake of fish fed with T5 diet may have accounted for the strong enzymatic activities.

Abundant zymogen granules in the pancreas of T5 fish may have accounted for the strong intestinal enzymatic activities.

Effects of the Different First Food Diets on the Some Enzymes

Alkaline phosphatase has a wide distribution in developing tissues and consistent localization is found within intestinal segments in several fish species (Baglole *et al.*, 1998).

It is found primarily in cell membranes where active transport takes place (Baglole *et al.*, 1998).

In this study, presence of this enzyme in the intestinal brush borders of mucosa of Nile tilapia *Oreohromis niloticus* identifies this tissue as a site of active nutrient absorption.

Effects of the Different First Food Diets on the Some Enzymes

Esterase activity in several fish species has been correlated with fat digestion and lipid absorption (Baglole *et al.*, 1998).

High crude fat content (*Moina*, 8.7%; fry booster, 12.0%) of the T5 diet suggests more fat digestion and abundant lipid vacuoles in the anterior intestine suggest greater lipid absorption.

The digestion of fats occurs completely in the intestine, under the action of pancreatic lipase.

Effects of the Different First Food Diets on the Some Enzymes

Amylase is a widely distributed enzyme in the plant and animal kingdom.

High enzyme activity may be closely related to the ability of digesting carbohydrates occurring in microalgae, which are used as food for zooplankton (Kumar and Chakrabarti, 1998).

High amylase activity in the gut of different fish species (*C. punctuatus* and carps) and also in sea bass larvae feeding on carbohydrate-rich diet suggest extensive amylase synthesis (Sarkar *et al.*, 1999).

Effects of the Different First Food Diets on the Some Enzymes

The digestibility of starch (carbohydrate) is affected not only by the source and nature of carbohydrate but also by the level of its incorporation.

Wheat and other grains contain albumins, which inhibit the α-amylase activity in fish (Al-Ogaily *et al.*, 1996). This may suggest the weak amylolytic activity in Nile tilapia fed with T2 diet.

Weak enzymatic activites may be due to poor nutrition. In conditions of extreme malnutrition, pancreatic acinar cells and other active protein-secreting cells undergo atrophy and lose much of their endoplasmic reticulum and the production of digestive enzymes is hindered (Junqueira *et al.*, 1995).

CONCLUSIONS

- **Fish fed with *Moina* + fry booster (T5)showed better growth results supported by organ histology, electron microscopy and enzyme histochemistry.**

- **Fish fed with fish meal + rice bran showed poorest growth performance and development.**

RECOMMENDATIONS

- ✓ other organ systems
- ✓ other enzyme tests
- ✓ other supplemental diets

THANK YOU and GOOD DAY!