THE EFFECTS OF DIETARY SAPONIN AND TANNIN ON GROWTH PERFORMANCE AND DIGESTION IN *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS*

Thesis submitted for the degree of Doctor of Philosophy

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Declaration

I hereby declare that this thesis has been achieved by myself and is the results of my own investigations. It has neither been accepted, nor is being submitted for any other degree. All sources of information have been duly acknowledged.

Dedicated

to

My mother, my wife and my children

In the name of God, the most gracious the most merciful

ACKNOWLEDGEMENTS

Abstract

In order to investigate the antinutritive effects produced by the intake of saponin and tannin (hydrolysable and condensed), ten experiments were conducted on *Oreochromis niloticus* and *Clarias gariepinus* utilising casein/gelatin purified diets. Fish were fed (3% body weight/day) four isoenergetic isoproteic diets containing 0.02/0.01, 0.08/0.09, 0.42/0.47 and 0.87/0.91% saponin, (for *O. niloticus* and *C. gariepinus* respectively), 0.05, 0.08, 0.27 and 0.71% tannic acid or 0.06, 0.16, 0.73 and 1.66% catechin which represent diets I, II, III and IV respectively in each study.

In all studies fish fed diet I showed the maximum weight gain, highest specific growth rate (SGR), the best feed conversion (FCR), the best protein efficiency ratio (PER) and the best apparent protein digestibility (APD) in comparison with all other diets; whereas no significant differences ($P < 0.05$) were observed in dry matter digestibility among the diets in all studies. Fish fed diet IV showed the poorest values (SGR, 0.31/0.21; FCR, 9.51/4.75; PER, 0.39/0.69; PPV, 4.37/-0.35 and APD, 84.04/80.63 with saponin), (SGR, 1.89/2.01; FCR, 1.36/1.05; PER, 2.31/2.97; PPV, 36.93/54.76 and APD, 86.49/95.33 with tannic acid) and (SGR, 2.16/1.85; FCR, 1.19/1.03; PER, 2.55/2.92; PPV, 37.88/50.93 and APD, 89.59/91.31 with catechin) in *O. niloticus* and *C. gariepinus* respectively in comparison with others. In terms of carcass composition, there were an increases in moisture and ash contents in fish fed diets III and IV of the study on saponin whereas lipid content was markedly decreased in proportion to the

level of saponin in the diets. However, fish in all treatments did not show any significant difference (P< 0.05) in their body protein content. In the studies with tannic acid and catechin, carcass composition of fish showed no significant difference (P< 0.05) in all treatments.

Histological examination showed cytoplasmic vacoulation of intestinal cells, disruption of straited borders and infiltration of *lamina propria* especially in *O. niloticus* and fatty degeneration in liver of fish fed diets III and IV of the study of saponin. However, fish in other studies (tannic acid and catechin) showed no abnormalities.

Digestive enzyme activities were inhibited by the inclusion of dietary tannic acid or catechin, and the inhibition was in proportion to the level of the antinutrients. However, both forms of tannins had no effect on lipase activity of *O. niloticus* while the opposite was observed in *C. gariepinus*. It was shown that the activities of proteolytic enzymes were more inhibited by the inclusion of tannic acid or catechin than the activities of α-amylase and lipase.

The results of the present thesis show that saponin and tannin (hydrolysable and condensed) acted as neutral or negative factors with respect to growth and feed performance, digestive enzyme activities and histology of intestine and liver.

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CHAPTER I

GENERAL INTRODUCTION

1   Introduction

1.1   Definition and History of Aquaculture

Aquaculture is a term used to describe the growing of aquatic animals and plants in fresh, brackish and seawater under controlled or semi-controlled conditions, for profit and/or human consumption. Aquaculture may be conducted in earthen ponds, concrete ponds, tanks, raceways, cages suspended in the open seas or lakes or in impounded coastal waters (Parker, 1995). Aquaculture has been practised for centuries, particularly in the countries of Asia, ancient Egypt and in central Europe, where it has been conducted on for over a thousand years. The earliest aquaculture occurred in China, beginning about 2500 B.C. with the common carp *Cyprinus carpio* (Ling, 1977). From there, it was introduced into several countries of Asia and the Far East by Chinese immigrants, and to Europe during the Middle Ages for culture in monastic ponds (Pillay, 1993). Carp were not the only fish of interest to early culturists. Tilapia were raised in ponds in ancient Egypt and mullet were cultivated in Italy in Roman times (Landau, 1992).

1.2   Importance of Aquaculture

Aquaculture has become recognised as a growth area of economic importance in many countries and has attracted the attention of both the private and public sectors. The development plans of most producing countries are aimed at increasing fish supplies from aquaculture for local and export markets, and at increasing the sector's contribution to food security in rural areas. According

to Newman (1992) and Pillay (1993) aquaculture has many important roles and objectives such as:

a)- increasing production, especially of animal proteins, and achieving self-sufficiency in aquatic product supplies by serving as source for human food.

b)- producing food near consuming centres in rural areas, thus contributing to improvement of human nutrition.

c)- supplementing or replacing capture fishery production of over-exploited fish and shellfish stocks.

d)- creating jobs and economic activity in rural areas and preventing the migration of people from rural to urban areas.

e)- earning foreign exchange through export or saving foreign exchange through import substitution.

f)- using waste lands productively and using organic wastes for food production and environmental management.

g)- promoting agro-industrial development, which could include demanding for grain crops for feeding fish, processing and marketing of fishery products, feeds and equipment for aquaculture.

h)- providing recreational activities, including sport fishing and home and public aquaria.

i)- conservation of endangered stocks and maintenance of biodiversity.

1.3   Status of World Aquaculture

Aquaculture has grown rapidly during the last decade (Fig. 1.1). Between 1986 and 1995 production expanded 126%. Global aquaculture production in 1995 was estimated at 27.8 million metric tons, 25% of the total fisheries production. The total value of this production is approximately US$ 42.3 thousand million , an increase of around 9.6% and 5.2% over 1994 production in quantity and value, respectively (FAO, 1997).

According to FAO (1997) global aquaculture production continues to be dominated by Asia (Fig. 1.2), which in 1995 accounted for over 90% of world output. While Europe's share is 5.1% of global aquaculture production, Africa, South America and North America contribute only 3.8%. The low contribution from such continents is attributed to the fact that Europe, South America and North America do not have long histories of aquaculture like Asia. For example, in China the aquaculture industry goes back thousands of years and has not only become an important industry to that country but has also become part of the nation's culture. However, statistical data (Fig. 1.3) reported by FAO (1997) show that aquaculture production expanded rapidly from 1986 to 1995 in Africa (113%), Europe (34%), South America (446%) and North America (29%).

World aquaculture production will surely increase to meet the increasing demand for animal protein. The growth of the human population has led to an increased search for methods of producing animal protein other than those of
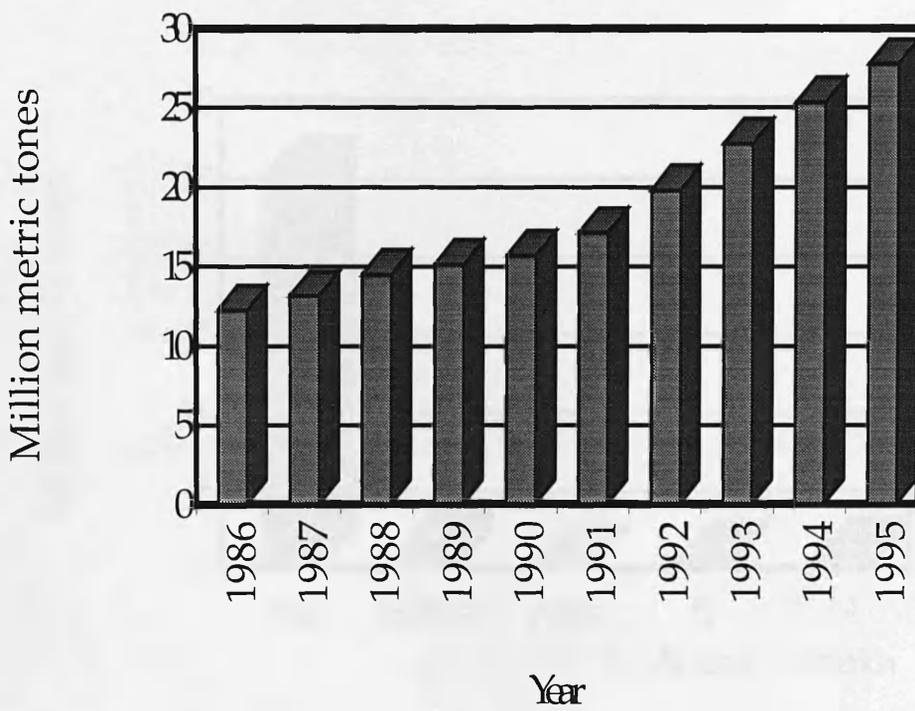
Fig. 1.1 World Aquaculture Production (FAO, 1997)
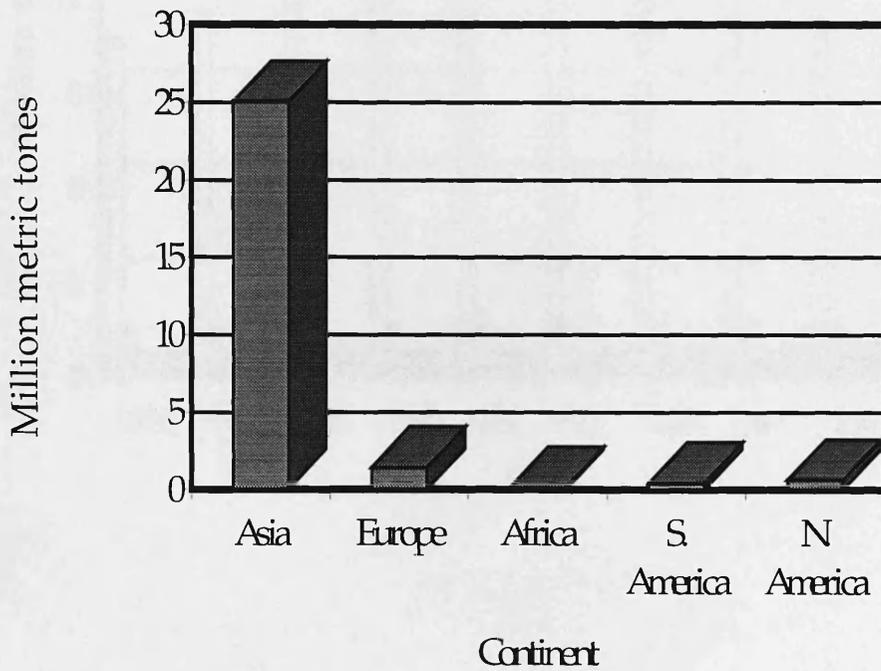
Fig. 1.2 World Aquaculture Production in 1995 by Continent (FAO, 1997).
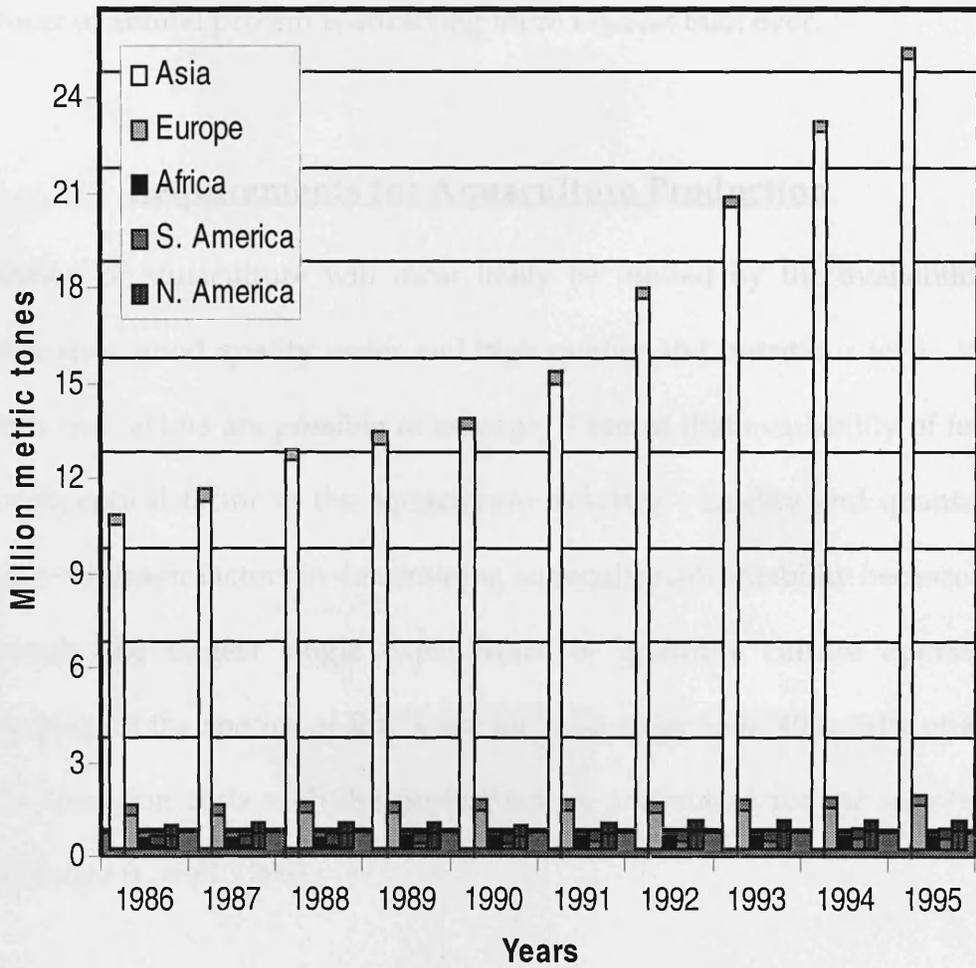
Fig. 1.3 Expansion of Aquaculture Production from 1986 to 1995 by Continent (FAO, 1997).

animal livestock and capture fisheries as both face limits in production performance. Therefore the potential of aquaculture as a supplementary producer of animal protein is attracting more interest than ever.

1.4   Requirements for Aquaculture Production

Expansion of aquaculture will most likely be limited by the availability of suitable sites, good quality water and high quality and nutritious feed. While the first two factors are possible to manage, it seems that availability of feed is the most critical factor in the aquaculture industry. Quality and quantity of feed are the major factors in determining aquaculture profitability because feed represents the largest single expenditure in intensive culture operations. Depending on the species of fish, costs for feed range from 40 to 70% of a fish farm's operation costs with the protein source accounting for the majority of the feed cost (Lovell, 1996).

At present, marine animal proteins such as fish meal, shrimp meal and squid meal represent the major protein sources used in most commercial feeds for aquaculture. Among these, fish meal is the major component of aquaculture feeds. It is the main source of protein and may constitute up to 60% of the total diet (Fig. 1.4). As well as being a rich source of balanced amino acids, fish meal has a relatively high energy content, is rich in long-chain n-3 polyunsaturated fatty acids and in important minerals such as calcium, phosphorus, as well as being highly digestible, and highly palatable for most fish (Goddard, 1996).

Fish meal is produced mainly from species of fish which are either not suitable
for direct human consumption or for which there is a limited demand. These
include principally small bony pelagic fish such as anchovy, horse mackerel,
capelin, menhaden, sand eel, sardine and pilchard or so-called 'industrial' fish
(Sedgwick, 1988).

The global catch of fish for fish meal production increased steadily during the
1950's and 1960s, but has since remained relatively constant at approximately 30
million metric tons from the total catch (Hardy, 1997). Over the past 10 years
6-10 million metric tons of fish meal were manufactured each year (Pike,
1998). It is not expected that fish meal production will increase beyond this
during this time. New (1997) estimated that a shortfall in fish meal would
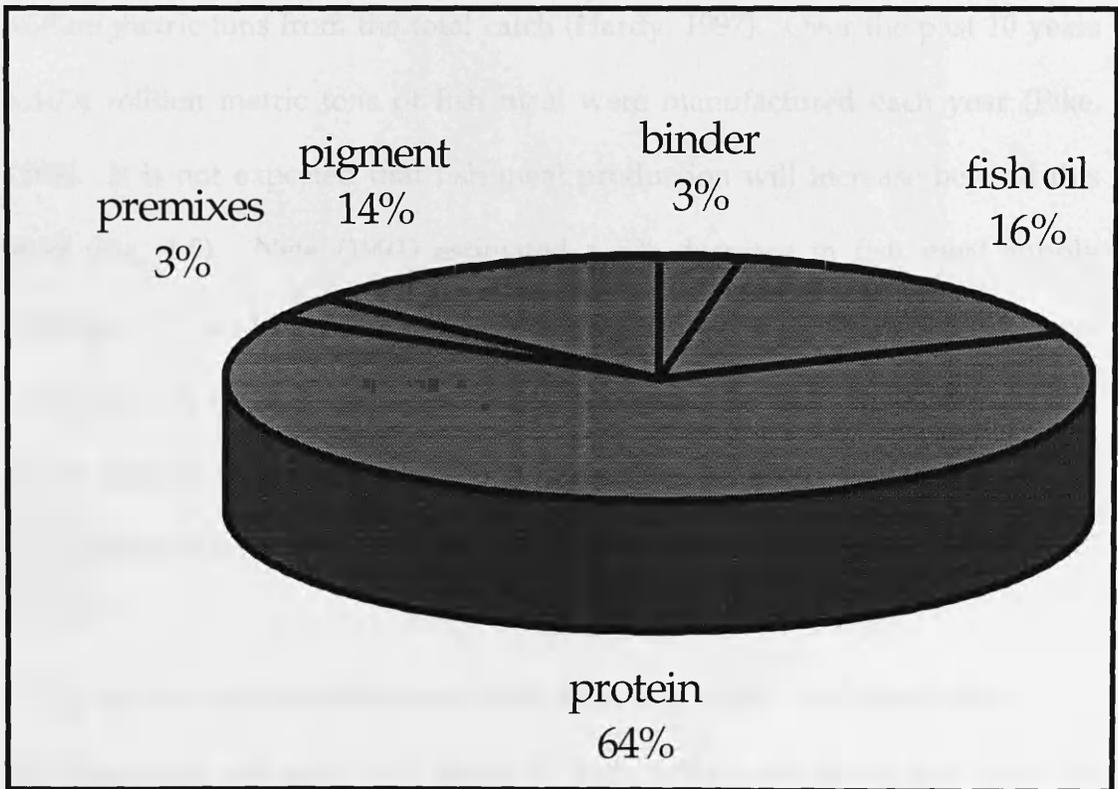
Premixes (minerals and vitamins)

Fig. 1.4 Feed cost breakdown (Prendergast et al., 1994).

Fish meal is produced mainly from species of fish which are either not suitable for direct human consumption or for which there is a limited demand. These include principally small bony pelagic fish such as anchovy, horse mackerel, capelin, menhaden, sand eel, sardine and pilchard or so called 'industrial' fish (Ruiter, 1995).

The global catch of fish for fish meal production increased steadily during the 1960s and 1980s, but has since remained relatively constant at approximately 30 million metric tons from the total catch (Hardy, 1997). Over the past 10 years 6.3-7.4 million metric tons of fish meal were manufactured each year (Pike, 1998). It is not expected that fish meal production will increase beyond this level (Fig. 1.5). New (1991) estimated a 5% decrease in fish meal supply between 1990 and 2000 while demand should increase. Moreover, fish meal use will likely increase in pet foods and specially livestock (Fig. 1.6) feeds and direct utilisation in the human diets due to the tremendous increase in the world population (Rumsey, 1993).

Many factors could contribute to future decreases in fish meal production:

a)- exploiting industrial fish stocks to their sustainable limits as a result of overfishing, environmental degradation and habitat loss.

b)- expansion of the aquaculture industry which will put more pressure on feed availability.
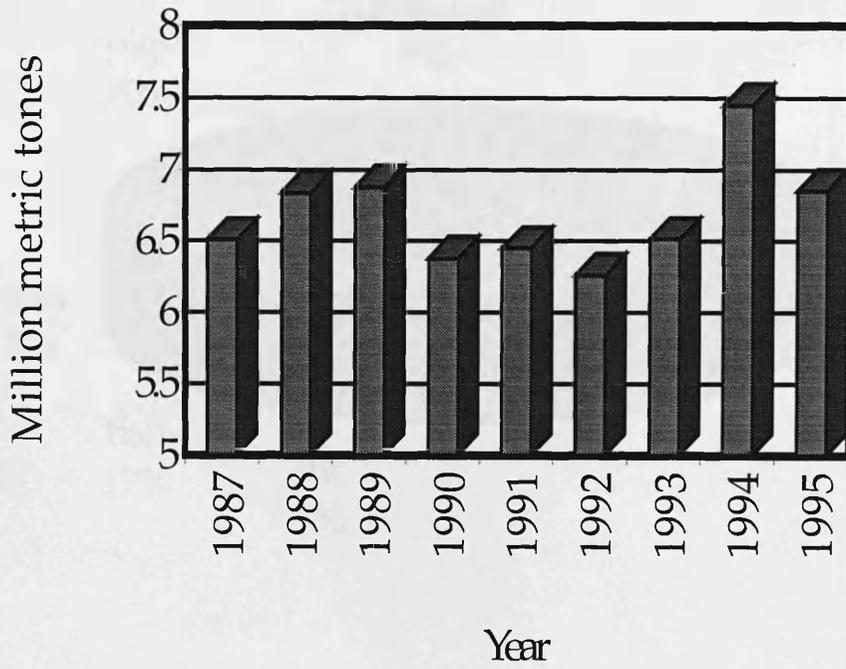
Fig. 1.5 World fish meal Production (Pike, 1998).
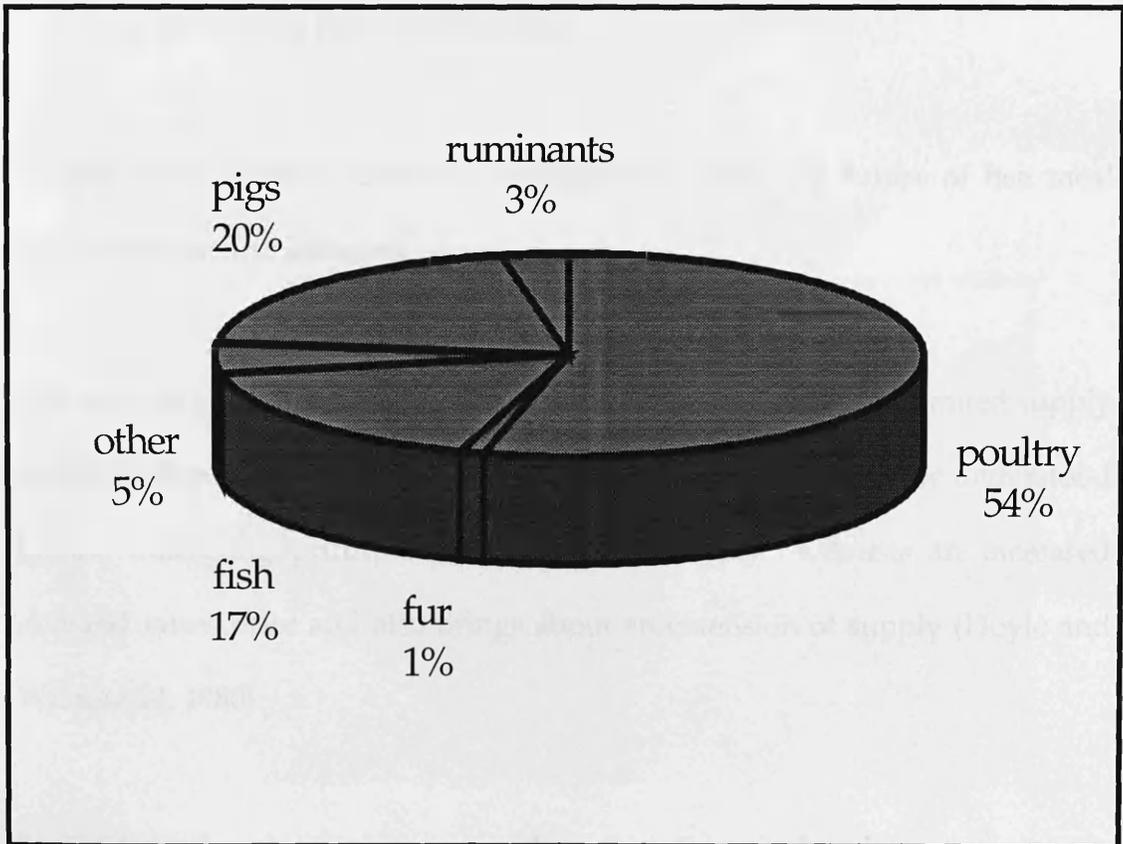
Fig. 1.6 World usage of fish meal of 1994 (United Fish Products Ltd., 1997).

c)- changes in the global climate as result of *El Nino* and other large-scale phenomena which affect the location of fish and the length of the fishing season.

d)- growth of the human population which may demand consumption of fish currently used to produce fish meal.

All the above factors collectively or separately make the future of fish meal both uncertain and insecure.

Fish meal prices are increasing significantly (Fig. 1.7) as result of limited supply and high demand for this material. Pricing of fish meal can best be understood by the marketing principle " demand and supply" whereas an increased demand raises price and also brings about an extension of supply (Hoyle and Whitehead, 1980).

In light of the above, it is very clear that fish meal industry requires a continuously increasing supply of raw material to meet the needs of the growing aquaculture production, livestock and poultry industries. As long as this is not the case, alternative ingredients must be identified, developed and brought into production to achieve the targets set for the aquaculture industry.

1.5   Replacing Fish meal in Aquaculture Feeds

The expansion of world aquaculture will create a significant demand for feeds. Currently there is a limited quantity of fish meal in the world (Pike, 1998) and
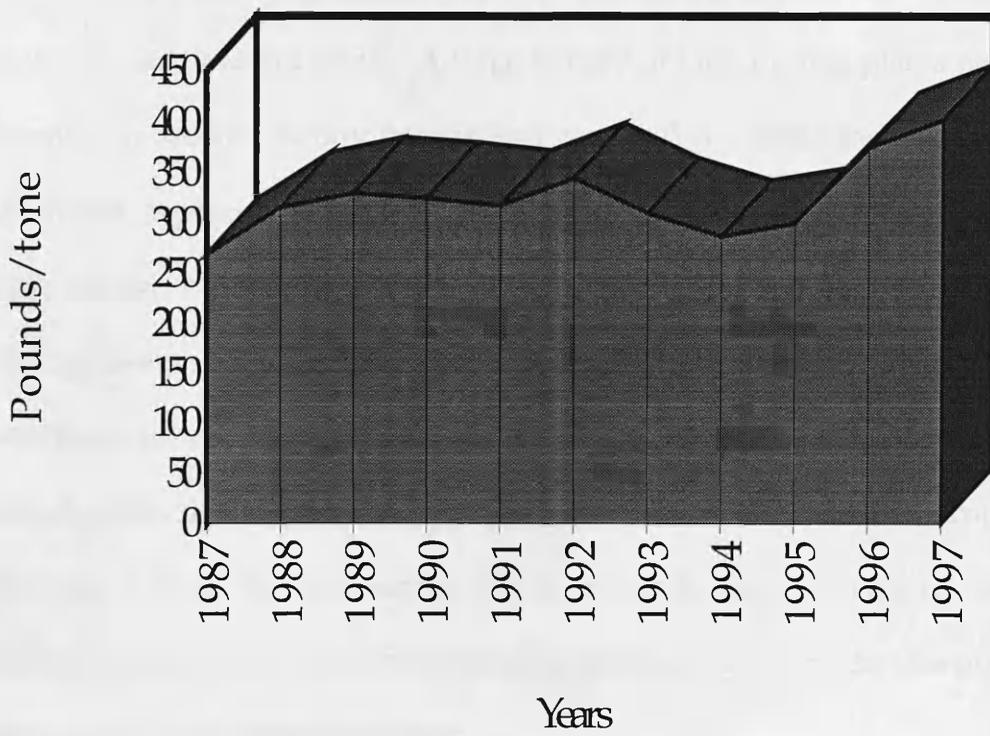
Fig. 1.7 Fish meal prices £ /tone (United Fish Products Ltd., 1997).

the amount available for feed mills will be inadequate in the future. New (1991) reported that during the past 20 years world fish meal supplies have increased about 27% and between 1990 and by the year 2000 world fish meal production is projected to decline by about 5%. Therefore, it will be necessary to find other sources of protein components in order to replace the shortage in fish meal and to satisfy growth of the aquaculture industry.

Proteins of international significance that will be the key for aquaculture are the major plant protein meals. A large variety of high protein plants have the potential to become dietary protein sources for fish. These include soybean, cottonseed, rapeseed, sunflower, peanut, copra, leaf protein concentrate and many others. Animal by-products also are of great interest to consider as fish meal replacers in the feed industry. However, their potential, diversity, and abundance are not as great as for plant protein. Moreover, feed supply to the animal production industry is dependent on fish meal as this industry consume 78% (Fig. 1.6) of the total world fish meal production. Therefore, finding suitable alternatives to fish meal from plant protein sources might also promote other animal production industries.

1.6   Antinutritional Factors

Attempts to replace fish meal partially or totally with plant protein meals in fish diets have met with variable success. Reduced palatabilities, poor feed efficiency, low nutrient digestibility and reduced growth have been observed (Table 1.1). Various hypotheses have been put forward to explain the variable

Table 1.1 Selected experiments employing plant proteins as fish meal replacers.

Ingredients	Levels	Species	Result	Reference
Alfalfa LPC *Medicago sativa*	113, 188, 263, 338, 413 g/kg	Tilapia mossambica, *O. mossambicus*	Fish fed diets containing 338 and 413 g/kg of alfalfa exhibited a reduction in feed consumption rate and a significant deterioration in growth and feed utilisation. A level of 413 g/kg of alfalfa in the diet affected protein digestibility. A reduction in carcass lipid with increasing level of alfalfa was also recorded.	Olvera et al., 1990
Canola meal *Brassica spp*	120–480 g/kg	Channel catfish, *Ictalurus punctatus*	Fish fed 480 g/kg canola had low weight gains, feed intake and feed conversion ratio compared to the control group.	Webster et al., 1997
Copra *Cocos nucifera*	500 g/kg	Mossambica tilapia, *O. mossambicus*	Fish fed diet contains 50% copra meal showed very low growth compared to fish fed a fish meal diet.	Jackson et al., 1982
Copra *Cocos nucifera*	341, 682 g/kg	Tilapia mossabica, *O. mossabicus*	Fish fed copra meal at these levels exhibited poor growth and conversion ratios and reduced specific growth rate.	Jackson et al., 1982

Ingredient	Inclusion level	Fish species	Effect	Reference
Cottonseed *Gossypium spp.*	250, 375, 520 g/kg	Channel catfish, *Ictalurus punctatus*	Fish fed glanded cottonseed or fullfat glandless cottonseed exhibited depressed growth and performance compared to fish fed other diets. Mortality was observed at levels of 375 and 520 g/kg.	Robinson et al., 1984; Robinson & Tiersch, 1995
De-oiled sal *Shorea robusta*	200-600 g/kg	Indian carp, *Labeo rohita*	Growth performance and feed utilisation were affected by inclusion of sal. Apparent protein digestibility showed progressive decline with increasing levels of ingredient incorporation.	Mukhopadhyay & Ray, 1996
Jack bean *Canavalia ensiformis*	132, 329, 472 g/kg	Tilapia mossambica, *O. mossambicus*	Fish fed jack bean at all levels exhibited depressed appetite, lethargic movements and subsequently increased mortality.	Martines et al, 1988
Leucaena *Leucaena leucocephala*	304, 607 g/kg	Tilapia mossabica, *O. mossabicus*	Inclusion of leucaena meal resulted in very poor growth and reduced specific growth rate. Authors concluded that poor conversion ratios and growth rates could have been due either to the unpalatable nature of the diets or toxic factors.	Jackson et al., 1982
Leucaena LPC *Leucaena*	310, 610, 900 g/kg	Nile tilapia, *O. niloticus*	Growth performance was affected by the inclusion of leucaena leaf at all levels. Mortality and	Wee & Wang, 1987

			cataracts have been reported for fish fed 610 g/kg of leucaena.	
leucocephala				
Mustard *Brassica juncea*	288, 576 g/kg	Common carp, *Cyprinus carpio*	Fish fed mustard at both levels did not show growth comparable to fish fed fish meal. Authors concluded that mustard meal was unsuitable at a high inclusion level. Fish liver showed severe intracellular lipid deposition as a result of feeding high level of mustard.	Hasan et al., 1997
Mustard *Brassica juncea*	805 g/kg	Tilapia mossambica, *O. mossambicus*	Fish fed diet containing 805 g/kg mustard had low protein digestibility compared to other diets.	Hossain et al., 1992
Peanut meal *Arachis* *hypogaea*	300 g/kg	*Clarias isheriensis*	Peanut meal at 300 g/kg produced low apparent digestibility coefficient of dry matter, protein and energy.	Fagbenro, 1996
Potato protein *Solanum spp*	22-111 g/kg	Rainbow trout, *O. mykiss*	Decreased feed intake, specific growth rate , feed efficiency, protein productive values, condition factor and increased mortality. Decreased dry matter, protein and fat and increased ash contents of fish body.	Xie & Jokumsen, 1997ab
Rape-seed	150, 300, 400, 500,	Tilapia mossabica,	Increasing rapeseed inclusion level resulted in	Davies et al.,

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Ingredient	Species	Inclusion level	Performance	Reference
Brassica spp	*O. mossabicus*	600 g/kg	progressively reduced growth performance. Feed conversion ratio increased for the higher rapeseed whereas protein efficiency ratio decreased.	1990
Rice bran	*Clarias isheriensis*	300 g/kg	Rice bran at 300 g/kg produced low apparent digestibility coefficient of dry matter, protein and energy.	Fagbenro, 1996
Sesame *Sesamum spp*	Common carp, *Cyprinus carpio*	197, 394, 592 g/kg	Depressed growth and feed utilisation were observed on tested fish. Fish performance showed that sesame has poor palatability.	Hasan et al., 1997
Sesame *Sesamum spp*	Indian carp, *Labeo rohita*	781 g/kg	Fish fed sesame meal as a sole source of protein at 781 g/kg had low protein digestibility	Hossain et al., 1997
Sesbania LPC *Sesbania spp*	Nile tilapia, *O. niloticus*	78-389.9 g/kg	Fish fed all levels of sesbania showed high food conversion ratio, decreased weight gain and very low protein efficiency ratio. Fish did not exhibit aggressive feeding as the control group did. High mortality was found with the highest level of sesbania.	Burgos, 1995
Sesbania seed *Sesbania spp*	Tilapia mossambica, *O. mossambicus*	92, 137, 229, 327 g/kg	Weight gains, feed conversion, protein efficiency ratio, and apparent protein utilisation were	Olvera et al., 1988

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Plant	Level	Species	Findings	Reference
			adversely affected by inclusion of Sesbania seed. Reduction was correlated with the levels of Sesbania seed. High mortality was recorded at 327 g/kg level.	
Soybean *Glycine max*	155, 320, 490 g/kg	Longsnout catfish, *Leiocassis longirostris*	Growth rate and feed utilisation decreased as the level of dietary ingredient increased.	Xie et al., 1998
Soybean *Glycine max*	200 g/kg	Nile tilapia, *O. niloticus*	Fish fed soybean meal diet had inferior growth performance and feed conversion in comparison to the fish fed fishmeal diet.	Sintayehu et al., 1996
Soybean *Glycine max*	159, 318, 477, 637 g/kg	Rainbow trout, *O. mykiss*	Fish fed diet with 100% soybean protein exhibited the poorest growth and feed performance among the rest treatments.	Stickney et al., 1996
Soybean *Glycine max*	178, 355, 532, 708 g/kg	Red drum, *Sciaenops ocellatus*	Half of fish fed 100% soybean as sole protein (708 g/kg) died during the first 4 weeks of the growth trial and the remainder lost weight.	Reigh & Ellis, 1992
Sunflower *Helianthus annuus*	210, 420, 631 g/kg	Rainbow trout, *O. mykiss*	Growth rate and feed utilisation decreased as the level of dietary sunflower seed increased.	Stickney et al., 1996

success. One of these hypotheses is the presence of antinutritional factors in plant protein (Liener, 1980; Huisman et al., 1989; Krogdahl, 1989; NRC, 1993). Tacon (1995) stated that the presence of naturally occurring antinutrients within oilseeds and pulses is the single most important factor limiting their use as fish meal replacers.

1.6.1   Tannin

Tannins are water soluble phenolic compounds having molecular weights between 500 and 3000 dalton (Singleton and Kratzer, 1973). They are found in many vascular plants (Table 1.2) and are characterised by their ability to precipitate protein (Hagerman et al., 1992). The term tannin was derived from the Latin form of a Celtic word for oak and was used to describe any substance with the ability to convert animal hides and skins to leather (Griffiths, 1991). Tannins have been grouped into two classes, the hydrolyzable and the non-hydrolyzable or condensed tannins. Treatment of hydrolyzable tannins with acid or alkali splits them into sugars, usually glucose, and carboxylic acids (Fig. 1.8) (gallic acid or ellagic acid). However, condensed tannins do not readily break down in this manner, nor do sugars contribute to their overall structure (Marquardt, 1989). Condensed tannin is an oligomer of flavan-3-ol (Fig. 1.9) units (Haslam, 1979) which in hot strong acids depolymerise to yield monomeric unit (Davies et al., 1964). Tannic acid is typical of the hydrolysable tannins (Singleton and Kratzer, 1973) whereas catechin (Fig. 1.10) is typical of the condensed tannins (Vohra et al., 1966; Marquardt, 1989).

Condensed tannins have been identified as the most common tannins (Table 1.2) found in grain legumes (Salunkhe et al., 1990). They are more widely distributed than hydrolysable forms. They are present in 54% of all studied angiosperm genera, 74% of all studied gymnosperm genera and 92% of all studied ferns (Griffiths, 1991).

Tannins affect many aspects of the consumer's nutrition and metabolism, including dietary intake, dietary protein availability, digestive enzyme activity and growth performance. In deer *Odocoileus hemionus hemionus*, protein digestibility is reduced when plants containing tannin are ingested (Hagerman et al., 1992).
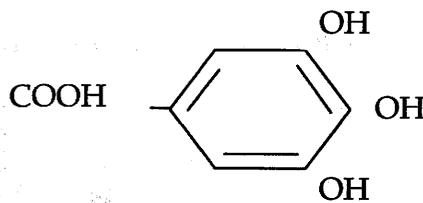
Fig. 1.8 Chemical structure of carboxylic acid (Harvey and McAllan, 1992).
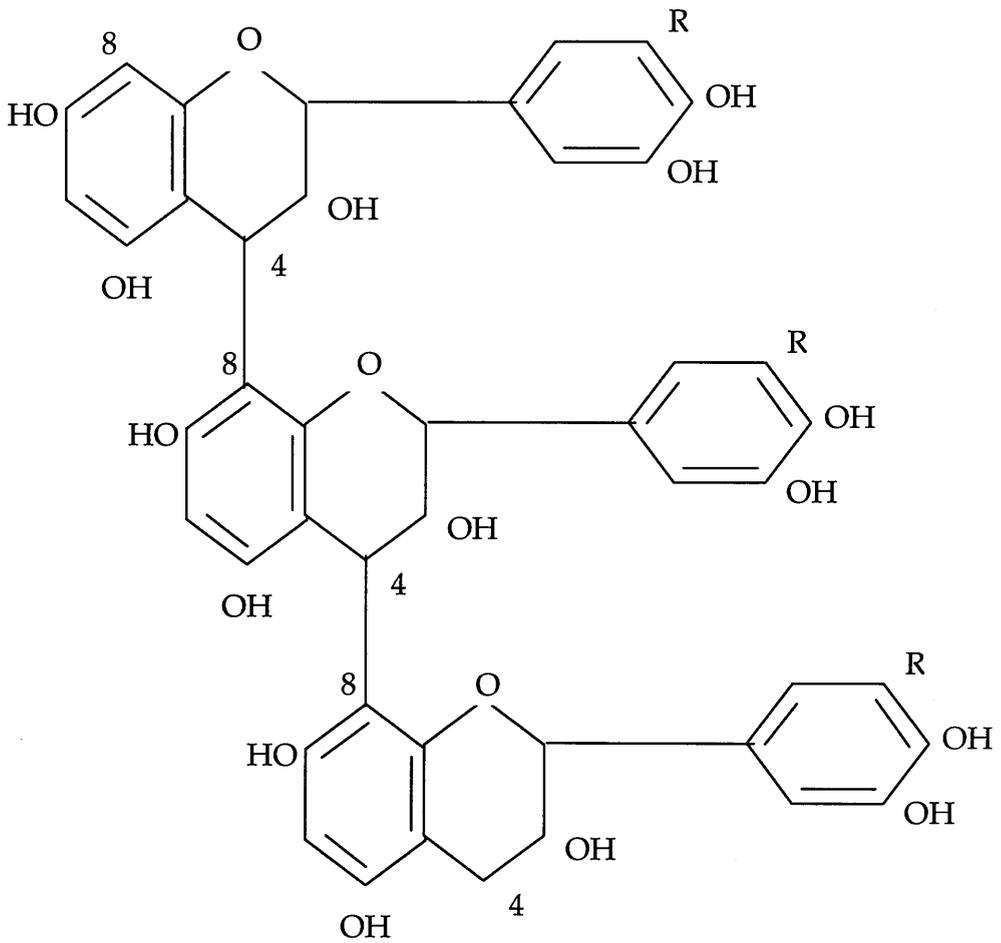
Fig. 1.9 Chemical structure of condensed tannin (Griffiths, 1991).
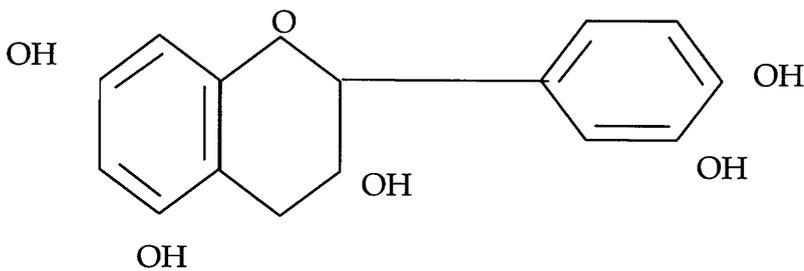
Fig. 1.10 Chemical structure of catechin (Salunkhe et al., 1990).

Table 1.2 A selection of tannin-containing plant materials.

Common name	Latin name	Reference
Barley[1]	*Oryza sativa*	Terrill et al., 1992
Carob[2]	*Ceratonia siliqua*	Joslyn et al., 1968
Copra[1]	*Cocos nucifera*	Terrill et al., 1992
Cottonseed[2]	*Gossypium hirsutum*	Chan et al., 1978; Terrill et al., 1992; Yu et al., 1996a; Yu et al., 1996b
Cowpea[1]	*Vigna unguiculata*	Laurena et al., 1984
Field bean[1]	*Vicia faba*	Griffiths, 1981; Reddy et al, 1985; Garrido et al., 1989; Bos and Jetten, 1989; Harvey and McAllan, 1992; Jansman et al., 1993; Ortiz et al., 1994
Lucerne[2]	*Medicago sativa*	Horigome et al., 1988
Lupin[1]	*Lupinus polyphyllus*	Terrill et al., 1992; Eggum et al., 1993
Mung bean[1]	*Vigna radiata*	Barroga et al., 1985
	Phaseolus aureus	Wiryawan et al., 1997
Mustard[3]	*Brassica juncea*	Hossain and Jauncey, 1989
Peanut[1]	*Arachis hypogaea*	Darchesy and Hemingw, 1986; Azaizeh et al., 1990; Grayer et al., 1992
Peas[1]	*Pisum sativum*	Griffiths, 1981; Buraczewska et al., 1989
Rapeseed.[1+2]	*Brassica spp*	Durke, 1971; Leung et al., 1979; Sarwar et al., 1981; Bell and Shires, 1982; Shahidi and Naczk, 1989;Terrill et al., 1992.
Sesame[1]	*Sesamum indicum*	Odumodu, 1992
Sesbania leaf[2]	*Sesbania sesban*	Kaitho et al., 1997
Sesbaniaseed[1]	*Sesbania cannabina*	Al-Kaisey et al., 1996
	S. sesban; S. goetzei	Wiegand et al., 1995

	S. bispinosa; Indgofera linifolia	Siddhuraju et al., 1995
Sorghum[1]	*Sorghum bicolor*	Price et al., 1978; Gujer et al., 1986; Putman and butler, 1989; Terrill et al., 1992
Soybean[1]	*Glycine max*	Rao and Prabhavati, 1982; Terrill et al., 1992; Odumodu, 1992
Sunflowerseed[2]	*Helianthus annuus*	Horigome et al., 1988
White clover[2]	*Trifolium repens*	Horigome et al., 1988

1- Condensed tannin
2- Hydrolysable tannin
3- Not determined

The anti affects of tannins cannot be reduced by heat treatment as they are

In poultry, tannin exhibits a wide variety of toxic effects when presented in their diets. Growth depression, reduced feed efficiency (Armstrong et al., 1973), and reduced protein digestibility (Rostagno et al., 1973) were observed. Elkin and Rogler (1990) reported depressed growth and feed efficiency of chicks and rats fed high tannin sorghum diet.

Tannins are known to impart an astringent or bitter taste and the level of tannins in the diet may therefore affect palatability. Ringrose and Morgan (1940; as cited in Connor et al., 1969) concluded that the reduced growth that resulted from feeding tannic acid to chicks was due to reduced feed consumption. Similar conclusions were reported for rats (Glick and Joslyn, 1970) and chicks (Vohra et al., 1966).

The toxic effects of tannins cannot be reduced by heat treatment as they are heat stable factors (Harvey and Mcallan, 1992). However, dehulling, soaking in alkali at 60 °C (Harvey and Mcallan, 1992), fermentation (Salunkhe et al., 1990), treatment with formaldehyde (Mohammed and Ali, 1988), and spraying with calcium hydroxide (Wah et al., 1977) were reported to reduce tannin levels.

1.6.2   Saponins

Saponins are high molecular weight glycosides that occur in higher plants (Table 1.3) and to a lesser extent in marine animals such as starfish and sea urchins. The name "saponin" comes from the Latin word *sapo* which means soap. Some saponin-containing plants have been employed for hundreds of

Table 1.3 A selection of saponin-containing plant materials.

Common name	Latin name	Reference
Alfalfa	*Medicago sativa*	Lindahl et al., 1957; Atta et al., 1961; Cheeke, 1971; Reshef et al., 1976; Oakenfull and Sidhu, 1985; Price et al., 1987; Fenwick et al., 1991; Ueda et al., 1996; Lee et al., 1996
Cowpea	*Vigna angularis*	Kitagawa et al., 1983
Jack bean	*Canavalia ensiformis*	Mukharya, 1985; Belmar and Morris, 1994; Udedibie and Carlini, 1998
Lentil	*Lens culinaris*	Applebaum et al., 1969; Fenwick and Oakenfull, 1983; Price et al., 1987; Ruiz et al., 1997
Mung bean	*Vigna radiata;*	Fenwick and Oakenfull, 1983; Waller et al., 1996; Lee et al., 1996a; Lee et al., 1996b;
	Phseolus aureus	Price et al., 1988
Oat	*Avena sativa*	Tschesche and Wulff, 1969; Fenwick and Oakenfull, 1983; Begley et al., 1986; Price et al., 1987; Onning and ASP, 1995; Onning and ASP, 1996
Peanut	*Arachis hypogaea*	Applebaum et al., 1969; Fenwick and Oakenfull, 1983; Price et al., 1987;

		Price et al., 1988
Peas	*Pisum sativum*	Curl et al., 1985; Price et al., 1987; Tsurumi et al., 1992; Khalil and El-Adawy, 1994; Ohana et al., 1998
Quinoa	*Chenopodium quioa*	Ruales and Nair, 1992, 1993, 1994; Jacobsen et al., 1997.
sesameseed	*Sesamum indicum*	Fenwick and Oakenfull, 1983
Sesbania	*Sesbania cannabina* *S. aculeata* *S. aegyptica* *S. sesban*	Al-Kaisey et al., 1996 Simes et al., 1959 Farooq et al., 1959 Dorsaz et al., 1988
Sorghum	*Sorghum bicolor*	Sodipo and Arinze, 1985
Soybean	*Glycine max*	Burrell and Wolter, 1935; Gestetner et al., 1966; Birk, 1969; Kitagawa et al., 1976; Oakenfull, 1981; Curl et al., 1985; Shimoyamada et al., 1990; Fuzzati et al., 1997; Hayashi et al., 1997; Koratkar and Rao, 1997; Bureau et al., 1998
Sunflower	*Helianthus annuus*	Kofler, 1927 (as cited by Oakenfull, 1981); Price et al., 1987; Fenwick et al., 1991

years as soaps and this fact is reflected in their common names; soapwort *Saponaria officinalis*, soapbark *Quillaja saponaria*, soapberry *Sapindus saponaria* (Hostettmann and Marston, 1995). More than one hundred plant families contain saponins varying in nature and amount according to plant part, physiological age and environment (Fenwick et al., 1991).

Chemically saponin consists of two portions: saccharide and non-saccharide. The non-saccharide portion or aglycone is called the *genin* or *sapogenin*. Depending on the type of genin present, the saponins can be divided into two major classes: triterpenoid (Fig. 1.11) glycosides and steroid (Fig. 1.12)
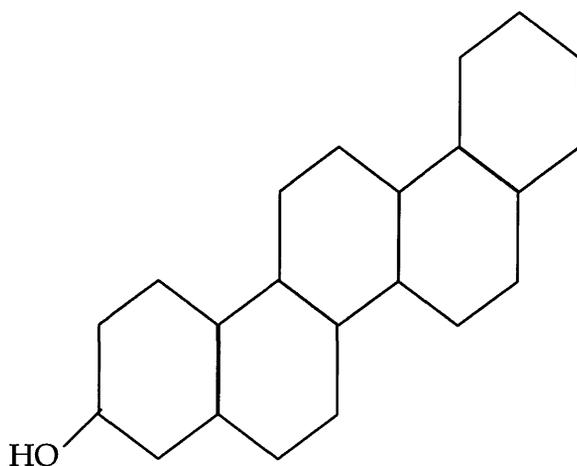
Fig. 1.11 Chemical structure of triterpenoid aglycone base (Hostettmann and Marston, 1995).
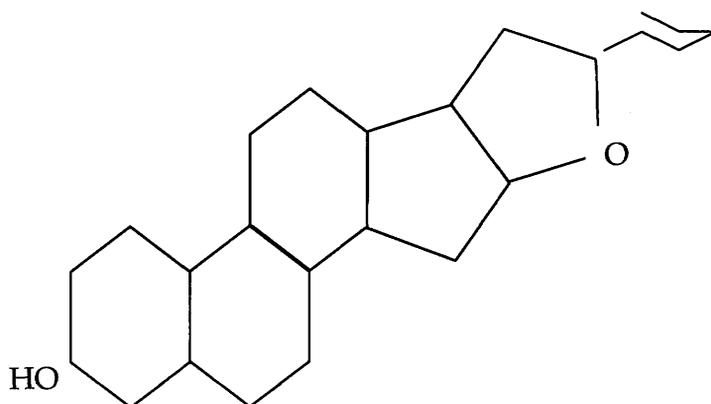
Fig. 1.12 Chemical structure of steroid aglycone base (Hostettmann and Marston, 1995).

glycosides (Hostettmann and Marston, 1995). In cultivated crops triterpenoid glycoside is predominant, whereas steroid glycoside is commonly found in plants used as herbs (Fenwick et al., 1991).

Saponins have detergent properties, produce stable foams in water, show haemolytic activity, have a bitter taste and in water are toxic to fish (Hostettmann and Marston, 1995) their toxicity being related to their activity in lowering surface tension (Birk and Peri, 1980). Animal scientists have always considered saponins as deleterious. Heywang and Bird (1954) found that alfalfa saponin at levels of 0.2% or more in the diet of chicks retarded growth and restricted feed intake and feed efficiency. Pigs and poultry display an aversion to dietary alfalfa. For example, Leamaster and Cheeke (1979) showed

that at all dietary levels of alfalfa exceeding 0.5% of the diet, pigs preferred an alfalfa free control diet. Various avian species (goose, turkey, quail, chicken) likewise showed an aversion to low dietary levels of alfalfa meal (Cheeke et al., 1983). Topping et al. (1980) found that adding saponin, from *Saponaria afficinalis*, to the drinking water of pigs increased faecal bile acid excretion. Jenkins and Atwal (1994) showed that feeding *Gypsophila sp.* saponins reduced chick growth and feed intake at 0.3% of the diet. Igile et al. (1995) fed mice saponin extracted from *Vernonia amygdalina* at levels of 0.33, 0.7 and 1.07%. They observed severe changes in mice growth performance and a significant body weight reduction in all animals compared to the control ones. In humans, high saponin alfalfa has an irritant effect on the tissues of the mouth and throat (Cheeke, 1983).

However, a number of studies have shown beneficial effects of saponins. Ishaaya et al. (1969) could detect no adverse effect of high concentrations of soybean saponins fed to chicks, mice and rats. Yucca extracts containing sarsaponins are extensively used as feed additives in animal production to reduce the emission of ammonia from animal excreta. Wallace et al. (1994) examined the effect of yucca extract on rumen ammonia concentrations, ammonia binding capacity, and rumen microbiology. They observed a decrease in rumen ammonia with addition of yucca extract. In another study involving use of yucca extract, a growth promotant effect was observed in broilers (Johnston et al., 1981). From the foregoing reports it is evident that there are species differences in response to dietary saponin.

1.6.3   Enzyme Inhibitors (EI)

Substances that inhibit the digestive enzymes trypsin, chymotrypsin and amylase are widespread in many legume and cereal species. These inhibitors are proteins and with some exceptions are denatured and inactivated by adequate heating (Liener,1979). Trypsin inhibitors are probably the most widely distributed among the inhibitors of proteolytic enzymes (Reddy and Pierson, 1994). They cause growth inhibition by interfering with protein digestion, causing pancreatic hypertrophy and excessive secretion of pancreatic enzymes (Kakade et al., 1973; Bene et al., 1979).

Protease inhibitors (PIs) are widely believed to be the most important of the antinutrients present within soybeans (Tacon, 1995) and field bean (Abbey et al., 1979; Bene et al., 1979). Kakade et al. (1973) showed that PI were responsible for 40% of the growth depression observed when rats were fed on diets containing raw soybean meal. Krogdahl et al. (1994) reported a negative effect on protein and amino acid digestion when rainbow trout *Oncorhynchus mykiss* fed on diets containing purified soybean protease inhibitors. Results reported by Bene et al. (1979) demonstrated that consumption of PIs from field bean elicits physiological responses such as pancreatic hypertrophy, decreased pancreas levels of trypsin and chymotrypsin and increased intestinal levels of these enzymes in rats.

Liener and Kakade (1980) reported that the most frequent source of PIs is the seed of the plant, but their location is not restricted to this part of the plant. In

some legumes, such as the mung bean and field bean, a fairly high level of trypsin inhibitor is also found in the leaves as well.

According to Liener and Kakade (1980) PIs fall into two main types: Kunitz inhibitors with a molecular weight of 20,000-25,000 dalton and capable of inhibiting trypsin, and Bowman-Birk inhibitors having a molecular weight of 6,000-10,000 dalton and capable of inhibiting trypsin and chymotrypsin.

Amylase inhibitors are sub-group of enzyme inhibitors that exist in several grains, like wheat, oat and rye (Kneen and Sandstedt, 1946). They are classified into three molecular weight groups: 12,500 dalton which affects insect and other invertebrate amylases and 24,000 & 60,000 dalton which inhibit vertebrate amylases (Silano et al., 1975). Amylase inhibitors have been shown to inhibit 10-30% of the amylase activity in carp and rainbow trout (Hofer and Sturmbauer, 1985). Heating can denature such inhibitors but they are resistant to acid treatment (Sturmbauer and Hofer, 1985).

1.6.4   Phytate

Phytate, a cyclic compound (myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) containing six phosphate groups (Fig. 1.13), is a common constituent of plant tissue where it accounts for as much as 85% of the total phosphorus (Reddy et al., 1989). Phytates are present in a wide range of cereals such as barley (Jacobsen et al., 1971), wheat (Tanaka, et al., 1974) and sorghum (Adams and Novellie, 1975) and legumes such as soybean (Hartman,

1979), canola (Al-Asheh and Duvnjak, 1995) and brown beans (Gustafsson and Sandberg, 1995). Their presence is restricted to the seeds of plants and content may vary ranging from 0.14 to 2.22% in cereals and from 0.22 to 9.15% in beans (Reddy et al., 1989).

Phytate has been shown to form stable complexes *in vitro* with di- and trivalent metal ions such as calcium, magnesium, zinc, copper and iron (Nolan and Duffin, 1987). The formation of such complexes *in vivo* is thought to be the mechanism by which phytate reduces dietary metal availability (Davies and Reid, 1979). Different negative effects have been reported as a result of feeding phytate including growth depression and low feed conversion in rainbow trout *O. mykiss* (Spinnelli et al., 1982), growth depression, high mortality,
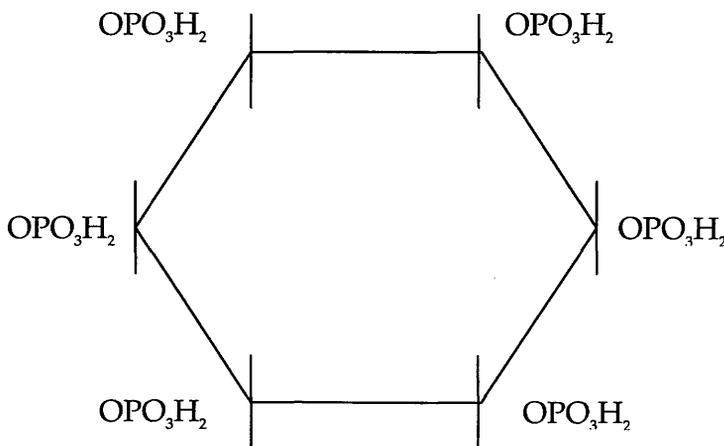
Fig. 1.13 Chemical structure of phytic acid (Reddy et al., 1989).

cataract formation, reduced zinc bioavailability in chinook salmon *Oncorhynchus tshawytscha* (Richardson et al., 1985), depressed protein utilisation and protein digestibility in common carp *Cyprinus carpio* (Hossain and Jauncey, 1991) and reduced zinc bioavailability in blue tilapia *Oreochromis aureus* (McClain and Gatlin, 1988), channel catfish *Ictalarus punctatus* (Gatlin and phillips, 1989) and white shrimp *Penaeus vannamei* (Davies et al., 1993).

Several methods have been reported by Reddy et al. (1989) which eliminate or reduce the content of phytate in seeds. These include: cooking, germination, fermentation, aqueous or alkali extraction and enzymatic hydrolysis.

1.6.5   Lectins

Lectins are carbohydrate-binding proteins or glycoproteins; they have the ability to agglutinate red blood cells so they are called hemagglutinins (Grant, 1991). Lectins are very widely distributed in the plant kingdom, particularly among the legumes, which are known to be very low in nutritive value unless subjected to some form of heat treatment (Liener, 1979). Lectins can be found at high levels in mature seeds, especially those of the legumes, where lectins may constitute as much as 10% of the total protein of the seed. Besides seeds, lectins are found in tubers, leaves and roots (Sharon and Lis, 1989).

Orally ingested lectins are highly toxic to humans and animals, perhaps because they bind specific receptor sites on the surface of the intestinal epithelial cells, seriously impairing the ability of cells to absorb nutrients from

the gastrointestinal tract, thus causing serious growth retardation and even death in extreme cases (Jaffe, 1980; Krogdahl, 1989).

Lectins are heat labile and can therefore be detoxified by heat treatment (Reddy and Pierson, 1994); also they are readily inactivated by pepsin in the stomach (Hendricks and Bailey, 1989).

1.6.6   Gossypol

Gossypol is a natural polyphenolic (Fig. 1.14) toxicant present in the pigment glands of leaves, stems, roots and seed of cotton plants (Berardi and Goldblatt, 1980). Pigment glands comprise between 2.4 and 4.8% of the seed (Singleton
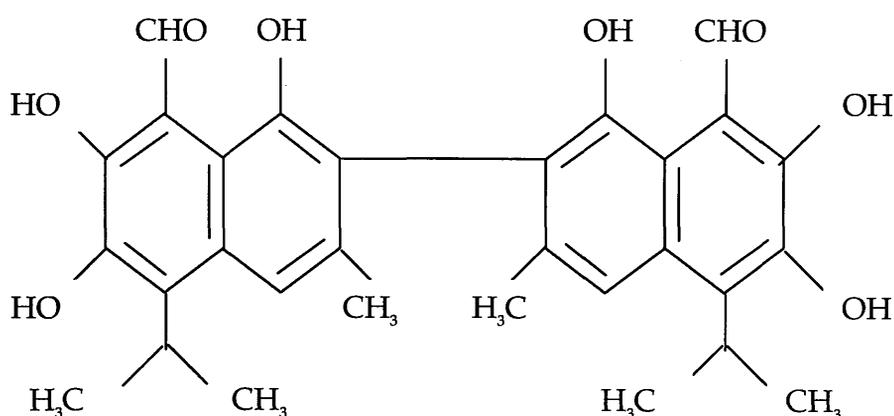
Fig. 1.14 Chemical structure of gossypol (Singleton and Kratzer, 1973).

and Kratzer, 1973) and gossypol is between 39 and 50% of the weight of the glands (Berardi and Goldblatt, 1980). Gossypol is a heat labile toxicant and can be detoxified by heat in the presence of moisture during which the free gossypol form will convert to a bound form (Singleton and Kratzer, 1973). The bound gossypol form is considered non-toxic to animals (Hendricks and Bailey, 1989).

Dietary gossypol has been shown to cause several negative effects in fish. Its presence in fish diets results in growth depression and decreased feed utilisation in channel catfish *Ictalarus punctatus* (Dorsa et al., 1982), Nile tilapia *Oreochromis niloticus* (Ofojekwu and Ejike, 1984; El-Sayed, 1990) and rainbow trout *Oncorhynchus mykiss* (Roehm et al., 1967), low protein digestibility in Nile tilapia *Oreochromis niloticus* (Sintayehu et al., 1996), histological abnormality in the liver and kidney and high mortality in rainbow trout *Salmo gairdneri* (Herman, 1970) and low growth, decreased feed intake and high mortality in white shrimp *Penaeus vannamei* (Lim, 1996).

1.6.7   Cyanogenic glycosides

The cyanogenic glycosides are compounds that yield hydrogen cyanide (HCN), which is a potent respiratory inhibitor, upon treatment with acid or appropriate hydrolytic enzymes (Davis, 1991). These compounds are not toxic *per se*, however, HCN which is released by enzymatic decomposition (Fig. 1.15) is believed to be toxic when the tissues of the plant are disrupted. Two inherent enzymes in two stages are responsible for decomposition of cyanogenic

glycosides to HCN, β-glucosidase and hydroxynitrile lyase (Conn, 1973). The cyanogenic glycosides are widely distributed in lima bean, kidney bean, chickpea, linseed, sorghum and tubers such as cassava and occur in all parts of the plant, leaves, stem, root and seed (Montgomery, 1980).

HCN toxicity in animals occurs when HCN is ingested and absorbed from the gastrointestinal tract and cellular respiration enzymes, notably cytochrome oxidase, are affected. When cytochrome oxidase is combined with cyanide it cannot react with O_2 and consequently aerobic cellular respiration ceases (Conn, 1973). However, the many factors that will affect the toxicity of HCN in the plant include the size and species of the plant consumer, the type of plant consumed, the rate of ingestion, the possibility of the plants' hydrolytic enzymes remaining active in the digestive tract of the species and the ability of the species to detoxify any HCN that it encounters (Conn, 1979).

In fish, feeding studies showed reduced growth and feed efficiency in common carp *Cyprinus carpio* (Hossain and Jauncey, 1989) and Nile tilapia *Oreochromis niloticus* (Ng and Wee, 1989) as a result of feeding cyanogenic glycoside-containing linseed meal and cassava leaf meal respectively.

1.6.8   Glucosinolates

Glucosinolates are sulphur-containing compounds found naturally in all cruciferous plants, including cabbage, broccoli, cauliflower and oilseeds such as
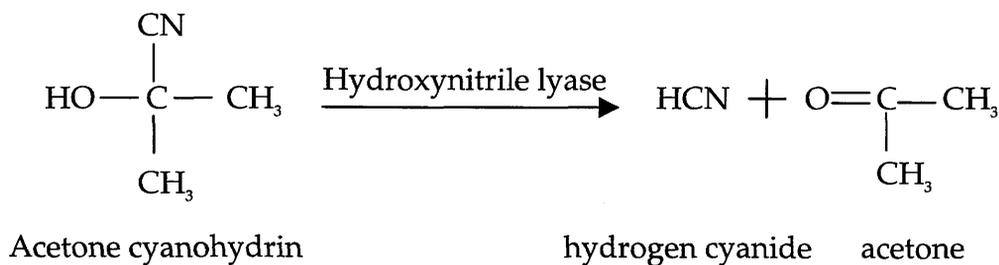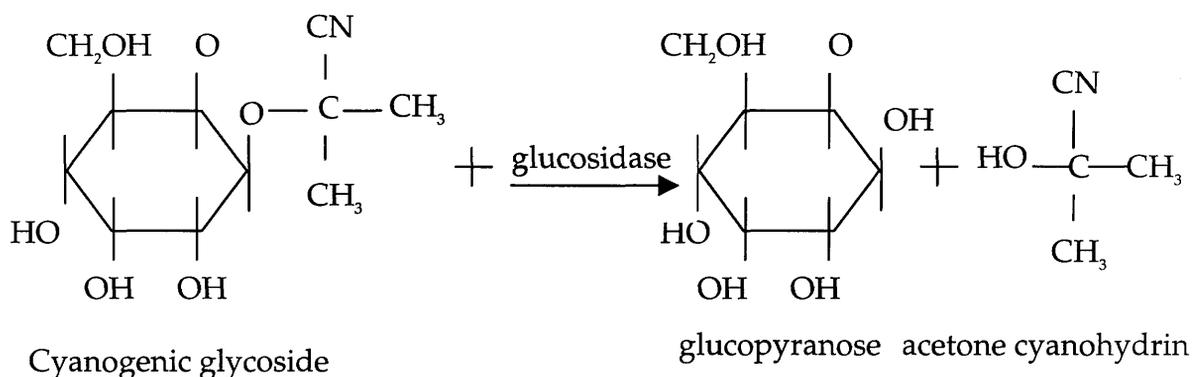
Fig. 1.15 The enzymatic decomposition of cyanogenic glycoside

(Conn, 1973).

rapeseed and mustard seed (NRC, 1993). They occur in all parts of the plant; however, their concentration varies throughout the plant with seed containing higher concentrations than other parts (Duncan, 1991).

Glucosinolates are always accompanied in plant tissue by the thioglucosidase enzyme, myrosinase, which is responsible for the hydrolysis of Glucosinolates

to a number (Fig. 1.16) of toxic metabolites, isothiocyantes, thiocyanate, goitrin and nitriles (Tookey et al., 1980). Collectively these metabolites are believed responsible for reduced feed intake, growth depression, thyroid enlargement and histological abnormalities of liver and kidney. Lee et al. (1984) added glucosinolate extracts to soyabean meal and demonstrated depression of the voluntary feed intake of growing pigs consuming the diet. In a further experiment, Vermorel et al. (1987) showed that administration of isolated glucosinolates, from rapeseed, to rats had little effect on dry matter intake and live weight gain. Feeding studies with rats fed nitrile and 5-vinyl-2-oxazolidinethione, a metabolite of isothiocyanate, showed increased thyroid weight and kidney enlargement (Duncan, 1991). Isothiocyanate has also been shown to increase thyroid gland weight in rats (Langer and Stolc, 1965).

The effects of glucosinolates on the performance of fish have been studied mainly in the context of feeding rapeseed meal and mustard seed meal to fish. It was suggested that the presence of allyl isothiocyanate in mustard oilcake is the reason for growth depression and histological abnormalities in the liver and thyroid tissues of common carp *Cyprinus carpio* (Hossain and Jauncey, 1989). Similar results, in terms of growth depression, were reported (Stickney et al., 1996) when rainbow trout *Oncorhynchus mykiss* fed on diets containing different levels of canola meal. Yurkowski et al. (1978) found that feeding rainbow trout *Oncorhynchus mykiss* rapeseed meal caused growth depression and thyroid hyperplasia.

S-glucose

$CH_2=CH-CH_2-C$

glucosinolate

$NOSO_3$

H_2O

myrosinase

SH

$CH_2=CH-CH_2-C$ +   glucose

allyl thioglucoside $NOSO_3$

$CH_2=CHCH_2CN$ $CH_2=CHCH_2NCS$ CH_2-CHCH_2CNS $CH_2=CHCH_2SCN$
nitriles   isothiocyanate   goitrins   thiocyanate
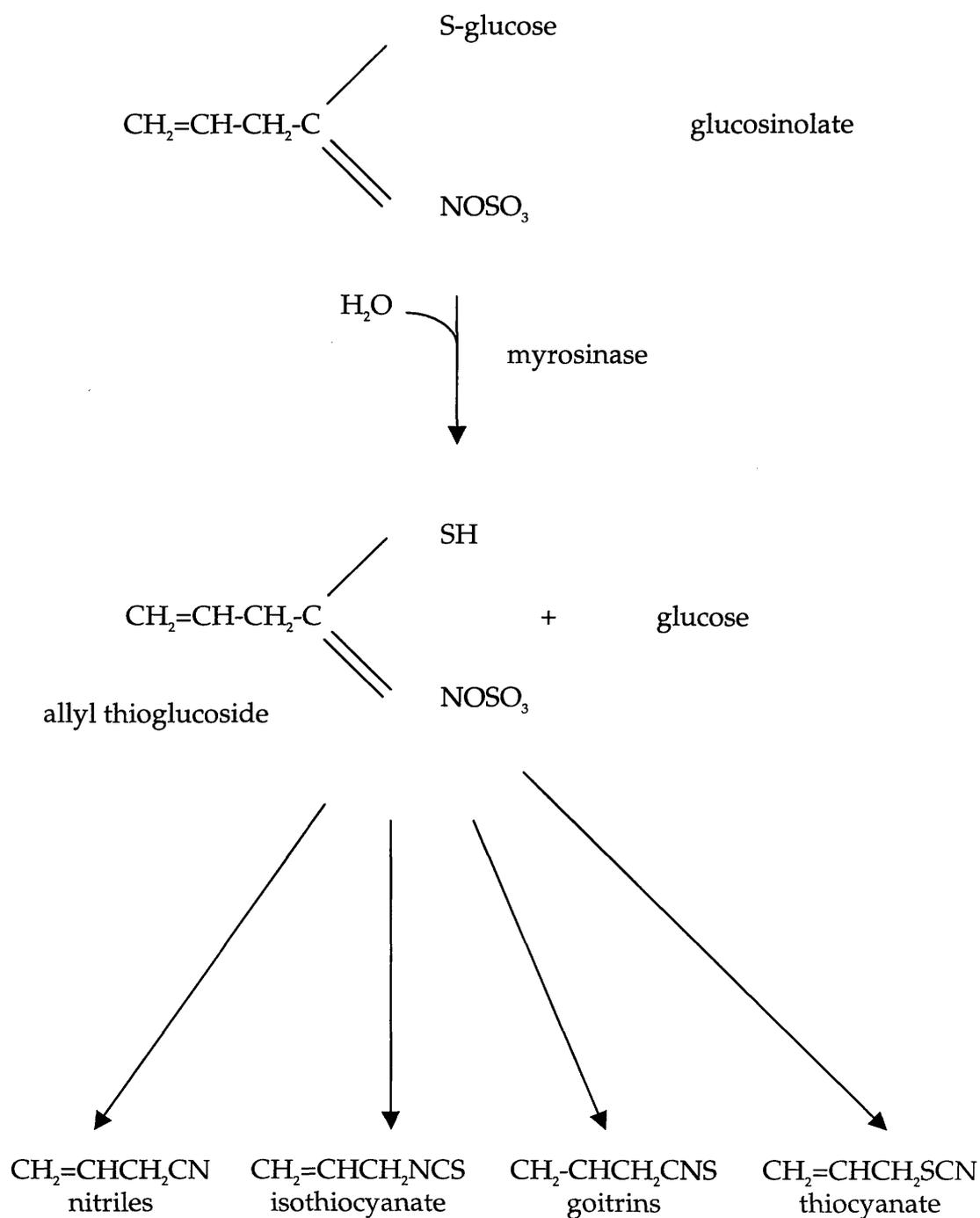
Fig. 1.16 The enzymatic decomposition of glucosinolates (Duncan,

1991).

Glucosinolates can be detoxified by heat treatment in which the enzyme myrosinase, that hydrolyses the glucosinolates to their toxic metabolites, will be inactivated; however, heating will not eliminate the glucosinolates (NRC, 1993). Glucosinolates can also be eliminated or reduced by means of aqueous extraction. Yurkowski et al. (1978) found that extraction of rapeseed meal with water reduced 90% of the glucosinolate content and led to improved growth of rainbow trout *Oncorhynchus mykiss*.

1.6.9   Antivitamins

Antivitamins are compounds that diminish or destroy the biological activities of vitamins and are found in a variety of plants (Somogyi, 1973). The presence of these compounds in some beans and meals may cause negative effects for the consumers. Flax seed has been shown to retard growth in chicks, an effect which is reversed by providing pyridoxine (Klosterman et al., 1967). Lipoxidase present in raw soybean can oxidise and destroy carotene (Liener, 1980). It has been shown that a high levels of raw soybeans, 30%, in the feed of calves can affect blood levels of carotene and retinol (Shaw et al., 1951). Factors have been shown in raw kidney bean, soybean and alfalfa which cause signs of vitamin E deficiency, liver necrosis in weanling rats and muscular dystrophy in chicks and lambs. The effects were overcome by supplementing with vitamin E and partly overcome by autoclaving the feed (Bender, 1987).

1.7   Objectives

From the foregoing discussion it is clear that one of the major problems faced by rapidly growing aquaculture in the world is the availability of feeds. In order to replace fish meal with plant protein meals, a full knowledge of antinutrients which are inherent in such meals is desired. It is believed that if the effect of these antinutrients on fish was fully understood, this may assist in the development of plant proteins as fish meal substitutes. The objective of the present study was to elucidate the effect of tannins and saponins on fish performance. According to Tacon (1995) there is no published information concerning the effect of dietary saponin and tannin on fish. Thus, the aims of the present research were to study:-

1- the use of commercially purified antinutrients, tannin and saponin, which are naturally present in some plant proteins, in purified diets to determine their effects on performance of Nile tilapia and African catfish fingerlings.

2- the effects of these antinutrients on protein digestibility.

3- the effects of these antinutrients on digestive enzymes.

4- the histopathological changes in fish intestine and liver resulting from ingestion of the various antinutrients under study.

Two fish species with commercial importance were used as biological models in this thesis. First the Nile tilapia *Oreochromis niloticus* (L) a highly and widely valued freshwater species, of great importance in commercial aquaculture. Second the African catfish *Clarias gariepinus* (B) a freshwater species with a wide natural distribution in Africa, Asia and Latin America.

CHAPTER II

GENERAL MATERIALS AND METHODS

2.   Materials and Methods

2.1   Experimental System

All experiments were conducted in a temperature controlled recirculation system (Fig. 2.1). The system consisted of twenty four self cleaning centrally drained circular tanks, each with a capacity of 30 L. Water was supplied to each tank at a rate of 1L min^{-1} from a 115L header tank. Water drained from the tanks through central stand pipes into a series of settling and biological filter tanks. The water was then pumped up to a 115L tank which was aerated using airstones and heated using 3 KW thermostatically controlled immersion heaters to maintain a temperature of $28 \pm 1^{0}C$ throughout the experimental period. Thirty per cent of total water was replaced biweekly with fresh water to adjust water quality (Table 2.1) and to avoid accumulation of faeces and uneaten feed or any excretory products. A constant photoperiod of 12 hours light and 12 hours dark was maintained.

2.1.1   Water Quality

2.1.1.1   Dissolved Oxygen

Dissolved oxygen of the water in the experimental system was monitored biweekly using an oxygen meter (YSI 57 Clandon, Ohio, USA).

2.1.1.2   pH

pH of the water in the experimental system was monitored biweekly using a pH meter (CG 840, Schott).

2.1.1.3   Temperature

Water temperature of the experimental system was thermoregulated with a 3
KW electric heater. The 3 KW electric heater was controlled by a Deem 10/1193
thermister which linked to an on/off controller set at $28\pm 1°C$.

2.1.1.4   Total Ammonia

Total ammonia of the water in the experimental system was determined using a
Tecnicon Auto Analyser II according to the method (SCA, 1981) adopted by the
Water Quality Laboratory, Institute of Aquaculture, University of Stirling.

Table 2.1 Water quality parameters as measured during the time of
the experiments

Parameter	Range
Dissolved oxygen (mg/L)	7.5-8.0
PH	6.6-7.3
Temperature (^{0}C)	27-29
Total ammonia (mg/L)	0.047-0.11

2.2   Experimental Fish

Nile tilapia *Oreochromis niloticus* (average weight 3.5-4g each) and African catfish *Clarias gariepinus* (average weight 4.5-5g) were obtained for the experimental work from stock held at the Institute of Aquaculture, University of Stirling. Fish were randomly assigned into groups of 25 fish and each group was placed in an individual aquarium (30 L).

2.3   Experimental Diets

2.3.1   Diet Formulation

Purified isoenergetic isonitrogenous diets were formulated to contain 32% crude protein and 11% lipid. Casein (Sigma 3400) and gelatin (Sigma 2625) were used as sources of protein whereas dextrin (Sigma 2131) was used as a source of carbohydrate. A blend of capelin and herring oil (BOCM Pauls Co., Glasgow) was used to achieve the desired level of lipid in the diets. α-cellulose (Sigma 8002) was used as a filler and carboxymethyl cellulose (Sigma 5013) was used as a binder at a rate of 2%. Antinutrient factors, saponin (Sigma 7900), tannic acid (Sigma 0125) and catechin (Sigma 1251) were added separately to the diets at levels of 0, 0.1, 0.5 and 1%. One per cent chromium (III) oxide (BDH 277574Q) was used as the inert indicator for protein digestibility. A mineral premix (Table 2.2) and vitamin premix (Table 2.3) were added to the diets at a rate of 4 and 2% respectively (Jauncey and Ross, 1982).

Table 2.2 Composition of mineral premixes used in experimental diets [1]

Minerals	Chemical Formula	g/kg	Source
Calcium Orthophosphate	$CaHPO_4.2H_2O$	727.7775	BDH 71766DD
Magnesium Sulphate	$MgSO_4.7H_2O$	127.5000	BDH 291172L
Sodium Chloride	$NaCl$	60.0000	BDH 26281
Potassium Chloride	KCl	50.0000	BDH 101984L
Iron Sulphate	$FeSO_4.7H_2O$	25.0000	BDH 101124V
Zinc Sulphate	$ZnSO_4.4H_2O$	5.5000	BDH 102994R
Manganese Sulphate	$MnSO_4.4H_2O$	2.5375	BDH 101534M
Copper Sulphate	$CuSO_4.5H_2O$	0.7850	BDH 10091
Cobalt Sulphate	$CoSO_4.7H_2O$	0.4775	BDH 27801
Calcium Iodate	$CaIO_3.6H_2O$	0.2950	BDH 27602
Chromic Chloride	$CrCl_3.6H_2O$	0.1275	BDH 27752

1- Jauncey and Ross (1982)

Table 2.3 Composition of the vitamin premix used in experimental diets[1]

Vitamins	mg/kg[2]	Source
Cyanocobalamin (B12)	1.25	Sigma V2876
Ascorbic Acid (C)	37500	Sigma A0278
Cholecalciferol (D)	4	Sigma C9756
Tocopherol Acetate (E)	7000	Sigma T3376
Menadione (K)	1500	Sigma V3501
Thiamine Hydrochloride (B1)	4250	Sigma T4625
Riboflavin (B2)	3000	Sigma R4500
Pyridoxine (B6)	1250	Sigma P9755
Calcium Pantothenate	5250	Sigma P9153
Niacinamide	12500	Sigma N3376
Biotin (H)	90	Sigma B4501
Folic Acid	1000	Sigma F7876
Choline Chloride	74050	Sigma C1879
myoInositol	25000	Sigma I5152
Ethoxyquin	200	Sigma E8260

1- Jauncey and Ross (1982)
2- This premix was made up to 1 kg with α-cellulose

2.3.2   Diet Preparation

Dry dietary ingredients were first mixed for about 30 minutes in a stainless steel food mixer bowl (Hobart Co. Ltd, London, England) to ensure that the mixture was well homogenised and then blended with oil for another 15 minutes. Water was added at 30% v/w to give a pelletable mixture. The resulting mixture was pressure-pelleted through a 2 mm die and air-dried for 12 h at 50°C. Dried feed was mechanically reduced using a manual grinder and sieved to obtain particle sizes between 1 and 2 mm which were stored at -20°C until use.

2.4   Experimental Practice

2.4.1   Acclimation and Weighing Procedures

Prior to initiation of each experiment, the fish underwent a 2-week conditioning period during which they readily adjusted to a purified casein-gelatin diet (Garling and Wilson, 1976). At the beginning of each experiment, fish were individually weighed under anaesthesia with 1% Benzocaine solution (Ross and Geddes, 1979). Fish were gently wiped dry with a soft tissue paper and weighed on a Mettler PM 6000 balance to the nearest 0.1g. Fish were bulk-weighed, once every week for tilapia and every two weeks for catfish, using the same mentioned balance. The weekly and biweekly bulk weights of fish were used to adjust the daily feed ration for the following week/weeks.

Experimental tanks

Header tank

Pump

Sump tank

Settlement and biofilter tanks
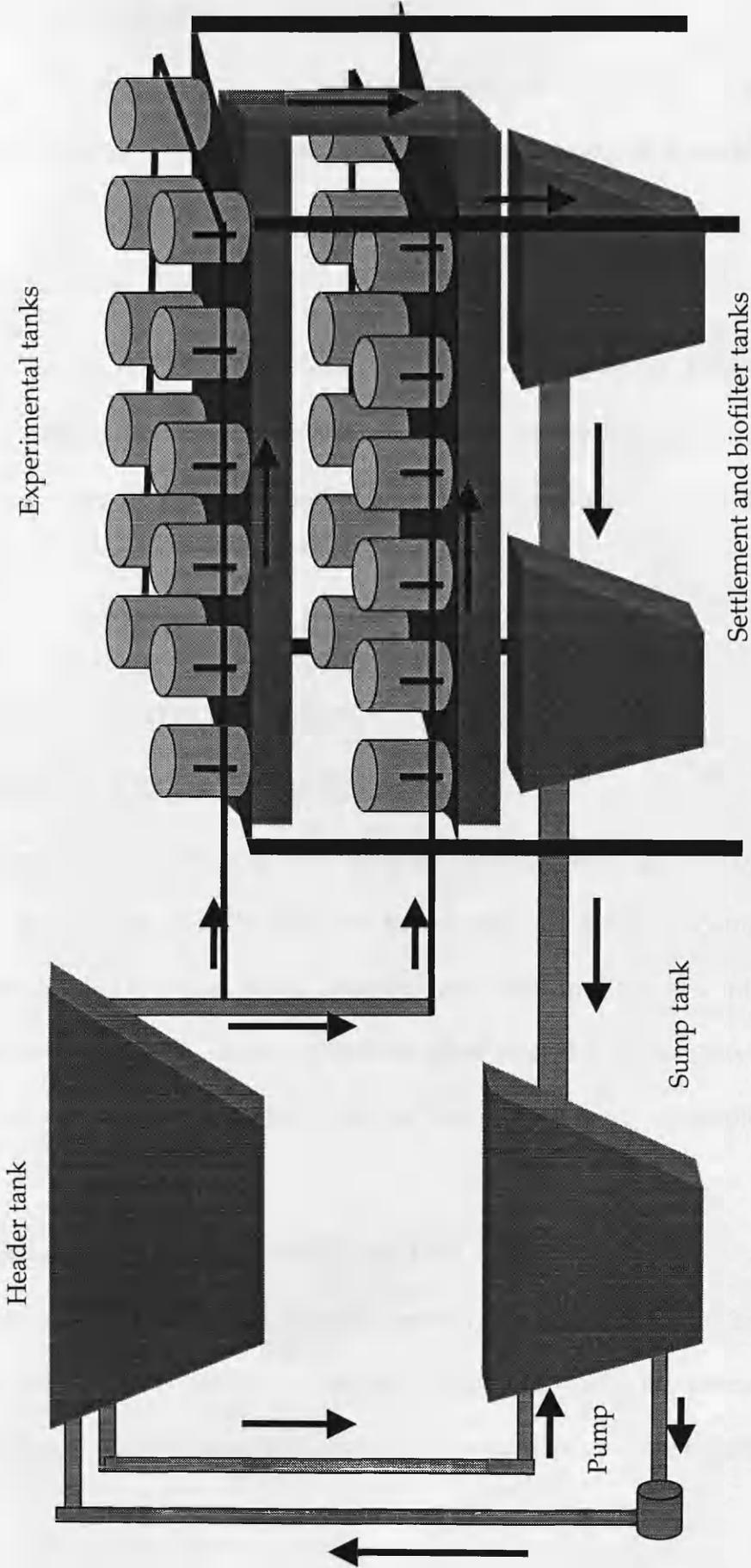
Fig. 2.1 Schematic three dimensional view of experimental system

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2.4.2   Experimental Period

Six growth performance experiments were conducted for 8 weeks each; whereas four digestive enzymes studies were conducted for 2 weeks each.

2.4.3   Fish Feeding

Fish were offered 3% of their body weight per day subdivided into three equal feeds at 10:00, 14:00 and 18:00 every day. Fish within a treatment group (three replicates per treatment) were fed one of the four experimental diets in each study.

2.4.4   Faeces Collection

2.4.4.1   Tilapia Faeces Collection

Faeces were collected daily from the growth system (in triplicate) by siphoning from the bottom of each tank two hours after the second feeding and were freeze-dried. The dried faecal samples were kept in air-tight containers until chemically analysed. Faeces collections were started from the second week of each study and were continued until sufficient faecal matter was collected.

2.4.4.2   Catfish Faeces Collection

Faeces were collected daily from the growth system (in triplicate) by siphoning from the bottom of each tank every morning at 8:30 and were freeze-dried. The dried faecal samples were kept in air-tight containers until chemically analysed.

Faeces collections were started from the second week of each study and were continued until sufficient faecal matter was collected.

2.5   Experimental Analyses

This comprised chemical analysis and biological evaluation of relevant nutrient components in diets, faeces and fish tissues together with growth performance and feed utilisation indicators.

2.5.1   Chemical Analysis

Chemical analyses of diets, fish and faeces were carried out using the following procedures (AOCA, 1990). All samples were analysed in triplicate except chromic oxide and protein in faeces which were analysed in duplicate.

2.5.1.1   Moisture

Representative portion of sample was dried to constant weight in an oven maintained at 110^0C (approximately 24 hours).

2.5.1.1.1   Procedures

One gram of sample was pre-weighed (W_1) on a foil tray and placed in the oven (Gallenkamp) at 110^0C for 24 hours. The sample was removed from the oven cooled in a dessicator and reweighed (W_2). Moisture percentage was calculated according to the formula:

$$\text{Moisture (\%)} = (W_1 - W_2/W_1) \times 100$$

2.5.1.2   Crude Protein (CP)

Crude protein was determined by the Kjeldhal method, the most widely used method employed for the determination of protein in organic substances. It is based on the fact that upon digestion with concentrated sulphuric acid and catalysts, the organic compounds are oxidised and the nitrogen is converted to ammonium sulphate. Upon making the reaction mixture alkaline, ammonia is liberated which is removed by steam distillation, collected and titrated.

2.5.1.2.1   Procedures

150 mg sample was placed in a Kjeldhal digestion tube. Two mercury kjeltabs (Fisher K/0130/80) and 5 ml concentrated sulphuric acid (BDH 45006) were added to the sample. The sample was digested at 400^0C (Digestion system 40 Tecator 1006 heating Unit) for 1 hour. Twenty ml of deionised water and 5 ml of 1.33N sodium thiosulphate were added to the sample after allowing it to cool. The sample was then distilled, ammonia liberated collected in standard boric acid and titrated against 0.2 M hydrochloric acid. Both distillation and titration were automated (Kjeltec Auto 1030 Analyser Tecator). A blank sample was prepared and treated in the same manner except that the tube was free of sample. Protein percentage was calculated according to the formula:

protein (%) = (sample titre - blank titre) $\times$ 0.2[1] $\times$ 14.007[2] $\times$ 6.25[3] $\times$ 100 / sample weight

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1- normality of hydrochloric acid; 2- molecular weight of nitrogen; 3- nitrogen factor, since protein is assumed to be 16% nitrogen.

2.5.1.3   Crude Lipid

Crude lipid was determined by extraction with petroleum ether solvent. The method depends upon the heating of petroleum ether which is allowed to pass through the sample to extract the lipid. The extract is collected in a cup and when the process is completed, the ether is evaporated and the remaining, crude lipid, is dried and weighed.

2.5.1.3.1   Procedure

One gram of sample was weighed into an extraction thimble and covered with absorbent cotton. 50 ml of petroleum ether was added to a pre-weighed cup which contained 5 glass balls. Both thimble and cup were attached to the extraction unit (Soxtec System 1043). The sample was subjected to boiling in petroleum ether for 30 minutes and then rinsed for 1 hour. Petroleum ether was evaporated from the cup to the condensing column. Cup and thimble were removed from the extraction unit and the cup was placed in an oven at 110^0C for 2 hours. At the end of this period the cup was removed from the oven, cooled in dessicator and weighed. Extracted lipid was expressed as percentage of the original sample and calculated according to the formula:

Crude lipid (%) = (extracted lipid / sample weight) × 100

2.5.1.4   Crude Fibre (CF)

The method depends upon digestion of a moisture free and ether extracted sample with weak acid solution and then with weak base solution.   The remaining residue is ashed and the difference of weight on ashing is considered crude fibre (hydrolysis resistant matter).

2.5.1.4.1   Procedures

One gram of defatted sample was placed in a glass crucible and attached to the extraction unit (Fibertec System 1020 hot Extractor).   150 ml of boiling 1.25% sulphuric acid solution and 10 drops of Octanol (anti-foaming agent) were added.   The sample was digested for 30 minutes and then the process was stopped, the acid was drained out and the sample washed with boiling distilled water.   After this, 150 ml boiling 1.25% sodium hydroxide solution and 10 drops of Octanol were added.   The sample was digested for 30 minutes then the process was stopped, the alkali was drained out and the sample washed with boiling distilled water.   Finally the crucible was removed from the extraction unit and oven dried at 110^{0}C overnight.   The sample was taken out the next day, cooled and weighed (W_1).   The sample was then ashed at 600^{0}C in a muffle furnace (Gallenkamp Muffle Furnace) for 2 hours, cooled in dessicator and reweighed (W_2).   Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula:

fibre (%) = (digested sample W_1 - ashed sample W_2) / sample weight

2.5.1.5   Ash

Ash content was determined by incineration of the sample at 600^0C.   The remaining inorganic materials which do not burn off are reduced to their most stable forms, oxides or sulphates and are considered ash.

2.5.1.5.1   Procedures

0.5 gram sample was weighed into a pre-weighed porcelain crucible and incinerated (Gallenkamp Muffle Furnace) overnight at 600^0C. The crucible was removed from the muffle furnace, cooled in dessicator and weighed. Incinerated sample was expressed as a percentage of the sample and calculated according to the formula:

$$\text{ash (\%) = (ash weight / sample weight)} \times 100$$

2.5.1.6   Nitrogen Free Extract (NFE)

Nitrogen free extract (carbohydrate) was calculated by subtracting the total of moisture, crude protein, crude lipid, ash, crude fibre and from 100%.

$$\text{NFE (\%) = 100 - (moisture + ash + fibre + ether extract + protein)}$$

2.5.1.7   Gross Energy

Gross energy was determined by Automatic Adiabatic Bomb Calorimeter (Gallen Kamp & Co Ltd, England). It is based on the fact that upon combustion

in a bomb chamber in a flow of pure Oxygen, the sample is burned and the resulting heat is measured by the increase in the temperature of the water surrounding the bomb.

2.5.1.7.1   Procedure

One gram of sample was firm pelleted by briquette press and reweighed in a crucible. The sample was connected to the firing wire, which was fitted between the electrodes, by cotton line. The electrode assemply was placed into the bomb then the bomb cap was tightened. The circuit was tested and the bomb was filled with oxygen to a pressure of 30 bar. The calorimeter vessel was filled with water at 21-23°C (total weight 3Kg) and the prepared bomb was placed inside the calorimeter vessel, then the calorimeter vessel was placed into the water jacket. The machine was switched on and left for a while (10 minutes) to warm up. Prior to firing the initial temperature of the water was checked and recorded and 10 minutes after firing the final temperature was recorded. Finally, the energy content was calculated according to the formula:

Energy (KJ/g) = [(final temp.-initial temp.)×10.82]-0.0896/sample weight (g)

2.5.1.8   Chromic Oxide

Chromic oxide was determined according to the method of Furukawa and Tsukahara (1966). The procedure depends upon the digestion of the sample by concentrated nitric acid and oxidising chromic oxide by 70% perchloric acid.

The orange colour formed by the oxidation of chromium III to chromium IV is read on a spectrophotometer (Uvikon 810) at 350nm against distilled water.

2.5.1.8.1   Procedure

50-100 mg of sample was weighed into a Kjldahl flask. 5 mL of concentrated nitric acid were added to the flask and the mixture was boiled gently for about 20 minutes (care taken not to boil dry). After cooling the sample, 3 ml of 70% perchloric acid was added to the flask. The mixture then was gently heated again until the solution turned from a green to an orange colour plus 5 minutes to ensure oxidation is complete. The solution was transferred to a 100 ml volumetric flask and diluted to volume. The absorbance of the solution was determined by a spectrophotometer (Uvikon 810) at 350nm and chromic III oxide was calculated according to the formula:

Chromic oxide (%) = [(absorbance - 0.0032 / 0.2089) / sample weight] × 100

2.5.1.9   Hydrolysable tannin (Tannic acid)

Hydrolysable tannin was determined according to the method of Allen (1989). The procedure depends upon the extraction of tannic acid from the feed by boiling water. The extracted sample is treated with Follin-Denis reagent to produce a blue colour. The blue colour intensity is proportional to tannic acid concentration in the sample. The colour formed is read on spectrophotometer (Uvikon 810) at 760nm against distilled water. The following reagent and standards were prepared for tannic acid determination.

2.5.1.9.1   Folin-Denis Reagent

Folin-Denis reagent was prepared by dissolving 50 gram of sodium tungstate (Sigma 0765) and 10 gram of phosphomolybdic acid (Sigma 7390) in 25 ml of orthophosphoric acid (BDH 10173AB) and 375 ml of water. The mixture was refluxed in a water bath at 80^{0}C for 2 hours. The mixture was cooled and diluted to 500 ml with distilled water.

2.5.1.9.2   Tannic acid Standard

The tannic acid standard was prepared by dissolving 50 mg of tannic acid (Sigma 0125) in 500 ml of distilled water. One ml of water contained 0.1 mg of tannic acid. A group of 4 volumetric flasks were numbered and 0, 1, 2, 3 ml of tannic acid standard were pipetted in flasks 1, 2, 3 and 4 respectively. Standards were treated with Folin-Denis, incubated and read as samples.

2.5.1.9.3   Procedures

0.1gram dry ground sample was weighed into a conical flask (100 ml). 50 ml of distilled water was added to the sample and left on hot plate for gentle boiling for 1 hour. The sample extract was filtered through Whitman No. 44 filter paper into a 50 ml volumetric flask and diluted to a volume. Three ml of extract was pipetted into a 50 ml volumetric flask; 2.5 ml of Folin-Denis reagent and 10 ml of sodium carbonate (BDH 301214L) were added then diluted to volume. The sample was incubated in a water bath at 25^{0}C for 20 minutes. The

colour formed was read on a spectrophotometer (Uvikon 810) at 760nm and absorbance compared to a tannic acid standard curve.

2.5.1.10   Condensed tannin (Catechin)

Condensed tannin was determined according to the method of Burns (1971). The procedure depends upon the extraction of catechin from the feed with methanol by swirling into a 100 ml conical flask for 24 hours. The extracted sample is treated with Vanillin-HCl reagent to produce yellow colour. The yellow colour intensity is proportional to condensed tannin concentration in the sample. The colour formed is read on a spectrophotometer (Uvikon 810) at 500nm against vanillin-HCl reagent. The following reagent and standards were prepared for catechin determination.

2.5.1.10.1   Vanillin-HCl Reagent

Vanillin-HCl reagent was prepared by combining equal volumes of 8% concentrated hydrochloric acid in methanol and 4% vanillin (Sigma 2375) in methanol.

2.5.1.10.2   Catechin Standard

The catechin standard was prepared by dissolving 50 mg of catechin (Sigma 1251) in 50 ml of methanol. Several dilutions with methanol were made to obtain a 1000, 500, 400, 300, 200, 100 and 50 µg concentration of catechin in 1 ml to construct standard curve. One ml of each dilution was treated as sample and

the colour formed was read on spectrophotometer (Uvikon 810) at 500nm against vanillin-HCl reagent.

2.5.1.10.3   Procedures

One gram dry ground sample was weighed into a capped bottle (100 ml). 50 ml of methanol was added to the sample and left on stirring magnetic plate for 24 hour. One ml of the methanol extract was pipetted into a 15 ml test tube and 5 ml of vanillin-HCl reagent were added with mixing. The sample was incubated at room temperature for 20 minutes. The colour formed was read on a spectrophotometer (Uvikon 810) at 500nm against vanillin-HCl reagent and catechin was used as a standard.

2.5.1.11   Saponin

Saponin was determined according to the method of Baccou et al. (1977). The procedure depends upon the extraction of saponin from the feed with methanol (Aderibigbe et al., 1997) by swirling in a 25 ml glass bottle for 24 hours. The extracted sample is treated with 99.5% ethyl acetate and concentrated sulphuric acid to produce a yellow colour. The yellow colour intensity is proportional to saponin concentration in the sample.   The colour formed is read on spectrophotometer (Uvikon 810) at 430nm against blank sample. The following reagent and standards were prepared for saponin determination.

2.5.1.11.1 Reagent

A 99.5% ethyl acetate reagent was prepared by adding 0.5 ml of anisaldehyde to 99.5 ml of ethyl acetate.

2.5.1.11.2 Saponin Standard

The saponin standard was prepared by dissolving 20 mg of saponin (Sigma 7900) in 20 ml of ethyl acetate. Several dilutions with ethyl acetate were made to obtain 20, 15, 10, 5, 4, 3 and 2 µg concentrations of saponin in 1 ml to construct a standard curve. One ml of each dilution was treated as sample and the colour formed was read on a spectrophotometer (Uvikon 810) at 430nm against blank.

2.5.1.11.3 Procedure

0.5 gram dry ground sample was weighed into a capped bottle (25 ml). 15 ml of methanol was added to the sample and left on stirring magnetic plate for 24 hour. 0.5 ml of the methanol extract was pipetted into a 15 ml test tube and 2 ml of ethyl acetate, 1 ml of ethyl acetate reagent and 1 ml of concentrated sulphuric acid were added with mixing. The sample was incubated at room temperature for 20 minutes. The colour formed was read on a spectrophotometer (Uvikon 810) at 430nm against blank sample and saponin was used as a standard. A blank sample was prepared in the same manner as test samples except that 0.5 ml of distilled water was used instead of extracted sample.

2.5.2   Biological Evaluation

2.5.2.1   Percentage weight gain (%)

$$\% \text{ weight gain} = (W_2 - W_1 / W_1) \times 100$$

Where W_1 is the mean initial fish weight and W_2 is the mean final fish weight.

2.5.2.2   Specific Growth Rate (SGR)

SGR is the rate of change in weight of fish calculated as the percentage increase in body weight per day over any given time interval as follows:

$$\text{SGR} (\% / \text{Day}) = [(\text{Ln}W_2 - \text{Ln } W_1) / (T_2 - T_1)] \times 100$$

Where W_1 is the initial fish weight (g) at time T_1 (day) and W_2 is the final fish weight (g) at time T_2 (day).

2.5.2.3   Food Conversion Ratio (FCR)

FCR is defined as the amount of dry weight of feed per unit wet weight gain. It was calculated as follows:

$$\text{FCR} = \text{g dry food fed} / \text{g live weight gain}$$

2.5.2.4   Protein Efficiency Ratio (PER)

PER is a measure of the wet weight gain per unit protein fed. PER gives an indication of the efficiency of protein utilisation. PER was calculated as follows:

$$\text{PER} = \text{weight gain (g)} / \text{protein fed (g)}$$

2.5.2.5   Protein Productive Value (PPV)

PPV is an improved measure of protein utilisation. PPV expresses the percentage of ingested protein that is retained by deposition in the fish carcass. PPV was calculated as follows:

$$\textbf{PPV} = \textbf{(P}_2\textbf{-P}_1\textbf{)/ protein fed (g)} \times \textbf{100}$$

Where P_1 is the protein in fish carcass (g) at the beginning of the study and P_2 is the protein in fish carcass (g) at the end of the study.

2.5.2.6   Protein Digestibility

The apparent protein digestibility was calculated according to Maynard et al. (1979) as follows:

% protein digestibility =

$$100\text{-}[(\%Cr_2O_3 \text{ in feed}/\%Cr_2O_3 \text{ in faeces}) \times (\% \text{protein in faeces}/\% \text{protein in feed})] \times 100$$

2.5.2.7   Dry matter Digestibility

The dry matter digestibility was calculated according to Windell et al. (1978) as follows:

$$\textbf{\% dry matter digestibility} = 100 - \left[100\ (\%\ Cr_2O_3 \text{ in feed} / \%\ Cr_2O_3 \text{ in faeces})\right]$$

2.6   Histological analysis

Histological analysis was performed to investigate differences or abnormalities in fish livers and intestines as a result of feeding the antinutrient factors, tannin

and saponin. The method of Drury and Wallington (1980) was adopted to perform the histological techniques which are also approved by the Institute of Aquaculture, University of Stirling.

At the end of each growth experiment, 4 fish from each tank were sacrificed and livers and intestines were dissected. Samples were fixed in 10% neutral buffered formalin for at least 24 hours. Samples were cassetted and processed automechanically for dehydration, clearing and wax impregnation. Processed samples were embedded in paraffin wax and sectioned by microtome (5 μm thickness). Samples then were stained with haematoxylin and eosin and examined under light microscope to evaluate any differences or abnormality among samples.

2.7   Enzymes Studies

Enzymes studies were designed to investigate the effect of anti-nutrients, tannic acid (hydrolyzable tannin) and catechin (condensed tannin), on digestive enzyme activities.

2.7.1   Collection of digestive tract

Fish were dissected on day 14/ four hours after the second feeding and digestive tracts (stomachs and intestines) were removed. After as much as possible of the remaining fat had been removed, digestive tracts were blotted

dry with tissue paper and kept in small plastic bags individually before freezing (-20^0C) until use for the estimation of enzymes.

2.7.2   Enzymes extraction

Frozen stomachs and intestines were weighed and homogenised separately in an electrical homogeniser (Ultra-Turrax-England) with ice-cold physiological saline (9 g sodium chloride/L). Two samples from each tank were pooled together prior to homogenisation in order to minimise individual differences and to obtain sufficient extract to run the enzyme assays. Samples were surrounded by ice during homogenisation, and homogenisation carried out at 2000 rev/minutes for 3 minutes. One ml of physiological saline was used for each 100 mg of frozen stomach or intestines. The homogenised samples were then centrifuged at 4500 rpm (MSE Mistral 2000R centrifuge, Sanyo model) for 15 minutes. at 4°C. The resulting supernatant was used for the enzyme assays.

2.7.3   Enzyme Assays

2.7.3.1   Pepsin (EC 3.4.23.1)

Pepsin activity was determined according to the method of Anson (1938) using haemoglobin as a substrate. The procedure depends upon the break down of haemoglobin to peptides and free amino acids by extracted enzyme solution. The amount of tyrosine liberated from the substrate is taken as a measurement of pepsin activity. The following substrate, reagent and standards were prepared for pepsin determination.

2.7.3.1.1   Haemoglobin substrate

The substrate was prepared by dissolving 2 grams of haemoglobin (Sigma 2625) in 100 ml of 0.06N HCl and concentrated hydrochloric acid was used to adjust the pH to 2.

2.7.3.1.2   Phenol Reagent

Phenol reagent was prepared by dissolving 3 grams of sodium tungstate (Sigma 0765), 0.8 gram of sodium molybdate (Sigma 6646) and 5 grams of lithium sulphate (Sigma 6370) in 3.5 ml of concentrated hydrochloric acid and 1.6 ml of concentrated orthophosphoric acid (BDH 10173AB). The mixture was refluxed in a water bath at 80^0C until all components were totally dissolved and then made up to 100 ml with distilled water.

2.7.3.1.3   Tyrosine Standard

The tyrosine standard was prepared by dissolving 20 mg of tyrosine (Sigma 3379) in 20 ml of 0.2N HCl. Several dilutions with 0.2N HCl were made to obtain a 20, 15, 10, 5, 4, 3 and 2 µg concentration of tyrosine in 1 ml to construct a standard curve. One ml of each dilution was treated as sample and the colour formed was read on a spectrophotometer (Uvikon 810) at 750nm against 0.2N HCl.

2.7.3.1.4   Procedures

Two replicates of 200 µl of enzyme extract from the stomach were mixed in 15 ml test tubes with 1 ml of haemoglobin substrate. Tubes were incubated in a water bath at 30^0C for 15 minutes. At the end of the incubation time 1 ml of acetic acid was added to each tube to precipitate undigested haemoglobin. Samples were then centrifuged for 5 minutes at 13000 rpm. One ml of supernatant was added with mixing to 2 ml of 0.5N sodium hydroxide (Sigma 505-8) and 0.6 ml of phenol reagent. Samples were left for 10 minutes to form the colour and then the colour formed was read on spectrophotometer (Uvikon 810) at 750nm against 0.2N HCl. Pepsin activity was expressed as the amount of tyrosine liberated by 200 µl of enzyme solution at pH 2 per minute at 30^0C.

2.7.3.2   Trypsin (EC 3.4.21.4)

Trypsin activity was determined according to the method of Erlanger et al. (1961) using the synthetic substrate, N-benzoyl-DL-arginine-p-nitroaniline (BAPNA). The procedure depends upon the liberation of nitroaniline from the substrate by extracted enzyme solution under standard conditions. The following substrate and standards were prepared for trypsin determination.

2.7.3.2.1   BAPNA Substrate

Substrate was prepared by dissolving 0.044 gram of BAPNA (Sigma 4875) in 100 ml 0.05 M-Tris buffer (pH 8.2) containing 0.02 calcium chloride(BDH 262244W).

2.7.3.2.2   *p*-Nitroaniline Standard

p-Nitroaniline standard was prepared by dissolving 20 mg of *p*-nitroaniline (Sigma 2128) in 20 ml of distilled water. Several dilutions with distilled water were made to obtain a 02, 15, 10, 5, 4, 3 and 2 μg concentrations of *p*-nitroaniline in 1 ml to construct a standard curve. One ml of each dilution was read on spectrophotometer (Uvikon 810) at 410nm against distilled water.

2.7.3.2.3   Procedures

Two replicates of 0.5 ml of enzyme extract from the intestine were mixed in 15 ml test tubes with 2 ml of BAPNA substrate. Tubes were incubated in a water bath at 30^0 C for 15 minutes. At the end of the incubation time 1 ml of acetic acid was added to each tube to terminate the reaction. Samples then were centrifuged for 5 minutes at 13000 rpm and one ml of supernatant was read on spectrophotometer (Uvikon 810) at 410nm against distilled water. Trypsin activity was expressed as the amount of *p*-nitroaniline liberated by 0.5 ml of enzyme solution at pH 8.2 per minute at 30^0C.

2.7.3.3   Protease

General proteolytic activity was determined according to the method of Kuntiz (1947). The principle of this method is the measurement of the concentration of split products (peptides and amino acids) of casein solution after digestion by an extracted enzyme solution. The Lowry method (Sigma protein assay kit-

procedure No. p 5656) was adopted to calculate the concentration of split product. The following substrate was prepared for protease determination.

2.7.3.3.1   Casein Substrate

Substrate was prepared by dissolving 1 gram of casein (Sigma 8654) in 100 ml of 0.1M phosphate buffer (pH 7.6). The suspension was heated in a water bath at 60°C for a complete solution of casein.

2.7.3.3.2   Procedures

Two replicates of 0.5 ml of enzyme extract from the intestine were mixed in 15 ml test tubes with 2 ml of casein solution. Tubes were incubated in a water bath at 30°C for 15 minutes. At the end of the incubation time 1 ml of acetic acid was added to each tube to terminate the reaction. Samples were then centrifuged for 5 minutes at 13000 rpm and one ml of supernatant was pipetted to determine protein content based on Lowry method. Protease activity was expressed as the amount of protein digested by 0.5 ml of enzyme solution at pH 7.6 per minutes at 30^0C.

2.7.3.3.3   Lowry Method

One ml of supernatant of digested sample was mixed with 1 ml of Lowry reagent (Sigma 1013) in a 15 ml test tube. The sample was left to stand at room temperature for 20 minutes and then 0.5 ml of Folin & Ciocalteu's reagent (Sigma 9252) was added with rapid and immediate mixing. The sample was

left to stand another 30 minutes at room temperature to develop the colour. The colour formed was read on a spectrophotometer (Uvikon 810) at 500nm against blank. The blank was prepared in the same manner except 1 ml of water was used instead of 1 ml of supernatant.

2.7.3.3.4   Protein Standard

Protein standard was prepared by dissolving 2 mg of bovine serum albumin (Sigma 7656) in 5 ml of distilled water. Several dilutions with distilled water have been made to obtain a 50, 100, 200, 300 and 400 µg concentration of protein in 1 ml to construct standard curve. One ml of each dilution was then treated as sample and blank.

2.7.3.4   Lipase (EC 3.1.1.3)

Lipase activity was assayed with the aid of a Sigma diagnostic test-kit (Procedure No. 800). The procedure depends upon the hydrolysis of triglycerides in olive oil into fatty acids and diglycerides. The amount of fatty acids formed, under the specific conditions of the test, is a measure of lipase activity in the sample. The fatty acids formed are determined by titration with .05N sodium hydroxide.

2.7.3.4.1   Procedures

Two replicates of 1 ml of enzyme extract from the intestine were mixed in 15 ml test tubes with 3 ml of Sigma lipase substrate (Sigma 800-1) and 1 ml of Trizma

buffer (Sigma 800-2). Tubes were incubated in a water bath at 30^0C for 3 hours. At the end of incubation time 3 ml of 95% ethanol were added to each tube to terminate the reaction. Samples then were subjected to titration with 0.05N sodium hydroxide. Prior to titration 6 drops of thymolphthalein indicator solution (Sigma 800-3) were added to the sample. A blank sample was incubated alongside the tested sample and treated in the same manner except that 1 ml of enzyme extract solution was added at the end of incubation time. The difference in titration volume between sample and blank was to determine the quantity of fatty acids liberated during incubation time by 1 ml of extracted enzyme solution.

2.7.3.5   α-Amylase (EC 3.2.1.1)

α-Amylase activity was determined according to the method of Tietz (1970) using soluble starch as a substrate. The procedure depends upon the liberation of maltose from the substrate by extracted enzyme solution under standard conditions. The following substrate, reagent and standards were prepared for α-Amylase determination.

2.7.3.5.1   Starch Substrate

Substrate was prepared by dissolving 1 gram of starch (Sigma 9765) in 100 ml of 0.1M phosphate buffer (pH 7.0). Concentrated HCl and 0.5N NaOH were used to adjust the pH.

2.7.3.5.2   Dinitrosalicylic acid Reagent

The reagent was prepared by dissolving 5 grams of 3,5-dinitrosalicylic acid (Sigma 0550) and 150 grams of sodium potassium tartrate (Sigma 6170) in 150 ml of distilled water and 200 ml of 1N sodium hydroxide. The mixture was refluxed in a water bath at 60^0C until all components were totally dissolved and then made up to 500 ml with distilled water.

2.7.3.5.3   Maltose Standard

The maltose standard was prepared by dissolving 20 mg of maltose (Sigma 9171) in 20 ml of distilled water. Several dilutions with distilled water were made to obtain a 1000, 500, 400, 300, 200, 100 and 50 µg concentrations of maltose in 1 ml to construct a standard curve. One ml of each dilution was treated as samples and the colour formed was read on spectrophotometer (Uvikon 810) at 546nm against blank sample.

2.7.3.5.4   Procedures

Two replicates of 200 µl of enzyme extract from the intestine were mixed in 15 ml test tubes with 1 ml of starch substrate. Tubes were incubated in a water bath at 30^0C for 15 minutes. At the end of the incubation time 2 ml of dinitrosalicylic acid reagent were added to terminate the reaction. Samples were then incubated in a boiling water bath for 5 minutes. Samples were then left to cool at room temperature and the colour formed was read on spectrophotometer (Uvikon 810) at 546nm against a blank sample. α-Amylase

activity was expressed as the amount of maltose liberated by 200 µl of enzyme solution at pH 7.0 per minute at 30^0C. A blank sample was incubated alongside the tested sample and treated in the same manner except that 200 µl of distilled water was used instead of extracted enzyme solution.

2.8   Statistical Analysis

All data in this study were analysed by analysis of variance (ANOVA) using Mini Tab statistical software for Windows (release 11.12, 1996). Comparison among treatment means was carried out by analysis of variance followed by Tukey's test and a significance level of 0.05 was applied in all tests. Standard deviation ($\pm$ SD) was calculated to identify the range of means.

CHAPTER III

THE EFFECT OF DIETARY SAPONIN ON PERFORMANCE OF *OREOCHROMIS NILOTICUS* (L.) AND *CLARIAS GARIEPINUS* (B.) FINGERLINGS

3.1   Introduction

Saponins are glycosides; that is, they are composed of carbohydrate and non-carbohydrate, or aglycone, portions; the aglycones are often referred to as sapogenins (Farnsworth, 1966). They are distributed in plants of agricultural importance, in particular in forage, legume and cereal species (Table 1.3).

Saponins have been shown to have diverse biological properties including haemolytic, mycostatic, virostatic, bacteriostatic, insecticidal, molluscicidal, piscicidal and miscellaneous industrial and pharmacological activities such as foaming and flavouring agents in many products (Price et al., 1987).

The antinutritional effects of saponins in the diets of monogastric animals have been investigated in a wide range of species including pigs (Cheeke, 1976; Topping et al., 1980), poultry (Heywang and Bird, 1954; Anderson, 1957; Cheeke et al., 1981; Ueda, 1992; Jenkins and Atwal, 1994), rats (Cheeke et al., 1977; Malinow et al., 1981; Petit et al., 1995), mice (Reshef et al., 1976; Igile et al., 1995), rabbits (LeaMaster and Cheeke, 1979), voles (Kendall and Leath, 1976) and monkeys (Malinow et al., 1982).

The role of saponins as growth depressants has been widely reported in animals fed diets containing alfalfa meal *Medicago sativa*. Cheeke (1971 and 1976) reported that feeding 10% or more of alfalfa meal to poultry and pigs depressed growth performance, while levels in excess of 40% caused complete elimination of growth. He concluded that the presence of saponins in alfalfa

meal was largely responsible for the poor responses of the animals. Heywang and Bird (1954) noted that levels of 0.2% or more alfalfa saponin in chick diets reduced growth rate and feed efficiency. Similar results have been reported by Anderson (1957) in chick.

The mechanism by which saponins reduced growth rate is largely attributed to its bitterness and astringent taste. Several studies have indicated that in some monogastric animals, saponins are unpalatable and may adversely affect feed intake. Kendall and Leath (1976) reported reduction in feed intake when alfalfa saponin was added to meadow vole diets. Similar results have been reported by Jenkins and Atwal (1994) who showed that feeding *Gypsophila sp.* saponins at 0.3% of the diet reduced feed intake of chicks. Ueda (1992) stated that depressed feed intake seems to be one of the major factors of growth limitation in chicks since consumption of *Gypsophila* saponin was always accompanied by a corresponding change in feed intake.

In studies with geese, turkeys, quail, and chicks fed high saponin (1.7%) and low saponin (0.4%) alfalfa meal, at levels 1-20% of the diet Cheeke et al. (1981) found that the only discrimination between the two alfalfa types was with geese fed 20% alfalfa. At this level they preferred the low saponin type. Rabbits showed no discrimination between the two types of alfalfa meal at levels up to 30% of their diet. However, at higher levels they preferred the low saponin alfalfa (LeaMaster and Cheeke, 1979). Cheeke et al. (1977) found that

rats preferred diets with low saponin alfalfa at all levels of alfalfa tested (10, 15, 20, 25 and 30%).

Saponins are widely used as a toxicants or disinfectants in fish and shrimp ponds (pond cleaner) to kill predators and unwanted organisms (Terazaki et al., 1980; Homechaudhuri and Banerjee, 1991; Shyam et al., 1993; Chen, et al, 1996). Saponins also are highly toxic to mammals when given intravenously causing local inflammation at the site of injection and, in larger doses, death as a result of the massive release of erythrocyte debris and the reduced oxygen carrying capacity of the blood (Price et al., 1987). Gestetner et al. (1968) reported that the toxicity of saponins administered orally is very much lower; that is partly because of their complete failure to cross the gut and enter the bloodstream. However, Igile et al. (1995) fed mice saponin extracted from *Vernonia amygdalina* (a small tree between 1 and 3 m in height that grows throughout tropical Africa) at levels 0.33, 0.7 and 1.07% of the diet. They observed a high mortality rate and severe changes in growth performance and a significant body weight reduction in all animals compared to the control ones. They concluded that none of the concentrations could be recognised as safe.

In contradiction to the above, numerous workers have considered saponin as a positive, or at least neutral, dietary factor. Inclusion of purified alfalfa saponin in Bengalgram (*Cicer arietinum*) diet of rats showed no interference with food intake or food efficiency ratio during the 47 days of a feeding experiment (Venugopalan and Srivastava, 1996). Saponin extracted from fenugreek seed

(*Trigonella foenum*) significantly increased feed intake and the motivation to eat in rats (Petit et al., 1995). Rats fed alfalfa saponins at a level of 1% of the diet for up to 6 months showed no ill effects (Malinow et al., 1981). Saponin-containing extracts of *Yucca mohaensis* introduced to rats at dietary level of 0.5% for 12 weeks produced no significant effects in terms of growth or food utilisation (Oser, 1966). Macaque monkey *Macaca fascicularis* fed on an undefined mixture of alfalfa saponin for up to 78 weeks with no adverse effects (Malinow et al., 1982). Johnston et al. (1981) administered material containing saponins at a level of 63 ppm to growing broilers in a attempt to reduce ammonia production in henhouse manure. No such reduction occurred, but the saponin treated group were approximately 3% heavier after 28 days.

From the foregoing discussion it seems that the effect of saponins on the performance of terrestrial animals has been studied extensively. However, in view of increasing demand to replace fish meal with plant protein, a better understanding of the effect of saponin on the performance of fish is needed in order to optimise the utilisation of such proteins. Therefore, a feeding trial was conducted for eight weeks to investigate the effect of saponin on performance of Nile tilapia and African catfish after the conflicting studies concerning the ambivalence of saponins toward growth rate in terrestrial animals.

3.2   Materials and Methods

3.2.1   Experimental System

The experimental system described in section 2.1 was used to conduct two separate experiments to evaluate the effect of saponin in fish.

3.2.2   Experimental Fish

Oreochromis niloticus and *Clarias gariepinus* as described in section 2.2 were used as models in this study.

3.2.3   Experimental Diets

The composition of the experimental diets and their proximate analysis are shown in Tables 3.1 and 3.2. Diets formulation and preparation were as described in sections 2.3.1 and 2.3.2.

3.2.4   Experimental Procedure

Fish acclimation to the casein and gelatin based diet and periodical weighing were as described in section 2.4.1. Fish feeding and faeces collection were as described in sections 2.4.3 and 2.4.4.

3.2.5   Experimental Analyses

Proximate analysis (moisture, protein, lipid, fibre, ash, energy and chromic oxide) of fish, diets and faeces were as described in sections 2.5.1.1, 2.5.1.2, 2.5.1.3, 2.5.1.4, 2.5.1.5, 2.5.1.7 and 2.5.1.8. Saponin was determined according to

the method described in section 2.5.1.11. Growth and feed performance, protein digestibility and dry matter digestibility were calculated according to the methods described in sections 2.5.2.1, 2.5.2.2, 2.5.2.3, 2.5.2.4, 2.5.2.5 and 2.5.2.6.

3.2.6   Histological Analysis

Histological analyses of fish livers and intestines were performed as described in section 2.6.

3.2.7   Statistical Analysis

Statistical analyses were carried out as described in section 2.8.

3.3   Results

3.3.1   Growth and Feed Performance

No mortality nor external disease symptoms occurred in any treatment during the period of this study. Feed consumption was significantly (F= 193.85, d.f.= 11-3 and F= 318.85, d.f.= 11-3 for Nile tilapia and African catfish respectively, P < 0.05) affected by the addition of saponin and there was a decrease in feed intakes (Tables 3.3 and 3.4) with each increase of saponin. Fish fed diets I, control, and II were active and voracious and all offered feed was completely consumed. However, fish fed diets III and IV were less active and exhibited obvious signs of rejection of the unpalatable diets and the daily offered ration was never completely consumed. Moreover, fish fed diets III and IV exhibited lethargy during the last two weeks of the study.

Table 3.1 Composition of the experimental diets fed to *Oreochromis niloticus* (L.) (g kg^{-1}, on dry matter basis).

ingredients	diet I 0% Saponin	diet II 0.1% Saponin	diet III 0.5% Saponin	diet IV 1% Saponin
Casein (bovine milk)	260	260	260	260
Gelatin (porcine skin)	40	40	40	40
Dextrin (corn)	315	315	315	315
Fish oil (capelin and herring, BOCM Pauls)	110	110	110	110
Saponin (*Quillaja saponaria*, Sigma)	0	1	5	10
Cellulose	185	184	180	175
CMC[1]	20	20	20	20
Cr$_2$O$_3$	10	10	10	10
Mineral mix[2]	40	40	40	40
Vitamin mix[3]	20	20	20	20
Proximate composition				
Moisture	35.8	31.2	32.3	38.4
Crude protein	275.9	275.9	277.3	287.3
Crude lipid	102.0	103.5	100.7	102.1
Ash	49.8	49.8	50.6	52.5
Crude fibre	141.8	136.2	134.7	132.9
Saponin	0.2	0.8	4.2	8.7
Cr$_2$O$_3$	10.4	11.2	10.8	10.8
N-free extract[4]	384.1	391.4	389.4	367.3
Gross energy (kJ g^{-1})	20.23	20.05	20.35	19.79

1- Carboxymethylcellulose-sodium salt, high viscosity.
2- As listed in Table 2.2.
3- As listed in Table 2.3
4- Obtained by subtraction.

Table 3.2 Composition of the experimental diets fed to *Clarias gariepinus* (B.) (g kg^{-1}, on dry matter basis).

Ingredients	diet I 0% Saponin	diet II 0.1% Saponin	diet III 0.5% Saponin	diet IV 1% Saponin
Casein (bovine milk)	280	280	280	280
Gelatin (porcine skin)	60	60	60	60
Dextrin (corn)	315	315	315	315
Fish oil (capelin and herring, BOCM Pauls)	110	110	110	110
Saponin (*Quillaja saponaria*, Sigma)	0	1	5	10
Cellulose	145	144	140	135
CMC[1]	20	20	20	20
Cr_2O_3	10	10	10	10
Mineral mix[2]	40	40	40	40
Vitamin mix[3]	20	20	20	20
Proximate composition				
Moisture	63.6	50.1	57.4	50.5
Crude protein	315.8	321.4	330.4	324.2
Crude lipid	102.6	101.7	102.5	103.9
Ash	43.3	42.2	40.2	44.5
Crude fibre	122.7	120.9	118.2	115.5
Saponin	0.1	0.9	4.7	9.1
Cr_2O_3	10.8	9.6	9.2	10.2
N-free extract[4]	341.1	353.2	337.4	342.1
Gross energy (kJ g^{-1})	20.31	20.52	20.30	20.43

1- Carboxymethylcellulose-sodium salt, high viscosity.
2- As listed in Table 2.2.
3- As listed in Table 2.3.
4- Obtained by subtraction.

Growth responses of fish fed the experimental diets are shown in Figures 3.1, 3.2, 3.3 and 3.4 and Tables 3.3 and 3.4. There was a trend of reduced growth performance with each increase in the level of saponin on the basis of final mean weight, percentage weight gain and SGR.

FCR (Tables 3.3 and 3.4) ranged from 1.37 to 9.51 and from 0.91 to 4.75 for Nile tilapia and African catfish respectively being highest for diet IV and lowest for diet I. No significant difference (F= 14.67, d.f.= 11-3 and F= 19.04, d.f.= 11-3, P< 0.05 for Nile tilapia and African catfish respectively) was observed in FCR except for fish fed diet IV which produced a higher value.

PER (Tables 3.3 and 3.4) ranged from 0.39 to 2.64 and from 0.69 to 3.48 for Nile tilapia and African catfish respectively being highest for diet I and lowest for diet IV. PER values significantly (F= 507.82, d.f.= 11-3, and F= 263.90, d.f.= 11-3, P< 0.05 for Nile tilapia and African catfish respectively) decreased with each increase of dietary saponin.

PPV (Tables 3.3 and 3.4) ranged from 4.37 to 43.49 and from -0.35 to 53.73 for Nile tilapia and African catfish respectively being highest for diet I and lowest for diet IV. PPV values significantly (F= 73.74, d.f.= 11-3, and F= 210.54, d.f.= 11-3, P< 0.05 for Nile tilapia and African catfish respectively) decreased with each increase of dietary saponin.

3.3.2   Protein and Dry matter Digestibility

Protein digestibility data are summarised in Tables 3.3 and 3.4.   Protein

digestibility values ranged from 84.04 to 93.25% and from 80.63 to 95.23% for

Nile tilapia and African catfish respectively being highest for diet I and lowest

for diet IV. Protein digestibility values tended to decrease (F= 78.60, d.f.= 11-3,

and F= 234.88, d.f.= 11-3, P< 0.05 for Nile tilapia and African catfish

respectively) as the level of dietary saponin increased.

Dry matter digestibility data are summarised in Tables 3.3 and 3.4. Dry matter

digestibility for all diets was similar and there were no significant differences

(P< 0.05) between diets except for diet III in Nile tilapia which was higher than

the rest.

3.3.3   Body Composition

Body composition data are summarised in Tables 3.5 and 3.6. Body moisture

percentages were highest in fish fed diets III and IV and lowest in fish fed diets

I and II. Body protein content declined with increasing saponin content but

there were no significant differences (F= 2.63, d.f.= 11-3, P< 0.05) in Nile tilapia;

however, body protein content of African catfish fed diet IV was significantly

lower (F= 3.21, d.f.= 11-3, P< 0.05) than that of fish fed diet I.   Body lipid

content was highest in fish fed diet I and lowest in fish fed diet IV. Moreover,

there was an overall trend of decreasing carcass lipid content with increasing

inclusion levels of dietary saponin. There was an inverse relationship between

moisture and lipid content. Body ash content of Nile tilapia was lower in fish
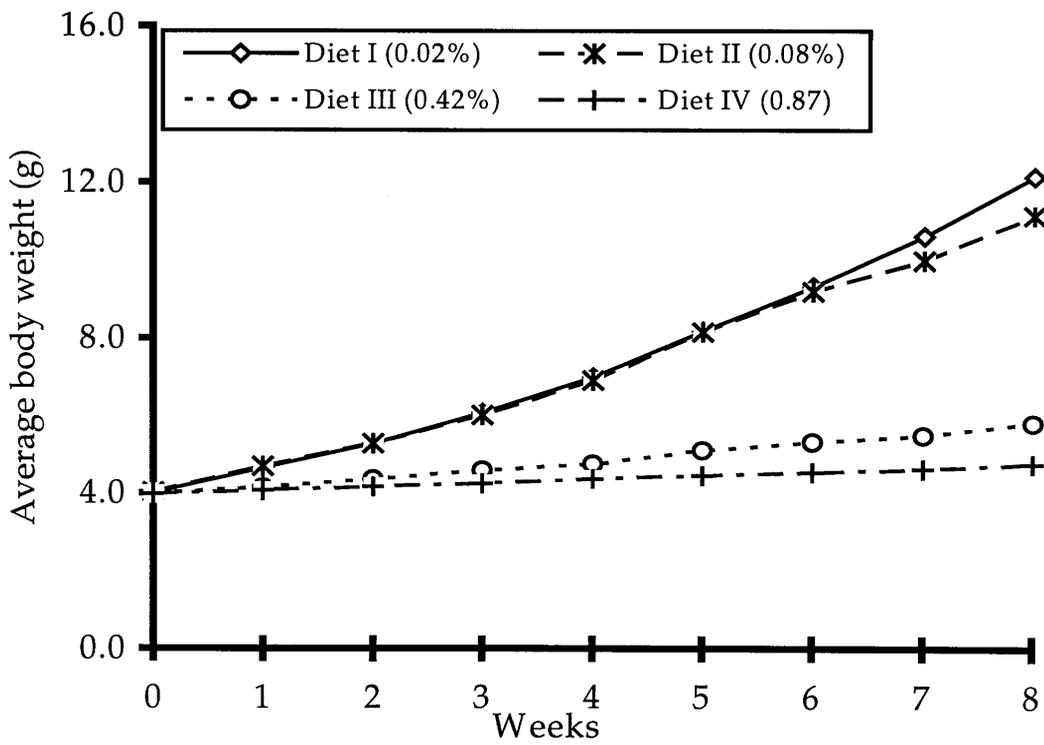
Fig. 3.1 Growth of Nile tilapia fed diets containing graded levels of
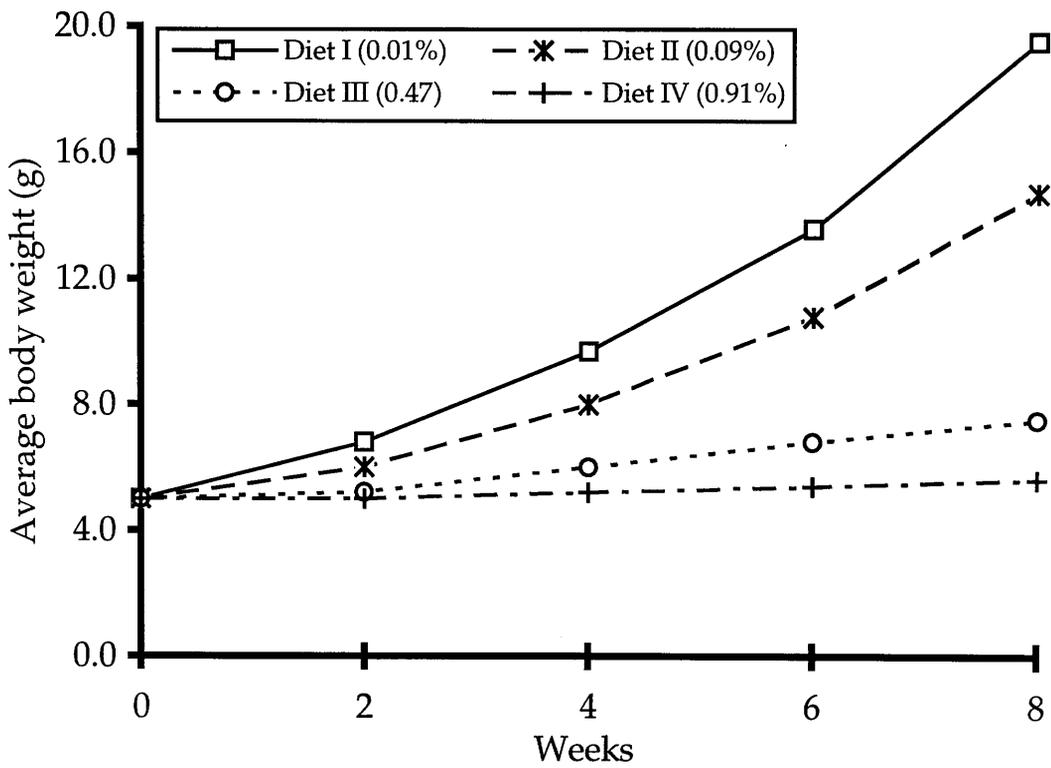
dietary saponin.

Fig. 3.2 Growth of African catfish fed diets containing graded levels

of dietary saponin.

Table 3.3 Growth and feed performance of *Oreochromis niloticus* (L.) fed graded levels of dietary saponin.

Parameters	diet I (0.02%)	diet II (0.08%)	diet III (0.42%)	diet IV (0.87%)
Initial mean wt (g)	4.0^a ± 0.00	4.0^a ± 0.06	4.0^a ± 0.06	4.0^a ± 0.00
Final mean wt (g)	12.1^a ± 0.25	11.1^b ± 0.15	5.8^c ± 0.20	4.8^d ± 0.23
Feed intake (g/fish/experiment)	11.2^a ± 0.27	10.9^a ± 0.31	7.4^b ± 0.40	6.8^b ± 0.06
Weight gain (%)	203.33^a ± 6.29	176.07^b ± 5.58	44.57^c ± 8.31	19.17^d ± 5.77
SGR (%/d)	1.98^a ± 0.04	1.81^b ± 0.03	0.66^c ± 0.10	0.31^d ± 0.09
FCR	1.37^a ± 0.03	1.53^a ± 0.04	4.06^a ± 0.29	9.51^b ± 3.37
PER	2.64^a ± 0.05	2.37^b ± 0.06	0.89^c ± 0.07	0.39^d ± 0.12
PPV	43.49^a ± 5.27	39.14^a ± 3.83	11.84^b ± 1.14	4.37^b ± 4.26
Protein digestibility (%)	93.25^a ± 1.15	93.02^a ± 0.23	88.70^b ± 0.96	84.04^c ± 0.76
Dry matter digestibility (%)	53.43^a ± 3.13	54.35^a ± 0.47	59.02^b ± 0.99	55.42^a ± 1.01

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Fig. 3.3 Growth and feed performance of *Oreochromis niloticus* (L.) fed graded levels of dietary saponin.
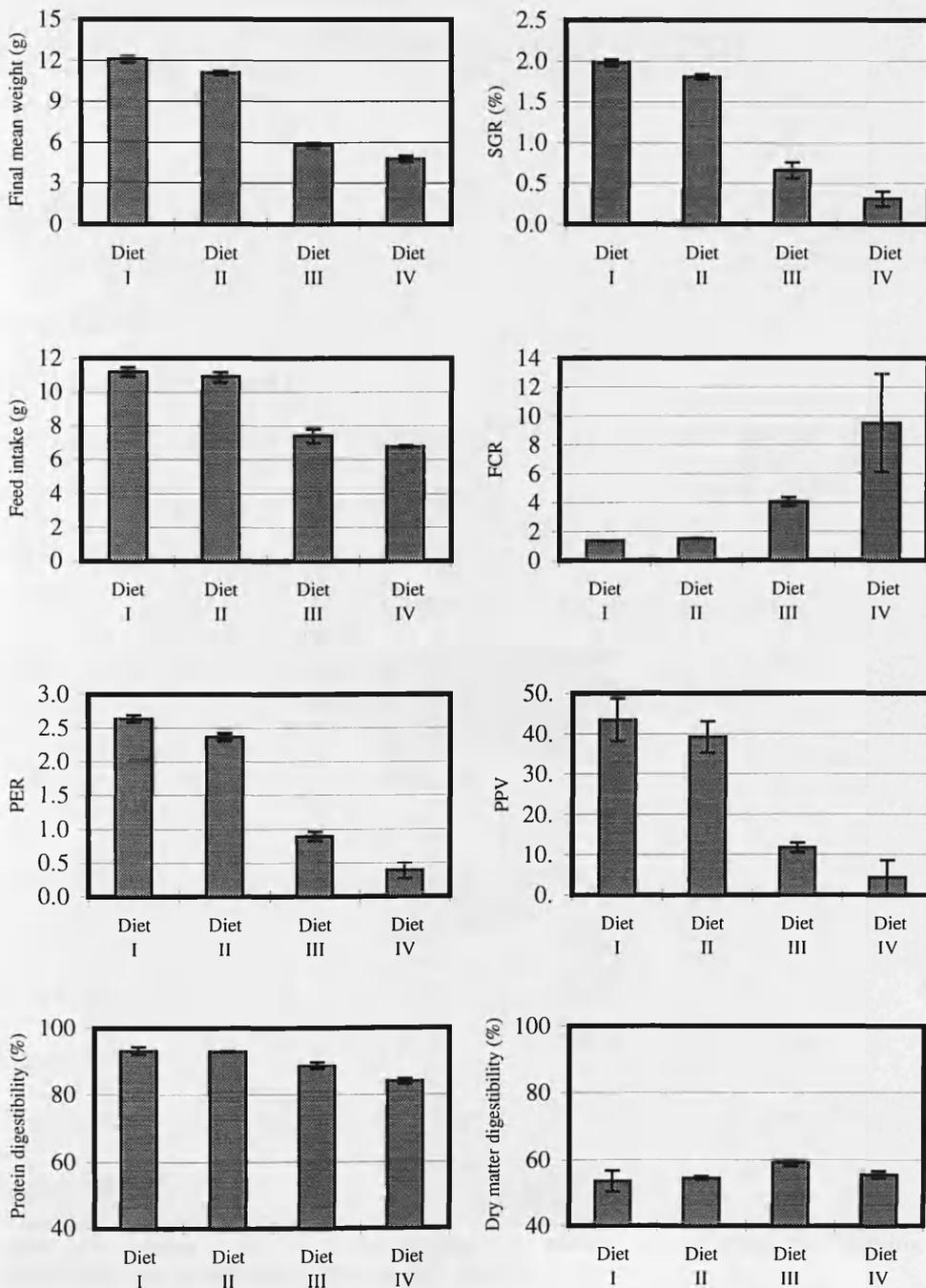
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.02, 0.08, 0.42 and 0.87% dietary saponin respectively.

Table 3.4 Growth and feed performance of *Clarias gariepinus* (B.) fed graded levels of dietary saponin.

Parameters	diet I (0.01%)	diet II (0.09%)	diet III (0.47%)	diet IV (0.91%)
Initial mean wt (g)	5.0[a] ± 0.00	5.0[a] ± 0.00	5.0[a] ± 0.00	5.0[a] ± 0.00
Final mean wt (g)	19.5[a] ± 0.60	14.7[b] ± 0.48	7.5[c] ± 0.07	5.6[d] ± 0.09
Feed intake (g/fish/experiment)	13.2[a] ± 0.52	9.8[b] ± 0.62	4.8[c] ± 0.36	2.9[d] ± 0.21
Weight gain (%)	290.00[a] ±12.00	193.33[b] ±9.61	49.33[c] ± 1.33	12.67[d] ± 1.76
SGR (%/d)	2.43[a] ± 0.05	1.92[b] ± 0.06	0.72[c] ± 0.02	0.21[d] ± 0.03
FCR	0.91[a] ± 0.03	1.02[a] ± 0.05	1.95[a] ± 0.16	4.75[b] ± 1.41
PER	3.48[a] ± 0.11	3.06[b] ± 0.14	1.59[c] ± 0.05	0.69[d] ± 0.21
PPV	53.73[a] ± 0.59	45.80[b] ± 1.52	18.05[c] ± 3.87	-0.35[d] ± 4.24
Protein digestibility (%)	95.23[a] ± 0.25	93.87[a] ± 0.56	86.27[b] ± 0.08	80.63[c] ± 0.64
Dry matter digestibility (%)	63.35[a] ± 0.28	64.50[a] ± 1.43	63.66[a] ± 1.49	62.81[a] ±1.68

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Fig. 3.4 Growth and feed performance of *Clarias gariepinus* (B.) fed graded levels of dietary saponin.
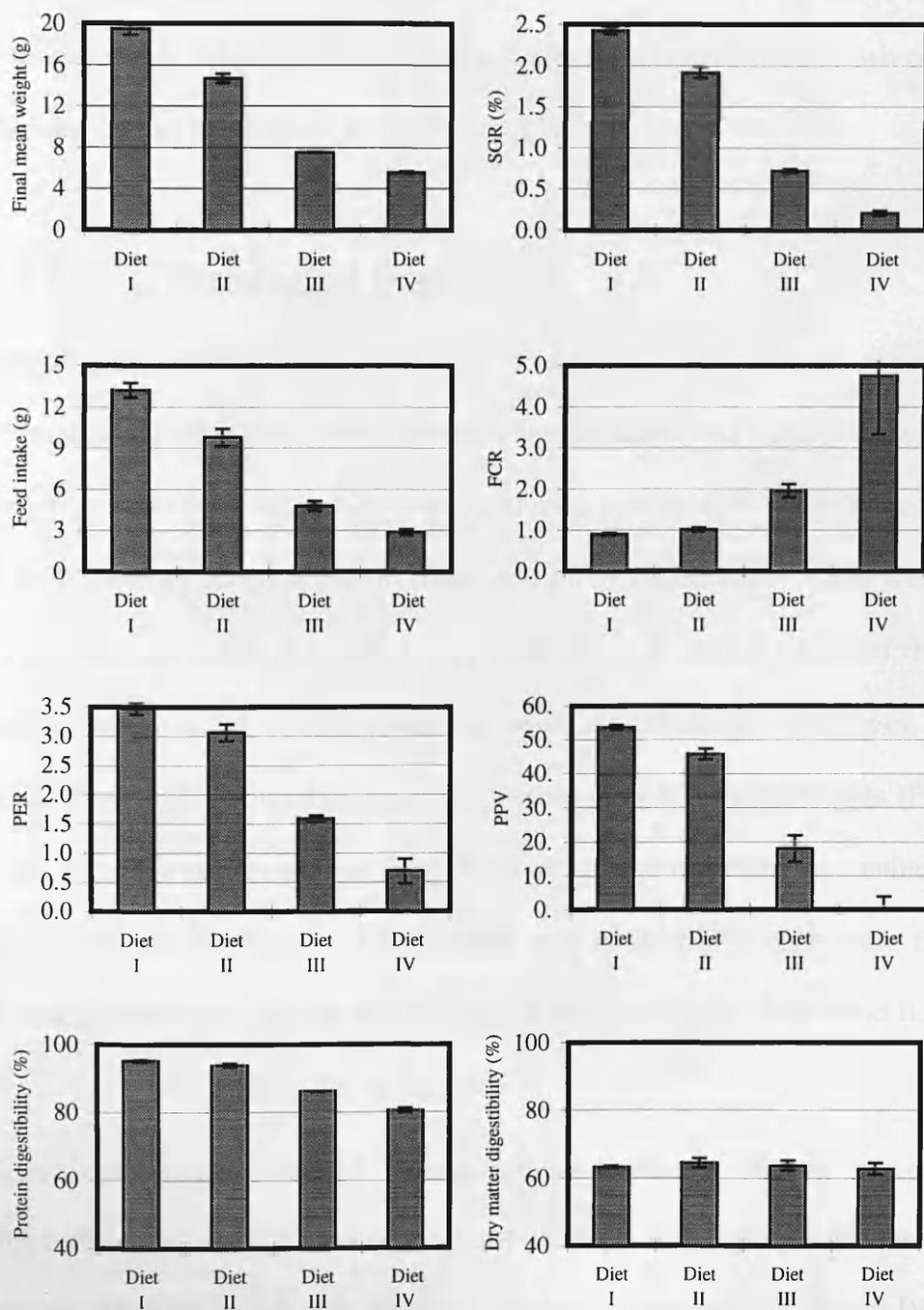
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.01,0.09,0.47 and 0.91% dietary saponin respectively.

fed diets I and II and higher in fish fed diets III and IV; however, fish fed diet IV had the highest (F= 9.96, d.f.= 11-3, and F= 6.70, d.f.= 11-3, P< 0.05 for Nile tilapia and African catfish respectively) body ash content among the rest. There was an inverse relationship between ash and lipid content. Body ash content of African catfish was higher in fish fed diet IV.

3.3.4   Histological Examination

Histological examination of intestines and livers of fish fed the experimental diets showed clear differences between fish fed diet I and those fed diets III and IV. Fish fed diet I exhibited well developed epithelial folding (Plates 3.1 and 3.3) and there was no apparent degeneration or vacuolation. Cells were full of cytoplasm and striated borders were well defined. The structure of the *lamina propria* was normal and there was no sign of infiltration. However, fish fed diets III and IV showed cytoplasmic vacoulation of intestinal cells (Plates 3.2 and 3.4), disruption of straited borders and infiltration of *lamina propria* especially in Nile tilapia. Goblet cells and neutrophilic cells were found in marked number in fish fed diets III and IV than in fish fed diets I and II.

Sections of liver also showed differences between treatments. Hepatic cells of fish fed diet I (Plates 3.5 and 3.7) were well defined in shape, well organised and there was no sign of shrinkage or cell wall breakage. However, fish fed diets III and IV showed alterations (Plates 3.6 and 3.8) from the normal histology which include shrinkage and breaking down of hepatic cells and disorganisation and crowding of hepatic cell nuclei.

Table 3.5 Body composition of *Oreochromis niloticus* (L.) fed graded levels of saponin.

%	Initial fish	Diet I (0.02%)	Diet II (0.08%)	Diet III (0.42%)	Diets IV (0.87%)
Moisture	75.23	73.07[a]	72.67[a]	77.00[b]	78.60[b]
		± 2.21	± 1.08	± 1.06	± 1.67
Crude protein	14.41	15.80[a]	15.75[a]	14.24[a]	13.93[a]
		± 1.35	± 0.86	± 0.77	± 1.14
Crude lipid	7.38	6.94[a]	7.26[a]	3.75[b]	1.94[c]
		± 0.35	± 0.28	± 0.12	± 0.36
Ash	2.98	4.18[a]	4.31[a]	5.01[b]	5.52[b]
		± 0.58	± 0.14	± 0.19	± 0.29

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Table 3.6 Body composition of *Clarias gariepinus* (B.) fed graded levels of saponin.

%	Initial fish	Diet I (0.01%)	Diet II (0.09%)	Diet III (0.47%)	Diets IV (0.91%)
Moisture	74.36	73.17^a ± 0.08	74.41^a ± 0.57	77.31^b ± 0.72	78.69^b ± 0.16
Crude protein	16.43	15.69^a ± 0.15	15.47^{ab} ± 0.43	14.71^{ab} ± 0.41	14.46^b ± 0.24
Crude lipid	5.38	7.28^a ± 0.19	6.48^b ± 0.22	4.33^c ± 0.25	2.54^d ± 0.14
Ash	3.83	3.86^a ± 0.12	3.64^a ± 0.08	3.65^a ± 0.19	4.31^b ± 0.05

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Plate 3.1 Section of intestine of Nile tilapia fed control diet showing normal appearance of epithelial folding and well defined of striated borders (H&E 260×).
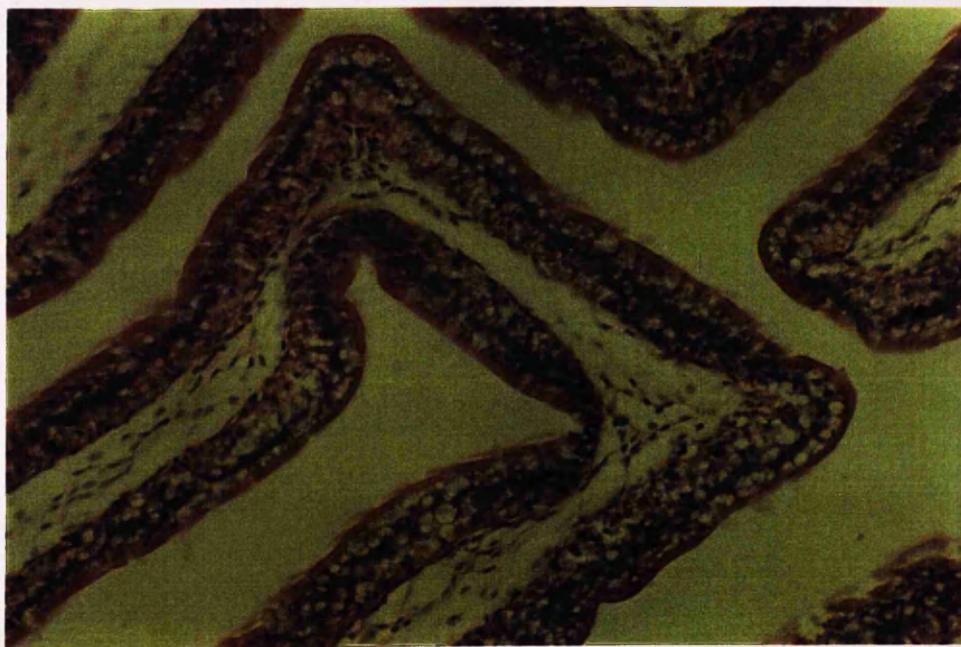
Plate 3.2 Section of intestine of Nile tilapia fed saponin containing diet showing cytoplasmic vacoulation of epithelial folding and disruption of straited borders (H&E 260×).
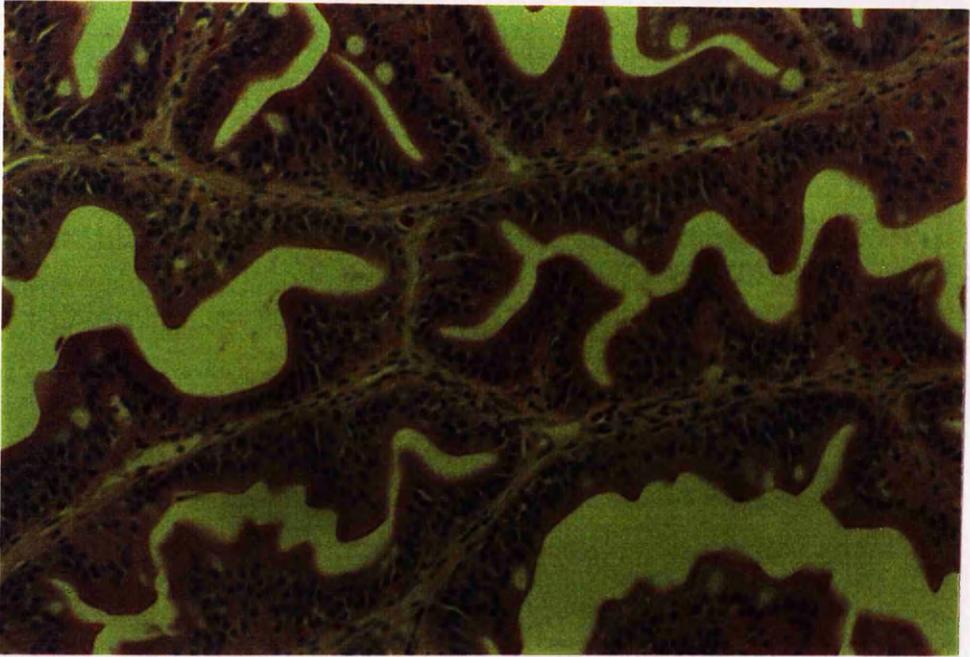
Plate 3.3   Section of intestine of African catfish fed control diet showing normal appearance of epithelial folding (H&E 260×).
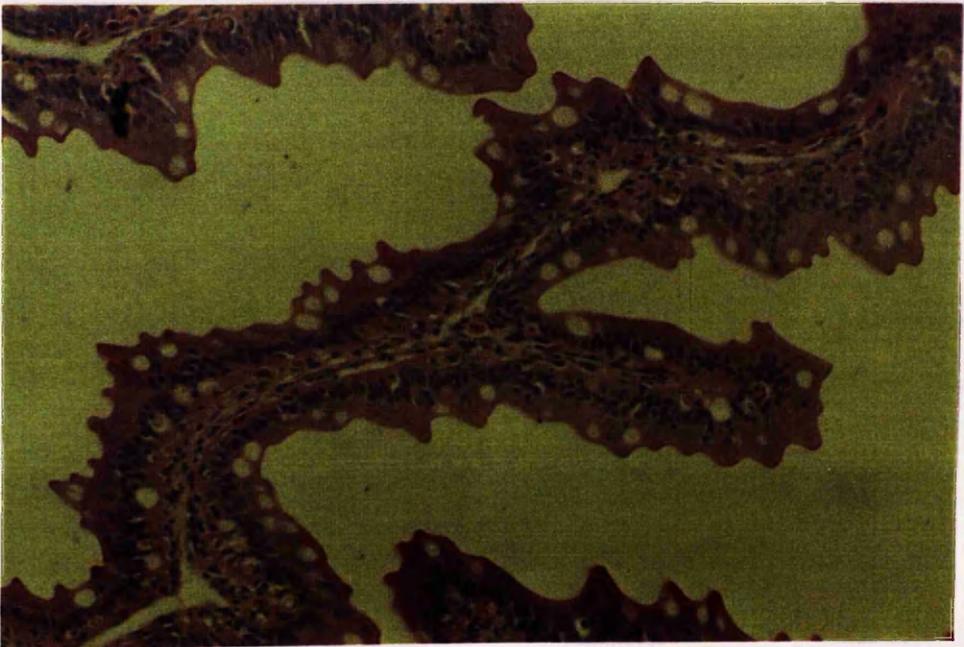
Plate 3.4   Section of intestine of African catfish fed saponin containing diet showing increasing number of goblet cells (H&E 260×).
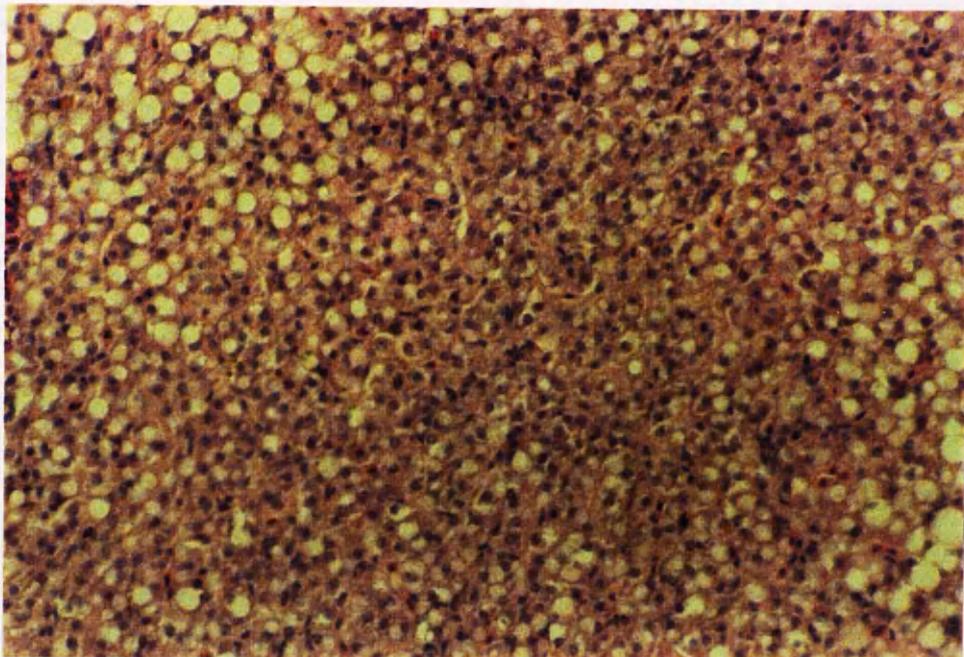
Plate 3.5 Section of liver of Nile tilapia fed control diet showing well defined in shape and well organised hepatic cells (H&E 260×).
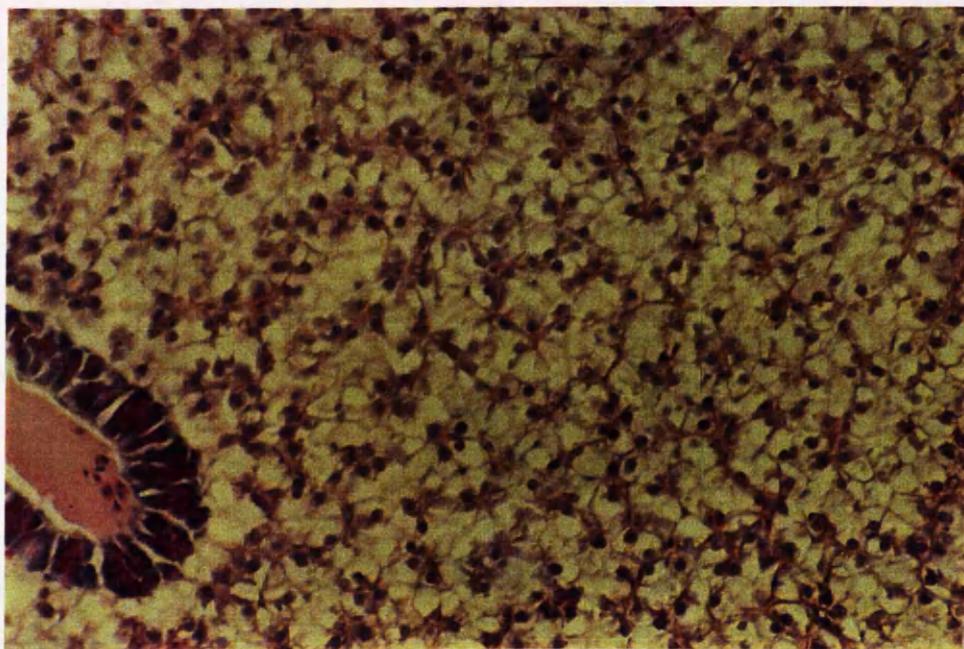
Plate 3.6 Section of liver of Nile tilapia fed saponin containing diet showing shrinkage and breaking down of hepatic cells (H&E 260×).
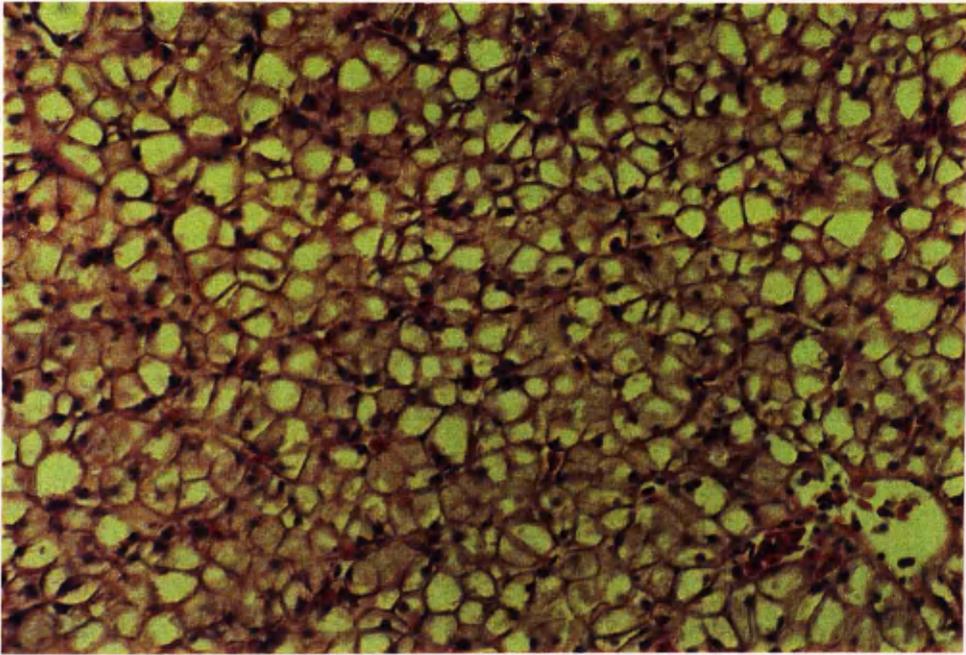
Plate 3.7 Section of liver of African catfish fed control diet showing well defined in shape and well organised hepatic cells (H&E 260×).
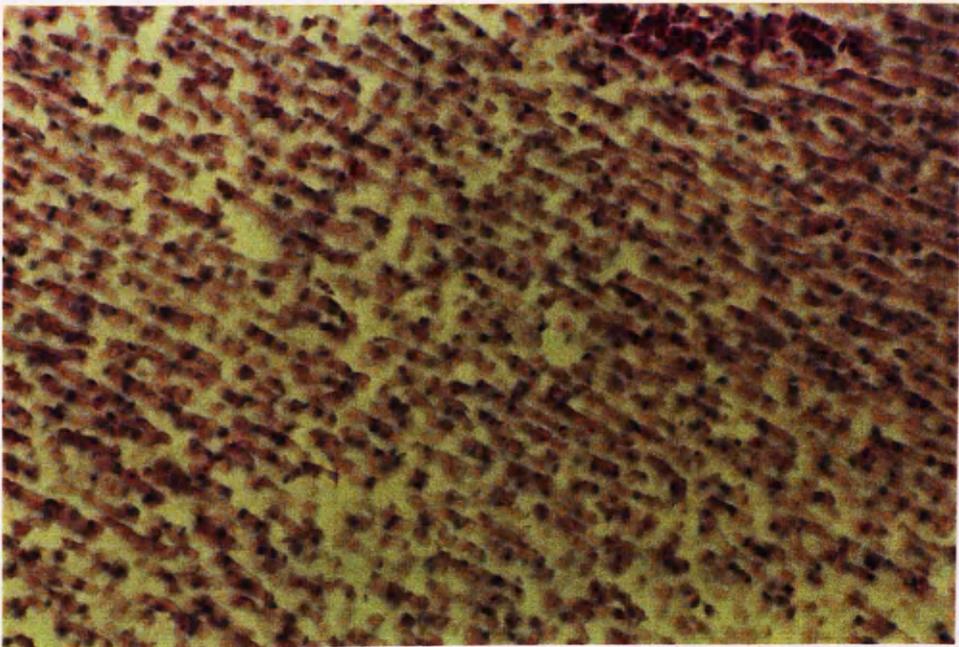
Plate 3.8 Section of liver of African catfish fed saponin containing diet showing breaking down of hepatic cells and disorganisation and crowding of hepatic cell nuclei (H&E 260×).

3.4   Discussion

3.4.1   Growth and Feed Performance

It is apparent from the results of this study that adding dietary saponin to the experimental diets considerably decreased feed palatability especially at inclusion levels of 0.42% and higher. Fish fed these diets exhibited inferior feeding activity to those fed a control diet. It is strongly believed that feeding unpalatable diets in this study was the major reason for growth depression due to restriction of feed intakes. The results of the present study are supported by Cheeke (1976) who suggested that the main factor limiting the direct use of alfalfa by monogastric animals is the fact that they reject the feed because of the bitter taste of the saponin. Pedersen et al. (1972) reported that the better growth response of chicks and rats to low saponin alfalfa (0.4%) than to high saponin (1.7%) may be due simply to greater palatability of the low saponin meal. A similar opinion was expressed by Kendall and Leath (1976) who used meadow voles *Microtus pennsylvanicus* and showed that meals of low saponin lines of alfalfa were generally more palatable than their corresponding high saponin lines.

Palatability is an extremely important consideration when formulating diets for aquaculture species. Many studies have reported that diets with high levels of plant protein inclusion are less readily accepted by fish and are accompanied by poor growth. For example red drum, *Sciaenops ocellatus,* have been reported to find soybean meal unpalatable and are not willing to consume diets without the inclusion of some fish meal (Reigh and Ellis, 1992). Mohsen and Lovell (1990) reported that the addition of animal byproducts to the plant protein

based diet, soybean, improved palatability in channel catfish *Ictalurus punctatus*. Similar results have been reported for Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* when fed a plant protein oat based diet (Arnesen et al., 1995; Arnesen and Krogdahl, 1995 respectively). It is possible that such findings may, in part at least, be a result of saponin presence in plant proteins (Table 1.3) used in those experiments.

It is well established that saponin is growth inhibitory to poultry and is largely responsible for the poor responses of birds fed levels of alfalfa meal exceeding 10% of the diet (Birk and Peri, 1980). Heywang and Bird (1954) found that alfalfa saponin at levels of 0.2% or more in the diet of chicks retarded growth and restricted feed intake and feed efficiency. Similarly, Anderson (1957) noted that levels of 0.1% or more alfalfa saponin in chick diets reduced growth and feed efficiency. The results of the present study confirm these findings as indicated by low growth performance (Tables 3.3 and 3.4) of Nile tilapia and African catfish. Based on the results presented in Figures 3.1 and 3.2, the negative effect of saponin, even at the lowest concentration (0.09%), on the growth of African catfish was clear from the first week. However, Nile tilapia took six weeks to exhibit a similar response to African catfish which may indicate that Nile tilapia is more tolerant to dietary saponin than African catfish. At higher concentrations all measured parameters (Tables 3.3 and 3.4) were influenced in both species similarly.

However, no growth inhibition was observed in young guinea pigs and rabbits receiving a dietary level of 2% alfalfa saponin (Lindahl et al., 1957). Moreover, Harris et al. (1981) have shown that rabbits are tolerant to bitterness and readily feed on high levels of alfalfa. Similarly, Binns and Pederson (1964) have reported that alfalfa saponin has no adverse influence on growth in cattle, and there is some evidence that saponin supplements in feeds may be beneficial to fattening lambs (Hale et al., 1961) and steers (Goodall et al., 1982). From these reports it is evident that there are species differences in response to dietary saponin, poultry and fish apparently being much more sensitive than other animals.

Olvera et al. (1990) evaluated alfalfa leaf protein as a fish meal replacer in diets for Mossambica tilapia *Oreochromis mossambicus* at inclusion rates of 33.8 and 41.3%. They observed low growth rates and poorer feed utilisation in fish fed these diets compared to those of fish fed a control diet. They concluded that the depression in growth was due to EAA deficiencies. Kodras et al. (1951) demonstrated that dehydrated alfalfa meal showed a typical growth depression in chicks when the alfalfa meal was incorporated in their diet at a 20% level. Cowlishaw et al. (1954) also demonstrated that alfalfa LPC was inferior to fish meal when added as the sole protein source to diets. However, these authors suggested that the deteriorative effect of alfalfa LPC was possibly due to the saponin contained in alfalfa. According to Ueda et al. (1996) a level of 33.8 and 41.3% alfalfa leaf protein, as used by Olvera et al. (1990), with a saponin content

of 0.95% diets would contain 3.21 and 3.92 g/kg saponin which is comparable to the saponin level of diet III in the present study.

Belmar and Morris (1994) have observed similar results to those reported in the present study in terms of low growth performance and poor feed utilisation when they incorporated jack bean in the diet of chicks. They concluded that the presence of saponin (present in a large amounts in this plant) is the reason for the negative effect of jack bean.

Despite its bitterness and its negative influence on growth and histology of intestines and livers, saponin did not cause any mortality during the period of the present study. This result is in disagreement with Gorski et al. (1984) and Ueda (1992) who observed mortality in mice and rats as result of feeding diets contained 0.54% *Midicago lupulina* saponin and 0.5% added *Gypsophila* saponin respectively. Two reasons might be suggested to explain this discrepancy. Firstly, it is believed that the toxic effect of saponin in the present study was reduced as a result of the leaching process when the diets were offered to the fish. Secondly, the types of saponins which were used in the studies of Gorski et al. (1984) and Ueda (1992) are different from that used in this study. The toxicity of saponins may depend mainly on the chemical structure of their aglycones (Price et al., 1987). However, histological examination in the present study showed a degree of a toxicity in intestines and livers of fish ingesting saponin at level 0.42% and higher.

3.4.2   Protein and Dry matter Digestibility

Protein digestibility was found to be dependent on the level of saponin. A comparison of the digestibility of diets showed that the saponin does have a negative effect (Tables 3.3 and 3.4) on protein digestibility at the level of 0.42% and higher. So, it is possible that the differences in growth performance of fish were, in part, a result of differences in protein digestibility.

Two assumptions might be suggested to explain the low protein digestibility in fish fed diets III and IV. Firstly, saponin may react with protein (substrate or enzymes) in the digestive system and form complexes of high molecular weight which are unlikely to be absorbed from the intestine. Secondly, saponin may cause a high loss of endogenous protein as a result of membranolysis or irritation. In fact, both assumptions have been supported. Potter et al. (1993) and Ikedo et al. (1996) showed that saponins (*Quillaja saponaria* and soyasaponin respectively) form complexes of high molecular weight when added to proteins in *in vitro* studies. Such complexes, *in vivo*, will be very large and , in turn, unlikely to be absorbed through the intestinal wall. Additionally, and based on histological results from the present study, saponin caused severe irritation and major structural lesions in the epithelial foldings and such lesions will increase the turnover rate of intestinal cells, thus increasing endogenous protein loss.

Protein digestibility in African catfish was more affected by inclusion of dietary saponin than in Nile tilapia. According to the results (Tables 3.3 and 3.4) of the

present study, levels of 0.42 and 0.87% saponin were responsible for a 4.88 and

9.88% reduction in protein digestibility in Nile tilapia respectively; whereas

levels of 0.47 and 0.91% saponin were responsible of 9.41 and 15.33% reduction

in protein digestibility in African catfish. This finding gives an indication that

Nile tilapia is more adapted to consume and digest saponin-containing diets

than African catfish. Naturally, Nile tilapia depends mainly on diets of plant

origin (Jauncey and Ross, 1982) of which saponin may constitute a part.

However, African catfish is a freshwater species that depends on diets largely

of animal origin (Weerd, 1995) which are known to be effectively saponin-free

(Section 1.6.2).

Many studies have reported that inclusion of alfalfa meal in animal diets results

in reduced protein digestibility. For example, Olvera et al. (1990) found a

significant reduction in protein digestibility when they incorporated various

levels of alfalfa leaf protein concentrates in the diet of Mossambica tilapia

Oreochromis mossambicus. Similar results have been reported with chicks

(Muztar and Slinger, 1980) and with rats (Hegsted and Linkswiler, 1980). It is

well documented (Table 1.3) that the presence of saponin within the alfalfa

meal is the reason for the low protein quality of such meal. Cheeke (1976)

reported that saponin could impair protein digestibility and could contribute to

the low digestibility of alfalfa protein in rats and pigs.

Dry matter digestibility (Tables 3.3 and 3.4) in both species was not affected by

the addition of saponin; although dry matter digestibility in African catfish was

higher than in Nile tilapia. Results reported in the present study were in agreement with those reported by Olvera et al. (1990) in Mossambica tilapia *Oreochromis mossambicus* fed various levels of alfalfa meal. Similarly, Martinez et al. (1988) found no significant differences in dry matter digestibility, although they were higher than those reported in the present study, in Mossambica tilapia *Oreochromis mossambicus* fed various levels of jack bean, which is known to contain saponin (Table 1.3). However, Jenkins and Atwal (1994) showed a reduction in chick dry matter digestibility from 90 to 81% when they fed saponin containing diets at levels comparable to those reported in this study.

Apparently, it is difficult to directly compare values obtained in different experiments because the degree of digestion of a specific diet may vary with dietary ingredients, their chemical composition and inclusion level (Jobling, 1994; De Silva and Anderson, 1995). Further, factors such as water temperature, feeding rate and animal model may also affect feed digestibility (Jobling, 1994; De Silva and Anderson, 1995). Several of these factors, alone or in combination, may have contributed to the relatively low dry matter digestibility reported in this study.

3.4.3   Body Composition and Histological Examination

The results of the present study indicated significant differences between dietary treatments. These differences in body composition were attributed to differences in feed intake as a result of feeding unpalatable diets. All body

composition parameters showed symptomatic starvation conditions in fish fed diets III and IV. Moisture content in fish carcass fed diets III and IV increased at the end of experiment. Increases in moisture levels in fish carcass fed diets III and IV was accompanied by concomitant decreases in lipid levels.

Results obtained in this study are similar to those reported from previous experiments in which saponin-containing plants were substituted for fishmeal in diets for fish. Attempts by Reigh and Ellis (1992) to incorporate 75% of the dietary protein as soybean meal for red drum *Sciaenops ocellatus* led to similar carcass composition changes. Olvera et al. (1990) reported that Mossambica tilapia *Oreochromis mossambicus* fed purified alfalfa leaf protein at rate of 55% of total protein showed high content of moisture accompanied with low content of lipid in fish carcass. Love (1980) reported that depletion of body lipid results in an increase in the water content of the muscle and Sargent (1976) explained this inverse relationship as a result of lipid mobilisation during fasting in fish.

During fasting or deprivation, fish must utilise stored energy supplies for metabolic processes. So, according to this hypothesis, it is clear that depletion of carcass fat in this study is a result of using such fat for biological functions in Nile tilapia and African catfish. In this study, fish fed diet IV showed the highest reduction in fat content followed by fish fed diet III and diet II respectively. This result probably reflects the degree of saponin bitterness in the diets and gives an explanation of why fish fed diet IV had the lowest level of feed intake.

In contrast to fat levels, carcass protein did not differ with increasing dietary saponin content in Nile tilapia; however, carcass protein of African catfish did differ slightly but significantly (P< 0.05) between diet I and diets III and IV. It appears that protein is spared during periods of fasting. Phillips et al. (1960) reported that whole body protein levels in brook trout *Salvelinis fontinalis* tended to increase or remain the same after 12 weeks of fasting. Satoh et al. (1984) also stated that whole body protein levels of fasted Nile tilapia *Oreochromis niloticus* were similar after 60 days fasting compared to fish before fasting. Similar results were reported for white sturgeon *Acipenser transmontanus* (Hung et al., 1997).

Ash contents of both Nile tilapia fed diets III and IV and African catfish fed diet IV was affected in the present study as a result of feeding saponin-containing diets. Similar results have been reported by Reigh and Ellis (1992) and Olvera et al. (1990) when they incorporated plant protein sources, known to contain saponin, in the diets of red drum *Sciaenops ocellatus* and Mossambica tilapia *Oreochromis mossambicus* respectively. However, results reported in this study were also comparable to those reported with starved fish. Pandian and Raghuraman (1972) reported that Mossambica tilapia *Oreochromis mossambicus* starved for 60 days exhibited a considerable increase in the ash content. Niimi (1972) also observed an increase in the ash content of starved largemouth bass *Micropterus salmoides*.

The histological examination at the end of the experiment showed clear changes in the intestine of fish fed diets III and IV compared to the intestine of fish fed diet I. These changes might be due to the presence of saponin in the diets. Cellular infiltration of the *lamina propria* was clear in fish fed diets III and IV and is a symptom of feeding irritant substances to animals (Roberts and Bullock, 1989). This observation is in agreement with Birk (1969) who reported gut inflammation of rats on ingestion of saponin and Mulky (1976) who reported that acute inflammation of the intestine can occur if Madhuca saponins were administrated to rats in a single dose.

Based on microscopic observation, neutrophils were present in marked numbers in the intestine of Nile tilapia fed diets III and IV. Neutrophils are commonly found at sites of inflammation (Roberts and Bullock, 1989) in order to destroy the ingested materials (organisms or toxicants) that caused the inflammation (Abdulfatah, 1983). Therefore, it is possible that saponin in the present study was absorbed through the wall of the intestine and caused such inflammation of *lamina properia*. Johnson et al. (1986) reported that most saponins are highly surface-active and rapidly increase the permeability of the intestinal tissue of rats *in vitro* leading to increased uptake of poorly permeable substances and a loss of normal function. Gee et al. (1993) also reported that quinoa saponins were shown to be membranolytic against cells of the small intestine of rats and to cause an increase in mucosal permeability *in vitro*.

The abnormalities found in the intestinal epithelial cells of fish fed diets III and IV are in agreement with Baeverfjord and Krogdah (1996) and Bureau et al. (1998) who reported cytoplasmic vacoulation in Atlantic salmon *Salmo salar*, chinook salmon *Oncorhynchus tshawytscha* and rainbow trout *Oncorhynchus mykiss* fed diets containing a solvent extracted soybean meal. Ingh et al. (1991) also reported that the use of soybean meal in diets for Atlantic salmon *Salmo salar* is known to induce pathological changes in the mucosal lining of the intestine.

Histological observation of liver sections revealed cell degeneration, shrinkage of cytoplasm and nuclei crowding in fish fed diets III and IV. These observations are in agreement with Annongu et al. (1996) who found severe areas of congestion in liver as a result of feeding sheabutter kernel cake saponin to chicks. Similar observations to those obtained in the present study were also reported in starved fish. Love (1980) reported that liver cells of common carp *Cyprinus carpio* diminish in size during starvation resulting in the nuclei crowding together. Similar results have been reported for jack mackerel *Trachurus symmetricus* (Theilacker, 1978), milkfish *Chanos chanos* (Storch and Juario, 1983) and Japanese flounder *Paralichthys olivaceus* (Chantanachookhin et al., 1990). From these findings it is possible to suggest that the alteration in the liver cells of fish fed diets III and IV might be due to the presence of saponin in the diet and/or feed deprivation. During starvation fish will use stored liver lipid to maintain biological functions and this will make cells appear depleted and diminished.

CHAPTER IV

THE EFFECT OF DIETARY HYDROLYSABLE TANNIN (TANNIC ACID) ON PERFORMANCE OF *OREOCHROMIS NILOTICUS* (L.) AND *CLARIAS GARIEPINUS* (B.) FINGERLINGS

4.1   Introduction

Hydrolysable tannin is widely distributed in the plant kingdom and occurs at high levels in various feeds. It is generally confined to certain legumes, leaf protein concentrates, fodders, browses, shrubs and hays (Table 1.2). It is defined as any polyphenolic substance with a molecular weight between 500 and 3000 daltons (Singleton and Kratzer, 1973) which on hydrolysis by acids or alkalis gives phenolic acid and a sugar moiety (Haslam, 1979). Tannic acid is reported to be typical of hydrolysable tannin (Singleton and Kratzer, 1973).

The negative effect of hydrolysable tannin on the growth of terrestrial animals is considered to be one of the factors that limit the nutritive value of plant proteins. Joslyn and Glick (1969) fed rat different levels of tannic acid, 2-10%. They found a proportional decrease in growth with increasing tannic acid level. A comparative study (Joslyn and Glick, 1969) between tannic acid and gallic acid (a metabolite of tannic acid) revealed that tannic acid was more toxic and caused higher mortality within a shorter period of time at dietary levels of 5, 8 and 10%. Mitjavila et al. (1971) also found significant growth depression in rats with increasing levels of tannic acid in the diet and feed efficiency ratio was increased by 50% compared to the control at levels of 6.4% tannic acid.

Meyer and Richardson (1993) found that preweanling prairie vole growth rate were unaffected by 4% tannic acid. Lindroth and Batzli (1984) found that weaned prairie vole young fed 3% and 6% tannic acid diets grew at the same

rate as young fed a control diet. In contrast, 4% tannic acid reduced growth in grey squirrel (MacCoubrey et al., 1997).

Vohra et al. (1966) reported that gain in weight and feed consumption in chicks decreased as the tannic acid content of the diet was increased and mortality increased as the level of tannic acid increased. In a number of other studies on chickens, levels of 0.2 and 2% added tannic acid have been shown to result in depressed dry matter intake, protein digestibility, reduced growth and higher feed efficiency ratios (Rostagno et al., 1973; Lacassagne et al., 1988).

Ringrose and Morgan (1940 as cited in Connor et al., 1969) concluded that the reduced growth that resulted from feeding tannic acid to chickens was due to reduced feed consumption whereas Vohra et al. (1966) suggested that the depression in growth of chicken cannot be explained entirely by feed intake. Glick and Joslyn (1970) supported the suggestion of Vohra (1966) by reporting that growth depression in rats was due to high faecal nitrogen losses occurring upon feeding tannic acid.

Bravo et al. (1994) reported that tannic acid at 1.92% of a rat diet was associated with increased faecal output of protein as compared with the control group. The increase of faecal protein excretion was correlated to a reduction of protein digestibility. Similarly, another study (Meyer 1989 as stated in MacCoubrey et al., 1997) has shown that feeding tannic acid at level of 4% reduced apparent protein digestibility in house mice *Mus musculus* and singing voles *Microtus*

miurus but not in snowshoe hares *Lepus americanus* or prairie voles *Microtus ochrogaster*.

In view of the absence of information concerning the effect of dietary hydrolysable tannin on fish and of possible presence of hydrolysable tannin in feed materials used for Nile tilapia and African catfish, a feeding trial was conducted for eight weeks with these species to establish the effects of dietary hydrolysable tannin.

4.2   Materials and Methods

4.2.1   Experimental System

The experimental system described in section 2.1 was used to conduct two separate experiments to evaluate the effect of hydrolysable tannin in fish.

4.2.2   Experimental Fish

Oreochromis niloticus and *Clarias gariepinus* as described in section 2.2 were used as models in this study.

4.2.3   Experimental Diets

The composition of the experimental diets and their proximate analysis are shown in Table 4.1. Diet formulation and preparation were as described in sections 2.3.1 and 2.3.2.

Table 4.1 Composition of the experimental diets (on dry matter basis) fed to *Oreochromis niloticus* (L.) and *Clarias gariepinus* (B) (g kg^{-1}).

ingredients	diet I 0% Tannic acid	diet II 0.1% Tannic acid	diet III 0.5% Tannic acid	diet IV 1% Tannic acid
Casein (bovine milk)	280	280	280	280
Gelatin (porcine skin)	60	60	60	60
Dextrin (corn)	315	315	315	315
Fish oil (capelin and herring, BOCM Pauls)	110	110	110	110
Tannic acid (Sigma)	0	1	5	10
Cellulose	145	144	140	135
CMC[1]	20	20	20	20
Cr_2O_3	10	10	10	10
Mineral mix[2]	40	40	40	40
Vitamin mix[3]	20	20	20	20
Proximate composition				
Moisture	45.2	60.6	86.1	76.4
Crude protein	320.5	305.2	302.3	319.9
Crude lipid	101.9	103.1	110	102.2
Ash	53.9	53.9	53.8	54.0
Crude fibre	115.5	117.9	117.5	114.2
Tannic acid	0.5	0.8	2.7	7.1
Cr_2O_3	10.9	11.9	11.3	11.5
N-free extract[4]	352.1	346.6	316.3	314.7
Gross energy (kJ g^{-1})	20.33	20.09	20.32	20.16

1- Carboxymethylcellulose-sodium salt, high viscosity.
2- As listed in Table 2.2
3-.As listed in Table 2.3
4- Obtained by subtraction

4.2.4   Experimental Procedure

Fish acclimation to the casein and gelatin based diet and periodical weighing were as described in section 2.4.1. Fish feeding and faeces collection were as described in sections 2.4.3 and 2.4.4.

4.2.5   Experimental Analyses

Proximate analysis (moisture, protein, lipid, fibre, ash, energy and chromic oxide) of fish, diets and faeces were as described in sections 2.5.1.1, 2.5.1.2, 2.5.1.3, 2.5.1.4, 2.5.1.5, 2.5.1.7 and 2.5.1.8. Hydrolysable tannin was determined according to the method described in section 2.5.1.9. Growth and feed performance, protein digestibility and dry matter digestibility were calculated according to the methods described in sections 2.5.2.1, 2.5.2.2, 2.5.2.3, 2.5.2.4, 2.5.2.5 and 2.5.2.6.

4.2.6   Histological Analysis

Histological analyses of fish livers and intestines were performed as described in section 2.6.

4.2.7   Statistical Analysis

Statistical analyses were carried out as described in section 2.8.

4.3   Results

4.3.1.   Growth and Feed Performance

No mortality nor external disease symptoms occurred in any treatment during the period of this study. Fish in all treatments accepted the diets from the first day of feeding and there was no sign of feed rejection observed. Fish exhibited good feeding activity and all offered feed was consumed in a short time period.

Nile tilapia mean weight gains (Figure 4.1), mean final weight (Table 4.2) and SGR (Table 4.2) all revealed a non-significant (F= 2.87, d.f.= 11-3, and F= 2.76, d.f.= 11-3, P< 0.05 for mean final weight and SGR respectively) trend of decreasing growth performance with increasing dietary tannic acid. Only percentage mean weight gains (Table 4.2) for diets I and IV differed significantly (F= 3.02, d.f.= 11-3, P< 0.05). However, the opposite was observed in African catfish in which mean weight gain (Figure 4.2), mean final weight (Table 4.3), SGR (Table 4.3) and percentage mean weight (Table 4.3) revealed a significant (F= 9.44, d.f.= 11-3, F= 6.38, d.f.= 11-3 and F= 8.24, d.f.= 11-3, P< 0.05) trend of decreasing growth performance with increasing dietary tannic acid. In general, fish in both species fed diet I produced the best growth performance while fish fed diet IV exhibited the poorest growth performance.

FCR ranged from 1.14 to 1.36 and from 0.93 to 1.09 for Nile tilapia and African catfish respectively being highest (F= 3.38, d.f.= 11-3 and F= 40.58, d.f.= 11-3, P< 0.05) for diet IV and III for Nile tilapia and African catfish respectively and
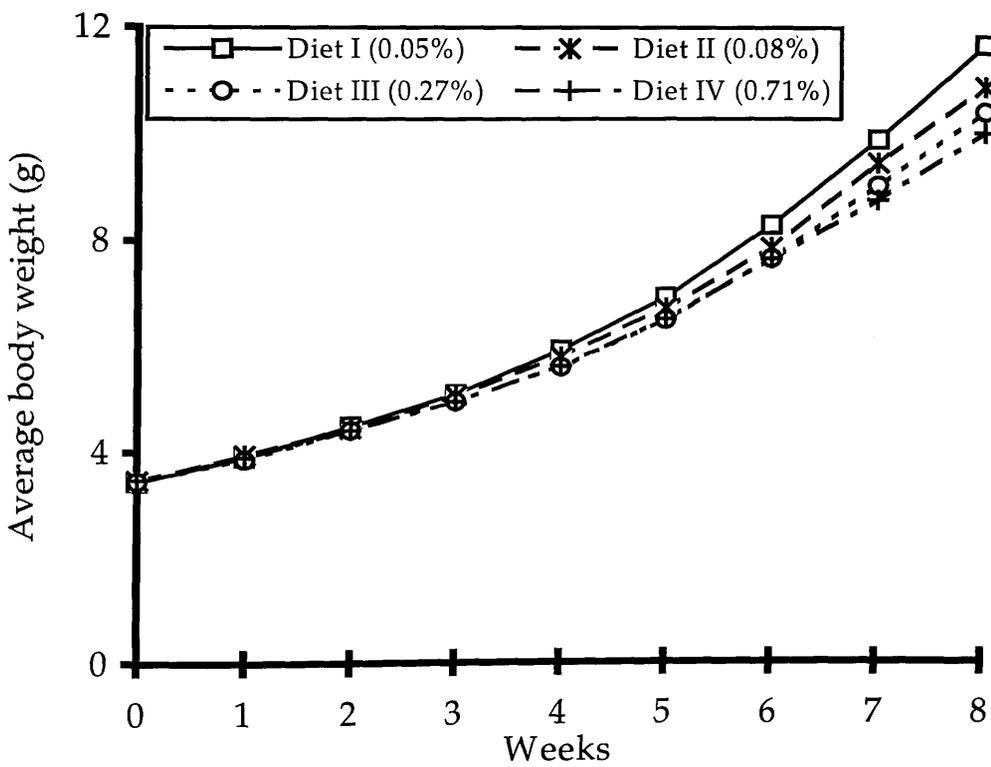
Fig. 4.1 Growth of Nile tilapia fed diets containing graded levels of
dietary tannic acid
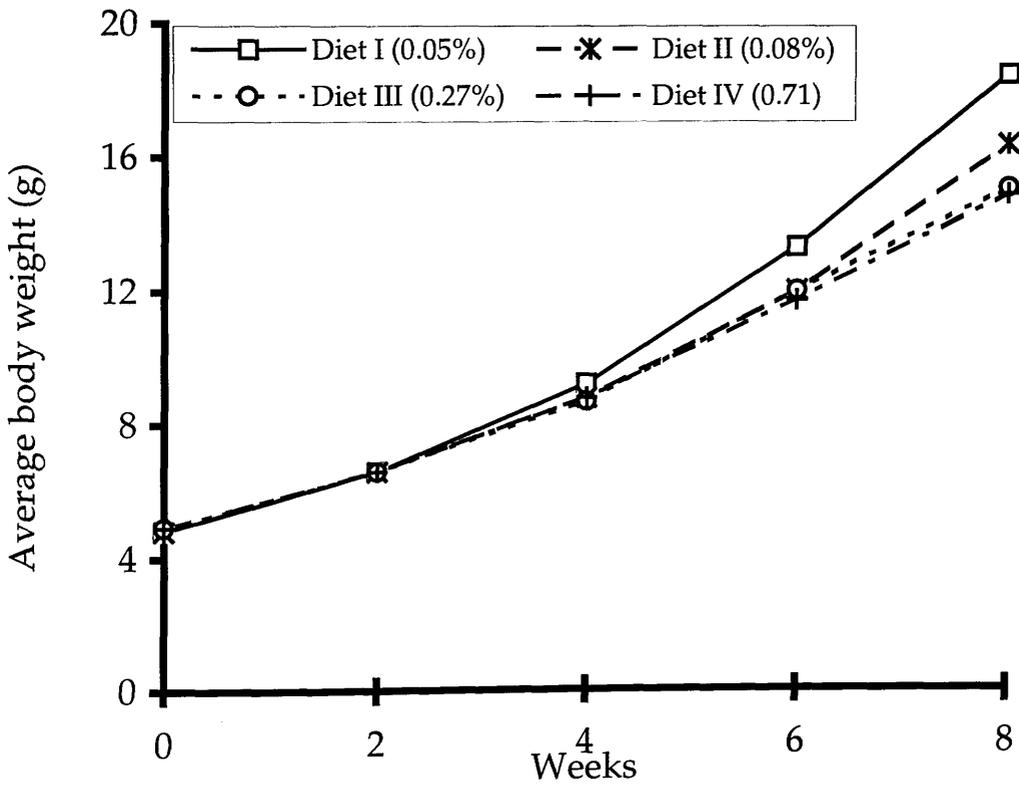
Fig. 4.2 Growth of African catfish fed diets containing graded levels
of dietary tannic acid

Table 4.2 Growth and feed performance of *Oreochromis niloticus* (L.) fed graded levels of dietary tannic acid.

Parameters	diet I 0.05%	diet II 0.08%	diet III 0.27%	diet IV 0.71%
Initial mean wt (g)	3.4[a] ± 0.00	3.5[a] ± 0.00	3.4[a] ± 0.06	3.5[a] ± 0.06
Final mean wt (g)	11.67[a] ± 0.90	10.90[a] ± 0.30	10.43[a] ± 0.67	10.00[a] ± 0.89
Feed intake (g/fish/experiment)	9.40[a] ± 0.36	9.20[a] ± 0.30	8.83[a] ± 0.31	8.80[a] ± 0.52
Weight gain (%)	243.14[a] ± 26.36	211.43[ab] ± 8.57	204.14[ab] ± 24.11	188.60[b] ± 27.28
SGR (%/d)	2.20[a] ± 0.13	2.03[a] ± 0.05	1.98[a] ± 0.15	1.89[a] ± 0.17
FCR	1.14[a] ± 0.08	1.24[ab] ± 0.01	1.27[ab] ± 0.09	1.36[b] ± 0.12
PER	2.74[a] ± 0.19	2.64[ab] ± 0.03	2.62[ab] ± 0.18	2.31[b] ± 0.19
PPV	45.87[a] ± 1.37	41.82[a] ± 3.77	42.17[a] ± 4.11	36.93[a] ± 4.54
Protein digestibility (%)	89.90[a] ± 1.24	87.92[ab] ± 1.67	87.82[ab] ± 1.12	86.49[b] ± 1.36
Dry matter digestibility (%)	64.16[a] ± 0.21	64.10[a] ± 1.18	64.61[a] ± 0.58	64.23[a] ± 2.06

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Fig. 4.3 Growth and feed performance of *Oreochromis niloticus* (L.) fed graded levels of dietary tannic acid.
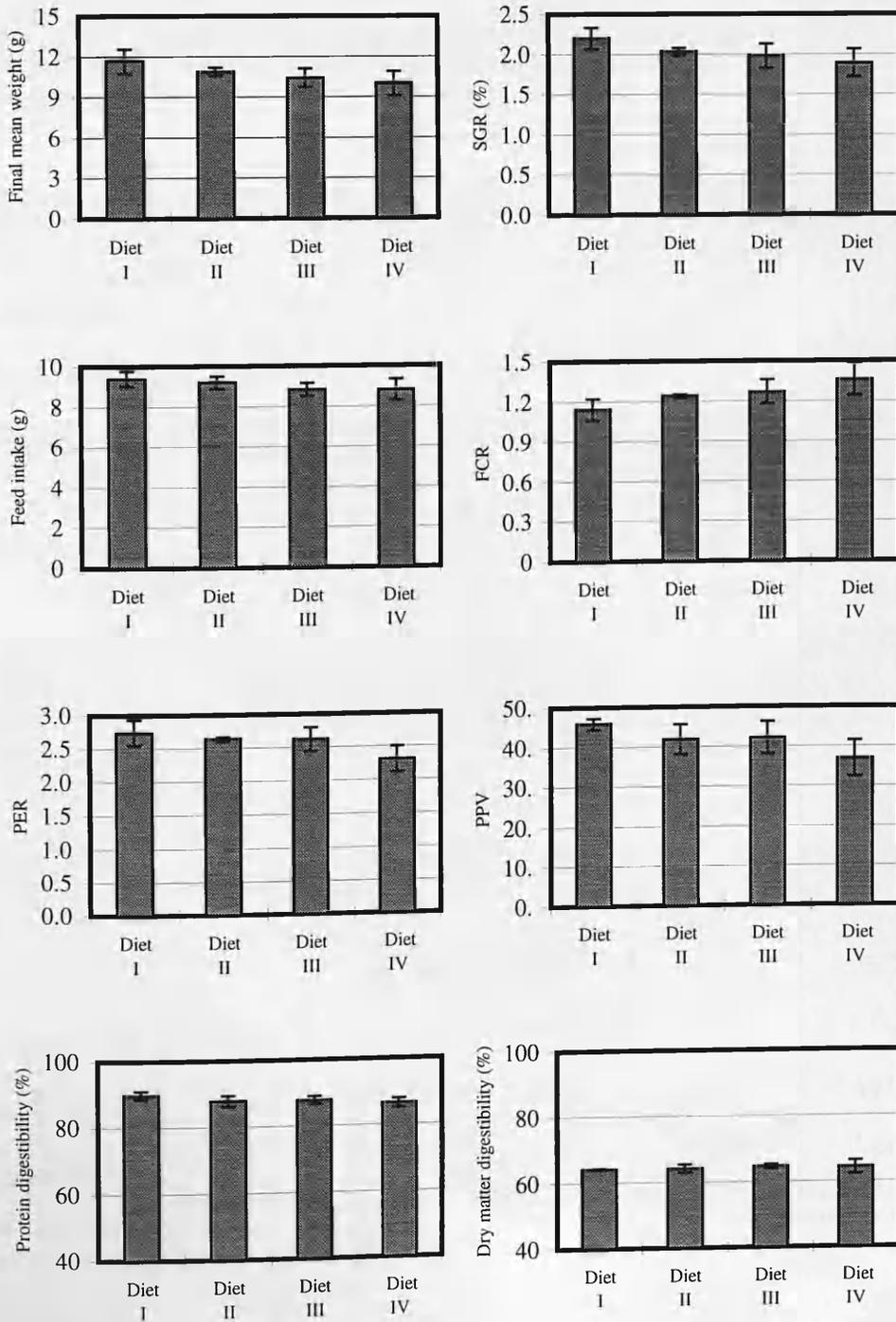
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.05, 0.08, 0.27 and 0.71% dietary tannic acid respectively.

Table 4.3 Growth and feed performance of *Clarias gariepinus* (B.) fed graded levels of dietary tannic acid.

Parameters	diet I 0.05%	diet II 0.08%	diet III 0.27%	diet IV 0.71%
Initial mean wt (g)	4.9[a] ± 0.06	4.8[a] ± 0.06	4.9[a] ± 0.00	4.8[a] ± 0.06
Final mean wt (g)	18.5[a] ± 0.50	16.40[b] ± 0.10	15.13[b] ± 0.59	14.93[b] ± 1.68
Feed intake (g/fish/experiment)	12.63[a] ± 0.12	11.97[ab] ± 0.06	11.17[ab] ± 0.50	10.60[b] ± 1.66
Weight gain (%)	280.10[a] ± 6.75	239.33[b] ± 2.48	208.84[b] ± 11.96	209.27[b] ± 38.13
SGR (%/d)	2.38[a] ± 0.03	2.18[ab] ± 0.01	2.01[b] ± 0.07	2.01[b] ± 0.23
FCR	0.93[a] ± 0.03	1.03[b] ± 0.00	1.09[c] ± 0.0	1.05[b] ± 0.02
PER	3.36[a] ± 0.09	3.17[b] ± 0.00	3.03[c] ± 0.05	2.97[c] ± 0.07
PPV	61.89[a] ± 1.03	58.41[b] ± 0.56	56.41[bc] ± 1.75	54.76[c] ± 1.54
Protein digestibility (%)	98.21[a] ± 0.04	97.54[b] ± 0.32	96.44[c] ± 0.23	95.33[d] ± 0.30
Dry matter digestibility (%)	57.83[a] ± 2.33	58.86[a] ± 5.23	58.09[a] ± 2.55	58.14[a] ± 4.36

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Fig. 4.4 Growth and feed performance of *Clarias gariepinus* (B.) fed graded levels of dietary tannic acid.
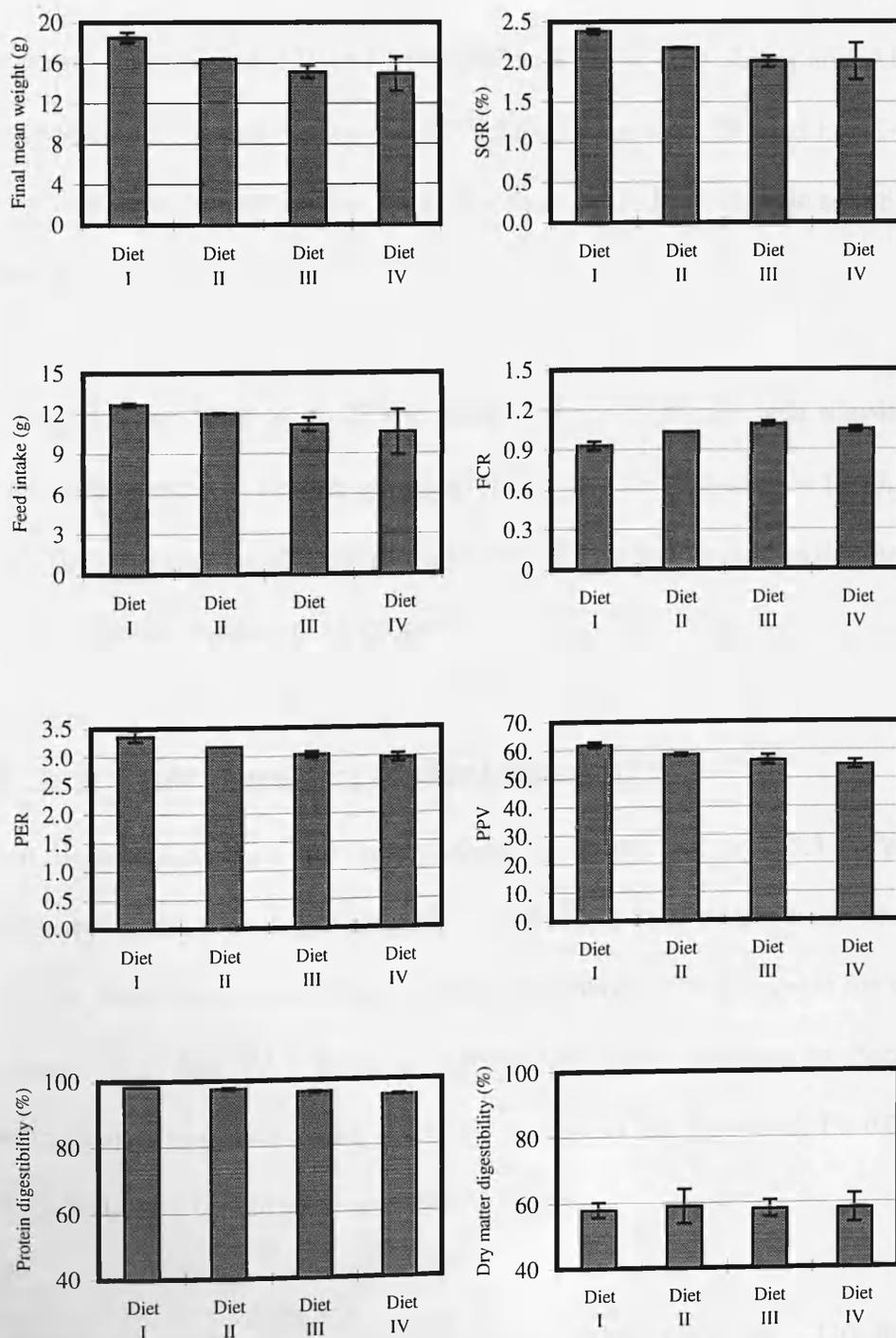
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.05, 0.08, 0.27 and 0.71% dietary tannic acid respectively.

lowest for diet I. In general FCR values tended to increase as the level of dietary tannic acid increased.

PER ranged from 2.31 to 2.74 and from 2.97 to 3.36 for Nile tilapia and African catfish respectively being highest (F= 3.77, d.f.= 11-3 and F= 25.20, d.f.= 11-3, P< 0.05) for diet I and lowest for diet IV. PER values tended to decrease as the level of dietary tannic acid increased.

PPV ranged from 36.93 to 45.87 and from 54.76 to 61.89 for Nile tilapia and African catfish respectively being highest (F= 3.01, d.f.= 11-3 and F= 16.63, d.f.= 11-3, P< 0.05) for diet I and lowest for diet IV. PPV values tended to decrease as the level of dietary tannic acid increased.

4.3.2 Protein and Dry matter Digestibility

Protein digestibility data are summarised in Tables 4.2 and 4.3. Protein digestibility values were high, ranging from 89.90 to 86.49% and from 98.21 to 95.33% for Nile tilapia and African catfish respectively being highest for diet I and lowest for diet IV. Protein digestibility values tended to decrease (sometimes significantly F= 3.20, d.f.= 11-3 and F= 77.39, d.f.= 11-3, P< 0.05) as the level of dietary tannic acid increased.

Dry matter digestibility data are summarised in Tables 4.2 and 4.3. Dry matter digestibility for all diets was similar and there were no significant differences

(F= 0.10, d.f.= 11-3 and F= 0.04, d.f.= 11-3, P< 0.05 for Nile tilapia and African catfish) between diets.

4.3.3   Body Composition

Body composition data are summarised in Tables 4.4 and 4.5. There were no significant (F= 0.65, d.f.= 11-3, F= 0.70, d.f.= 11-3, F= 0.28, d.f.= 11-3 and F= 0.82, d.f.= 11-3 for Nile tilapia; F= 0.33, d.f.= 11-3, F= 3.91, d.f.= 11-3, F= 0.89, d.f.= 11-3 and F= 0.02, d.f.= 11-3 for African catfish P< 0.05) differences between the four diets in terms of body moisture, protein level, lipid contents and ash contents of either species.

4.3.4   Histological Examination

Histological examination of intestines and livers of fish fed the experimental diets showed that tannic acid at all levels had no effect on the examined tissues. In all tested fish intestine was well developed with mucosal epithelium foldings and no apparent degeneration or vacuolation. Cells were full of cytoplasm and there was no sign of hypertrophy. Cells were columnar in shape and the nuclei were in the base of cells as usual. Striated borders were well defined and goblet cells were scattered between the epithelial cells. The structure of the *lamina propria* was normal and there was no sign of infiltration. Sections of livers showed no differences between treatments. Hepatic cells were well defined in shape and nuclei were near to the cell wall. Cells were well organised and there was no sign of shrinkage or cell wall breakage.

Table 4.4 Body composition of *Oreochromis niloticus* (L.) fed graded levels of dietary tannic acid.

%	Initial fish	Diet I 0.05%	Diet II 0.08%	Diet III 0.27%	Diets IV 0.71%
Moisture	74.44	71.73[a] ± 1.79	72.99[a] ± 1.47	72.51[a] ± 0.37	72.98[a] ± 0.96
Crude protein	14.32	16.17[a] ± 0.86	15.36[a] ± 0.97	15.59[a] ± 0.47	15.42[a] ± 0.67
Crude lipid	7.68	7.49[a] ± 0.77	7.24[a] ± 0.29	7.29[a] ± 0.55	7.12[a] ± 0.13
Ash	3.56	4.61[a] ± 0.23	4.41[a] ± 0.22	4.61[a] ± 0.03	4.48[a] ± 0.20

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Table 4.5   Body composition of *Clarias gariepinus* (B.) fed graded levels of dietary tannic acid.

%	Initial fish	Diet I 0.05%	Diet II 0.08%	Diet III 0.27%	Diets IV 0.71%
Moisture	75.67	71.31[a] ± 0.34	71.68[a] ± 0.54	71.50[a] ± 0.39	71.51[a] ± 0.53
Crude protein	14.55	16.25[a] ± 0.13	16.06[ab] ± 0.10	15.89[ab] ± 0.31	15.76[b] ± 0.14
Crude lipid	6.81	8.71[a] ± 0.24	8.54[a] ± 0.44	8.86[a] ± 0.21	9.00[a] ± 0.49
Ash	2.97	3.73[a] ± 0.12	3.73[a] ± 0.02	3.75[a] ± 0.14	3.73[a] ± 0.18

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

4.4   Discussion

4.4.1   Growth and Feed Performance

It is apparent from the present study that hydrolysable tannin (as tannic acid) is a growth inhibitor for both Nile tilapia and African catfish. The growth performance and feed utilisation efficiencies of both species fed diet I (the control) were better than for tannic acid supplemented diets. This can only be attributed to the presence of tannic acid in diets II, III and IV. It is well established that hydrolysable tannin is growth inhibitory to poultry and small mammals. Vohra et al. (1966) indicated that as little as 0.5% tannic acid in the diet significantly depresses growth of chicks. Rayudu et al. (1970) found that 1% tannic acid of chicks diet depressed growth significantly. Similarly, Ringrose and Morgan (1940, as cited in Connor et al., 1969) reported that tannic acid fed at 2% of the diet resulted in growth depression of chicks. Tannins extracted from sunflower seed meal and carob seed (Table 1.2) caused growth depression and lower feed efficiency when fed to mice at a level of 3% and to rats at level of 4% of the diet respectively (Liener, 1980; Tamir and Alumot, 1970 respectively). A similar result was reported by MacCoubry et al. (1997) who found that 4% tannic acid reduced growth in grey squirrel. Joslyn and Glick (1969) reported that tannic acid fed to rats at 5% of the diet gave weight gain of approximately 50% of the control. However, Lindroth and Batzli (1984) found that weaned prairie vole young fed 3 and 6% tannic acid diets grow at the same rate as young fed a control diet. Meyer and Richardson (1993) also found that preweanling prairie vole growth rates were unaffected by 4% tannic acid. Salunkhe et al. (1990) reported that rats could tolerate up to 5% tannic

acid, but higher levels caused marked growth depression. It would appear that fish are less tolerant than other species to the hydrolysable tannin and 0.08% dietary tannic acid was enough to exhibit a negative effect on growth performance.

It has been reported that tannins create palatability problems due to their astringent taste (Joslyn and Goldstein, 1964). For that reason Ringrose and Morgan (1940, as cited in Connor et al., 1969) concluded that the reduced growth that resulted from feeding tannic acid to chicks was due to reduced feed consumption. Based on visual observation during feeding time and behaviour of fish during feeding time as well, palatability or acceptability was not problem in this study. Fish consumed all the offered feed within a very short time which gives an indication that tannic acids did not affect palatability of diets. Similar results were reported by Tamir and Alumot (1970) who found that tannin extracted from carob did not affect feed consumption of rats; however, growth depression was observed. Vohra et al. (1966) also reported that feed consumption in chicks was the same in a control group and a group for which 5% tannic acid was added to the diet. Moreover, Bornstein et al. (1963) found that inclusion of carob (Table 1.2) to the diet of chicks increased feed consumption; however, weight gain was found to decrease considerably. Therefore, it is believed in the present study that the growth reduction observed cannot be explained by feed intake.

Mortality was not observed during the period of this study as a result of feeding dietary tannic acid. A similar result (Rayudu et al., 1970) was observed in chicks fed diets containing 1 or 2% tannic acid. However, the result of the present study is in disagreement with Joslyn and Glick (1969) who found 30, 70 and 100% mortality in rats fed diets containing 4, 8 and 10% tannic acid respectively. Similarly Glick and Joslyn (1970) reported 90% mortality in rats fed diet containing 8% tannic acid. Alexis (1990) also observed high mortality in rainbow trout *Oncorhynchus mykiss* fed a diet containing 576g/kg carob seed meal. It is probably that the low concentrations of tannic acid used in the present study are the reason behind the 100% survival rate.

It is well documented (Table 1.1) that the inclusion of plant protein sources in fish diets results in reduced feed intake and /or reduced nutrient digestibility that may result in reduced growth. It is believed that the presence of hydrolysable tannins in such sources (Table 1.2) is, in part, the reason for growth reduction. Several scientists have tried to replace fish meal with cottonseed meal at various levels; and they have generally observed low growth performance in fish fed cottonseed meal compared to the control group (Fowler, 1980; Ofojekwu and Ejike, 1984; Usmani et al., 1997). These researchers concluded that the presence of gossypol and the low availability of lysine in cottonseed meal were the principal reasons for poor growth in tested fish. However, Robinson et al. (1984) reported that the nutritive value of cottonseed meal for Blue tilapia *Tilapia aurea* is not as high as that of soybean and peanut meals even after the addition of supplemental lysine. They

concluded that the growth reduction observed in fish fed cottonseed meal could not be attributed to dietary gossypol as free gossypol levels were less than 0.005%. A similar conclusion has been reported by Robinson and Tiersch (1995) when they fed channel catfish *Ictalurus punctatus* various levels of cottonseed meal supplemented with lysine. Moreover, Roehm et al. (1967) demonstrated that rainbow trout *Oncorhynchus mykiss* can tolerate levels of gossypol acetate up to 0.025% of the diet. Dorsa et al. (1982) found that channel catfish *Ictalurus punctatus* can tolerate up to 0.09% free gossypol in their diets without any growth suppressive effect. Robinson et al. reported that up to 0.2% free gossypol can be safely added to the diets of Blue tilapia *Tilapia aurea*. Furthermore, El-Sayed (1990) found no difference in growth of Nile tilapia *Oreochromis niloticus* fed cottonseed meal with and without addition of lysine. Similar results have been reported by Robinson and Li (1994) and Sintayehu et al. (1996) in channel catfish *Ictalurus puncatatus* and Nile tilapia *Oreochromis niloticus* respectively. Accordingly, it is believed that the reduced growth of tested fish was not due to the presence of gossypol or the low availability of lysine but to the level of hydrolysable tannin present in cottonseed (around 4.2%, Yu et al., 1996b).

Attempts by Alexis (1990) to incorporate carob seed germ meal at levels of 20, 36.5 and 44.6% in diets of rainbow trout *Oncorhynchus mykiss* fingerlings led to results similar to the present study in term of growth performance. She concluded that tannin (the main antinutritional factor reported for carob) was the main reason for growth reduction in fish. Burgos (1995) reported that leaf

protein concentrates from sesbania, which is known to contain hydrolysable tannin (Kaitho et al., 1997), cannot be used as an alternative protein source for Nile tilapia *Oreochromis niloticus*; as the high content of polyphenols may negatively affect both protein quality and fish metabolism.

4.4.2   Protein and Dry matter digestibility

Protein digestibility in the present study was affected by addition of tannic acid to the diets (Tables 4.2 and 4.3). Diet I (the control) showed highest protein digestibility between all diets whereas the rest of the values showed a reduction as the content of tannic acid increased. The result of the present study is in agreement with Moulay et al. (1988), Santidrain and Marzo (1989) and Bravo et al. (1994) who reported a significant increase in faecal protein excretion in guinea-pigs, chicks and rats respectively fed diets containing tannic acid. It seems there might be an interaction between protein and tannic acid which might in turn be the reason for the differences in growth performance in the present study. McLeod (1974) showed that tannic acid has the ability to bind protein. This binding can reduce digestibility either by inhibiting the action of digestive enzymes or by rendering dietary proteins resistant to enzymatic breakdown.

In contrast, Hagerman et al. (1992) found that hydrolysable tannin in the form of tannic acid, did not decrease protein digestibility in deer or sheep. They concluded that hydrolysable tannin would be easily degraded in the gut to small phenolics which would not interact with protein and would finally be

absorbed. However, Buren and Robinson (1969) showed in an *in vitro* study that there was a reaction between tannic acid and protein (in the form of gelatin) and that this reaction formed insoluble complexes.

Sintayehu et al. (1996) found that the protein digestibility of cotton seed meal was inferior to sunflower seed meal and soybean meal (79.4, 89.8 and 93% respectively) in Nile tilapia *Oreochromis niloticus*. They assumed that the crude fibre content in cotton seed meal was the major reason for low protein digestibility. However, based on their proximate analysis sunflower seed meal contained higher crude fibre (29.4%) than cotton seed meal (20.4%) so that it alone was not the major reason for low protein digestibility for cotton seed meal. It is worth reporting that cotton seed contains a (4.2%; Yu et al., 1996b) higher level of hydrolysable tannin than sunflower seed (1.2 to 2.7%; Liener, 1980).

Dry matter digestibility in both species (Tables 4.2 and 4.3) was not affected by addition of tannic acid and was similar to values reported elsewhere. Reigh et al. (1990) and Fagbenro (1996) reported 64.8% and 46-54% dry matter digestibility for cottonseed meal in crayfish *Procambarus clarkii* and *Clarias isheriensis* respectively. Hilton and Slinger (1986) concluded that crude fibre content of the diet is the major factor limiting dry matter digestibility and that a high crude fibre content (11.1%) will produce low dry matter digestibility as fibre is negatively correlated with digestibility. Crude fibre contents of diets in the present study are in the range of 11.4 - 11.8% which are higher than

reported by Hilton and Slinger (1986). However, the results obtained from the present study (Tables 4.2 and 4.3) are higher than those reported for rice bran in red drum *Sciaenops ocellatus* (McGoogan and Reigh, 1996) and in hybrid striped bass (Sullivan and Reigh, 1995) and for sunflower seed meal in Nile tilapia *Oreochromis niloticus* (Sintayehu et al., 1996) in which crude fibre contents were similar to those reported in the present study.

4.4.3   Body Composition and Histological Examination

The results of the present study indicate that diets containing tannic acid at the tested levels did not have any effect in terms of dry matter, crude protein, ether extract and ash content of carcasses. No published data are available on the effect of feeding hydrolysable tannin on body composition of fish. However, results obtained from the present study are comparable to results reported elsewhere in which hydrolysable tannin-containing diets are adopted. Ofojekwu and Ejike (1984) and El-Sayed (1990) reported similar results of body composition to those reported in the present study for Nile tilapia *Oreochromis niloticus* fed various levels of cottonseed meal. However, Usmani et al. (1997) reported different results from those reported in the present study for *Labeo rohita* fed also various levels of cottonseed meal. These differences are expected as long as there are differences in the compositions of diets between the study of Usmani et al. (1997) and the present study in term of protein, lipid, carbohydrate and ash.

The results of histological examination of intestine and liver sections of all treatments showed no differences. It seems that hydrolysable tannin in the form of tannic acid does not cause any alteration or abnormality in intestines or livers of Nile tilapia or African catfish at the levels tested under these experimental conditions. However, Salunkhe et al. (1990) reported that chronic ingestion of large amounts of tannins can damage the gastrointestinal surface of certain species. Under such conditions, tannins might be absorbed and produce possible harmful effects. Tannic acid has been listed as a tentative carcinogen of category I under the carcinogen policy of the Occupational Safety and Health Administration (Salunkhe et al., 1990). According to Singleton and Kratzer (1973), the carcinogenicity of tannins may involve irritation and cellular damage resulting in a high malignancy rate.

Morphological alterations in the liver of rabbits were reported following subcutaneous injection of tannic acid (Arhelger et al., 1965). However, chronic doses of tannic acid fed to mice at a level of 1.5 mg/kg per month did not cause any detectable change in their livers (Glick and Joslyn, 1970). In the present study, the reported (Salunkhe et al., 1990) conditions that stimulate tannic acid to cause damage to fish gastrointestinal surface did not exist which might be the reason for observing no abnormalities.

CHAPTER V

THE EFFECT OF DIETARY CONDENSED TANNIN (CATECHIN) ON PERFORMANCE OF *OREOCHROMIS NILOTICUS* (L.) AND *CLARIAS GARIEPINUS* (B.)FINGERLINGS

5.1   Introduction

Condensed tannins are a second group of tannins considered as dimers or higher oligomers of variously substituted flavan-3-ols (Haslam, 1979). Condensed tannins are the most naturally abundant tannins in the plant kingdom and are more widely distributed than the hydrolysable form. They are present in 54% of all studied angiosperm genera, 74% of all studied gymnosperm genera and 92% of all studied ferns (Griffiths, 1991). These compounds may accumulate in the seed, stems, leaves or roots and as shown in Table 1.2 are frequently found in many commonly utilised constituents of both human and animal diets.

The antinutritional effects of condensed tannins in the diets of monogastric animals have been studied in a wide range of species including pigs (Rowan and Lawrence, 1986ab; Lizardo et al., 1995), poultry (Vohra et al., 1966; Elkin and Rogler, 1990; Rubio et al., 1990; Ortiz et al., 1994; Ravindran and Sivakanesan, 1996), rats (Elkin and Rogler, 1990; Ortiz et al., 1994), and squirrels (MacCoubrey et al., 1997).

The inclusion of high amounts of faba beans *Vicia faba* in diets has been reported to have a detrimental effect on the performance of pigs, rats and chicks. This has been attributed to the presence of condensed tannin (Marquardt, 1989) at a level of 2% (Reddy et al, 1985). Aherene et al. (1979) reported progressive decreases in weight gain and efficiency of feed utilisation as the dietary concentration of faba beans was increased from 0 to 30% in pig

diets. However, Mateos and Puchal (1981) reported that the use of up to 20% faba beans as a source of protein did not impair pig performance. Condensed tannin extracted from faba bean and fed to rats at a concentration of 0.4% of the total diet has been shown to significantly reduce weight gain, feed conversion ratio and protein efficiency ratio but had only a comparatively small negative effect on feed intake (Moseley and Griffiths, 1979). Ward et al. (1977) showed that diets containing purified faba bean tannin (1.7%) significantly decreased protein digestibility, depressed growth rate and feed conversion ratio in chicken.

Numerous studies have been carried out on the feeding value of sorghum grain and its content of condensed tannin. Almond et al. (1979) reported that the presence of condensed tannins in the sorghum kernel induced a decrease in weight gain and an increase in feed conversion ratio when diets based on sorghum were fed to chicks and pigs. Nutritional studies undertaken using chicks and rats have consistently shown reduced weight gains and feed efficiencies with observed deleterious effects increasing as the grain sorghum content increased (Marquardt, 1989).

Jambunathan and Mertz (1973) compared low (0.51%) and high (2.69%) condensed tannin isoproteic isoenergetic diets of grain sorghum for rats and found that a high tannin diet resulted in a low weight gain and reduced protein efficiency ratio when compared to the low tannin diet. Moreover, Armstrong et

al. (1973) found that dietary levels of sorghum tannins between 1.57 and 2.66% caused depression of growth in chicks.

Tolerance to condensed tannins varies between species of animals. Ford and Hewitt (1979) fed both sorghum and faba bean containing diets to both rats and chicks. They observed that the effect of sorghum was much greater than that of faba beans and that the chicks were much more sensitive to the effects of tannins than the rats. Similar observations have been reported by Ortiz et al. (1994) when they noticed high mortality in chicks, and an absence of mortality in rats, in groups of animals fed on diets containing faba bean tannin extract. Furthermore, the consumption of faba beans in a long-term trial was found to decrease the efficiency of feed utilisation and tended to increase mortality in chicks (Guillaume and Bellec, 1977).

In contrast, Lizardo et al. (1995) provided sorghum grain in diets of weaned piglets at 30% of the total protein content with a condensed tannin level of 0.28%. They found no deleterious effects either on animal performance or nutrient utilisation.

It appears that there are several causes of the growth depressing and toxic effects of condensed tannins, and the interplay between them and the experimental conditions may account for such variable observations. High tannin in the diet makes it astringent, and the animals must be starved to force them to eat it (Singleton and Kratzer, 1973). McCabe and Barry (1988)

conducted an experiment to assess two different tannin concentrations in sheep, goats and deer. Voluntary intakes of willow containing 2.9 or 6.6% condensed tannin (dry matter) were compared to lucerne hay. There were no significant differences between animal species in intakes of lucerne hay. However when the diet was changed to willow, there were marked decreases in the voluntary feed intakes in sheep. Deer showed little change in intake of willow and goats showed a marked increase in intake. There were tendencies for sheep and goats to ingest more of the lower tannin containing willow compared with the higher tannin ones whereas with deer the converse was observed. In another example, the voluntary feed intake by cattle of *Lespedeza cuneata* decreased by 70% as the tannin content of the forage increased from 4.8 to 12% (Griffiths, 1991).

The other possible cause of growth depression is the excretion of high levels of nitrogen. Several studies reporteded that high rates of nitrogen excretion result largely from the binding of dietary protein by tannin into an indigestible form. Chang and Fuller (1964) found reduced protein digestibility in chicks fed sorghum grains containing 0.6% tannin. Tamir and Alumot (1970) noticed increased quantities of insoluble nitrogen in the digestive system of rats fed tannin. They reported the effect to be due to the interaction of tannins with proteins. Rostagno et al. (1973) investigated protein digestibility of chicks fed diets based on low (0.33 and 0.59%) and high (1.10 and 1.41%) tannin sorghum. They observed that the protein digestibility was significantly lower in high tannin diets as compared to low tannin diets. The mean protein digestibilities

of the two low tannin cultivars were 79.8 and 48.2% while for two high tannin cultivars they were only 35.9 and 26.2%. Similarly, *in vitro* experiments on the influence of sorghum tannin on protein digestibility have shown that tannin significantly depressed protein digestibility (Ramachandra et al., 1977).

In fish, there is absence of information regarding the effects of dietary condensed tannin. Therefore, the objective of this study was to evaluate the influence of condensed tannin on the performance of Nile tilapia and African catfish fingerlings.

5.2   Materials and Methods

5.2.1   Experimental System

The experimental system described in section 2.1 was used to conduct two separate experiments to evaluate the effect of condensed tannin in fish.

5.2.2   Experimental Fish

Oreochromis niloticus and *Clarias gariepinus* as described in section 2.2 were used as models in this study.

Table 5.1 Composition of the experimental diets fed to _Oreochromis niloticus_ (L.) and _Clarias gariepinus_ (B.) (g*kg^{-1}, on dry matter basis)

ingredients	Diet I 0% Catechin	Diet II 0.1% Catechin	Diet III 0.5% Catechin	Diet IV 1% Catechin
Casein (bovine milk)	280	280	280	280
Gelatin (porcine skin)	60	60	60	60
Dextrin (corn)	315	315	315	315
Fish oil (capelin and herring, BOCM Pauls)	110	110	110	110
Catechin (Sigma)	0	1	5	10
Cellulose	145	144	140	135
CMC[1]	20	20	20	20
Cr_2O_3	10	10	10	10
Mineral mix[2]	40	40	40	40
Vitamin mix[3]	20	20	20	20
Proximate composition				
Moisture	54.1	43.3	41.5	37.0
Crude protein	328.5	330.2	323.2	331.5
Crude lipid	103.9	108.0	110.2	107.8
Ash	51.7	51.2	52.1	51.8
Crude fibre	107.9	109.7	108.6	102.9
Catechin (condensed tannin)	0.6	1.6	7.3	16.6
Cr_2O_3	12.1	12.3	13.3	13.6
N-free extract[4]	341.2	343.7	343.8	338.8
Gross energy (kJ*g^{-1})	21.09	21.57	20.86	21.23

1- Carboxymethylcellulose-sodium salt, high viscosity.
2- As listed in Table 2.2
3- As listed in Table 2.3
4- Obtained by subtraction.

5.2.3   Experimental Diets

The composition of the experimental diets and their proximate analysis are shown in Table 5.1. Diet formulation and preparation were as described in sections 2.3.1 and 2.3.2.

5.2.4   Experimental Procedure

Fish acclimation to the casein and gelatin based diet and periodical weighing were as described in section 2.4.1. Fish feeding and faeces collection were as described in sections 2.4.3 and 2.4.4.

5.2.5   Experimental Analyses

Proximate analysis (moisture, protein, lipid, fibre, ash, energy and chromic oxide) of fish, diets and faeces were as described in sections 2.5.1.1, 2.5.1.2, 2.5.1.3, 2.5.1.4, 2.5.1.5, 2.5.1.7 and 2.5.1.8. Condensed tannin was determined according to the method described in section 2.5.1.10. Growth and feed performance, protein digestibility and dry matter digestibility were calculated according to the methods described in sections 2.5.2.1, 2.5.2.2, 2.5.2.3, 2.5.2.4, 2.5.2.5 and 2.5.2.6.

5.2.6   Histological Analysis

Histological analyses of fish livers and intestines were performed as described in section 2.6.

5.2.7   Statistical Analysis

Statistical analyses were carried out as described in section 2.8.

5.3   Results

5.3.1.   Growth and Feed Performance

No mortality nor external disease symptoms occurred in any treatment during the period of this study. Fish in all treatments accepted the diets from the first day of feeding and there was no sign of feed rejection observed. In general fish exhibited good feeding activity and all offered feed was consumed. However, African catfish fed diet IV (1.66% catechin) exhibited obvious signs of rejection of the apparently unpalatable feed and the daily offered ration was never completely consumed.

Fish fed diets II, III and IV generally had significantly reduced mean weekly/biweekly weight gain (Figures 5.1 and 5.2), mean final weight, percentage weight gain and SGR (F= 2.01, d.f.= 11-3, F= 2.06, d.f.= 11-3 and F= 2.07, d.f.= 11-3 for Nile tilapia and F= 54.09, d.f.= 11-3, F= 57.58, d.f.= 11-3 and F= 53.99, d.f.= 11-3 for African catfish P< 0.05) (Tables 5.2 and 5.3) as compared to fish fed diet I. However, performance of Nile tilapia fed diet II was not significantly (P< 0.05) different from that of the control (diet I).

FCR ranged from 1.07 to 1.19 and from 0.89 to 1.03 for Nile tilapia and African catfish respectively being highest for diet IV and lowest for diet I. FCR values
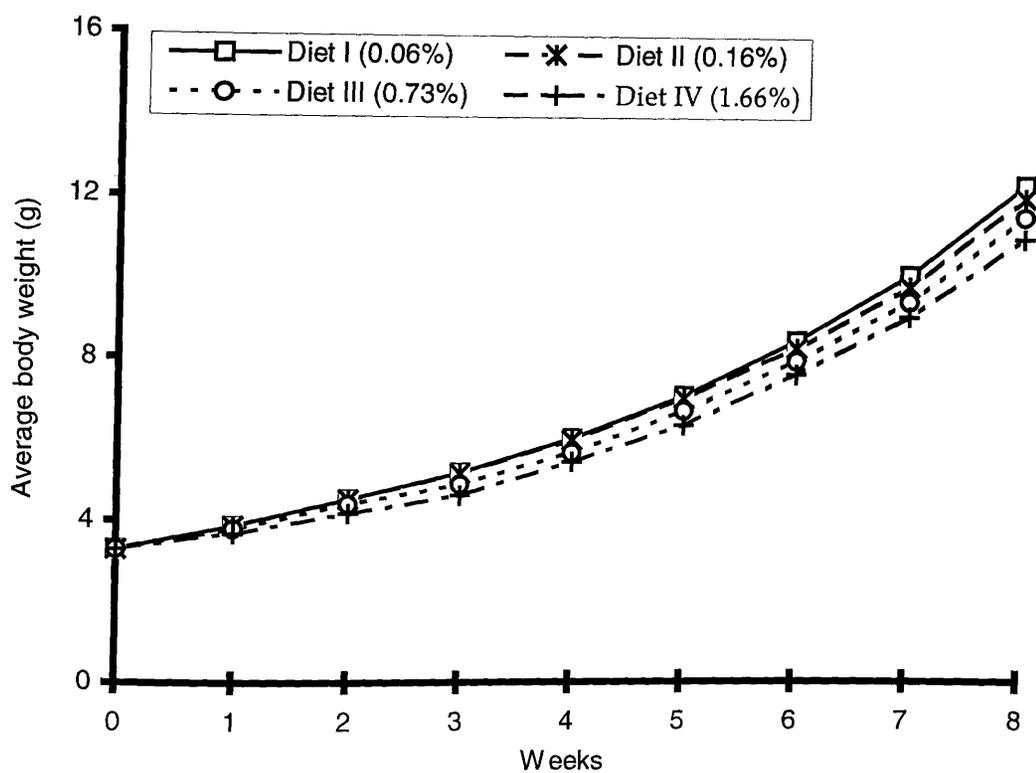
Fig. 5.1 Growth of Nile tilapia fed diets containing graded levels of dietary condensed tannin (catechin)
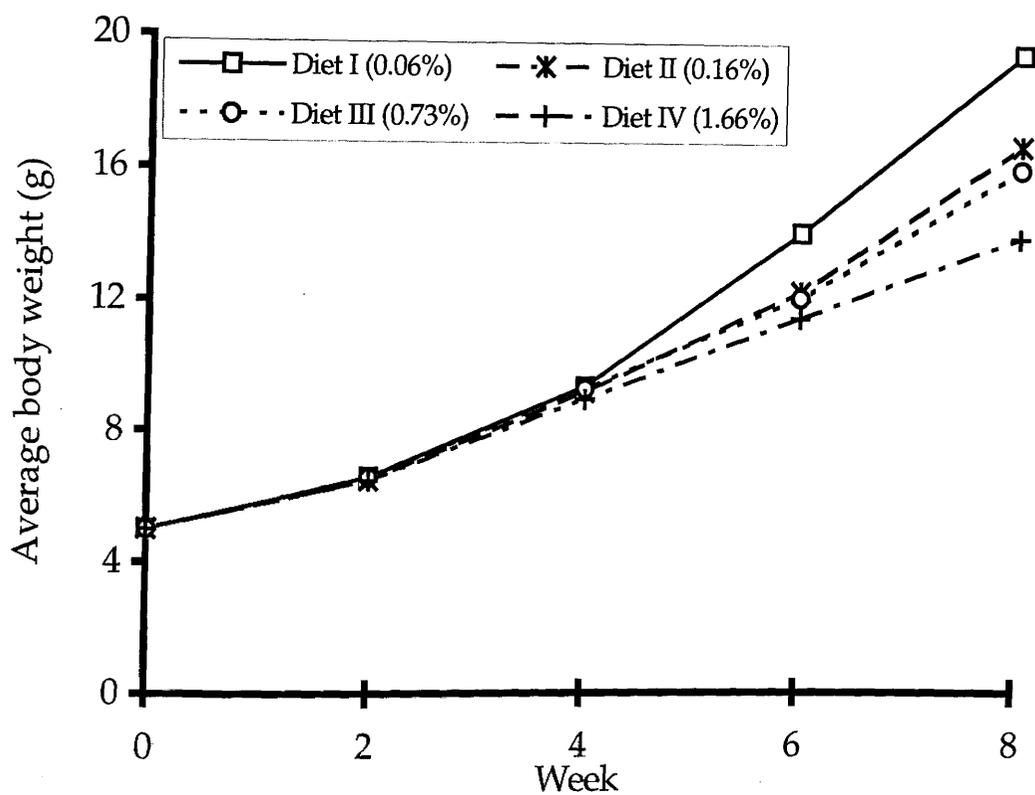
Fig. 5.2 Growth of African catfish fed diets containing graded levels of dietary condensed tannin (catechin)

Table 5.2 Growth and feed performance of *Oreochromis niloticus* (L.) fed graded levels of condensed tannin.

Parameters	Diet I (0.06%)	Diet II (0.16%)	Diet III (0.73%)	Diet IV (1.66%)
Initial mean wt (g)	3.3[a] ± 0.01	3.3[a] ± 0.01	3.3[a] ± 0.01	3.3[a] ± 0.02
Final mean wt (g)	12.4[a] ± 0.18	12.0[ab] ± 0.25	11.6[bc] ± 0.51	11.0[c] ± 0.30
Feed intake (g/fish/experiment)	9.7[a] ± 0.17	9.5[ab] ± 0.12	9.3[ab] ± 0.15	9.2[b] ± 0.14
Weight gain (%)	276.96[a] ± 5.49	266.15[ab] ± 6.65	251.65[bc] ± 14.59	236.35[c] ± 9.28
SGR (%/d)	2.37[a] ± 0.03	2.32[ab] ± 0.03	2.24[bc] ± 0.07	2.16[c] ± 0.05
FCR	1.07[a] ± 0.01	1.08[ab] ± 0.02	1.13[bc] ± 0.05	1.19[c] ± 0.03
PER	2.84[a] ± 0.04	2.80[a] ± 0.05	2.75[a] ± 0.13	2.55[b] ± 0.06
PPV	43.78a ± 1.59	41.70a ± 3.25	40.74a ± 1.72	37.88a ± 2.16
Protein digestibility (%)	92.02[a] ± 0.47	91.01[ab] ± 0.18	89.92[bc] ± 1.31	89.59[c] ± 0.45
Dry matter digestibility (%)	51.36[a] ± 2.91	49.85[a] ± 2.43	50.92[a] ± 2.63	50.75[a] ± 3.47

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Fig. 5.3 Growth and feed performance of *Oreochromis niloticus* (L.) fed graded levels of condensed tannin.
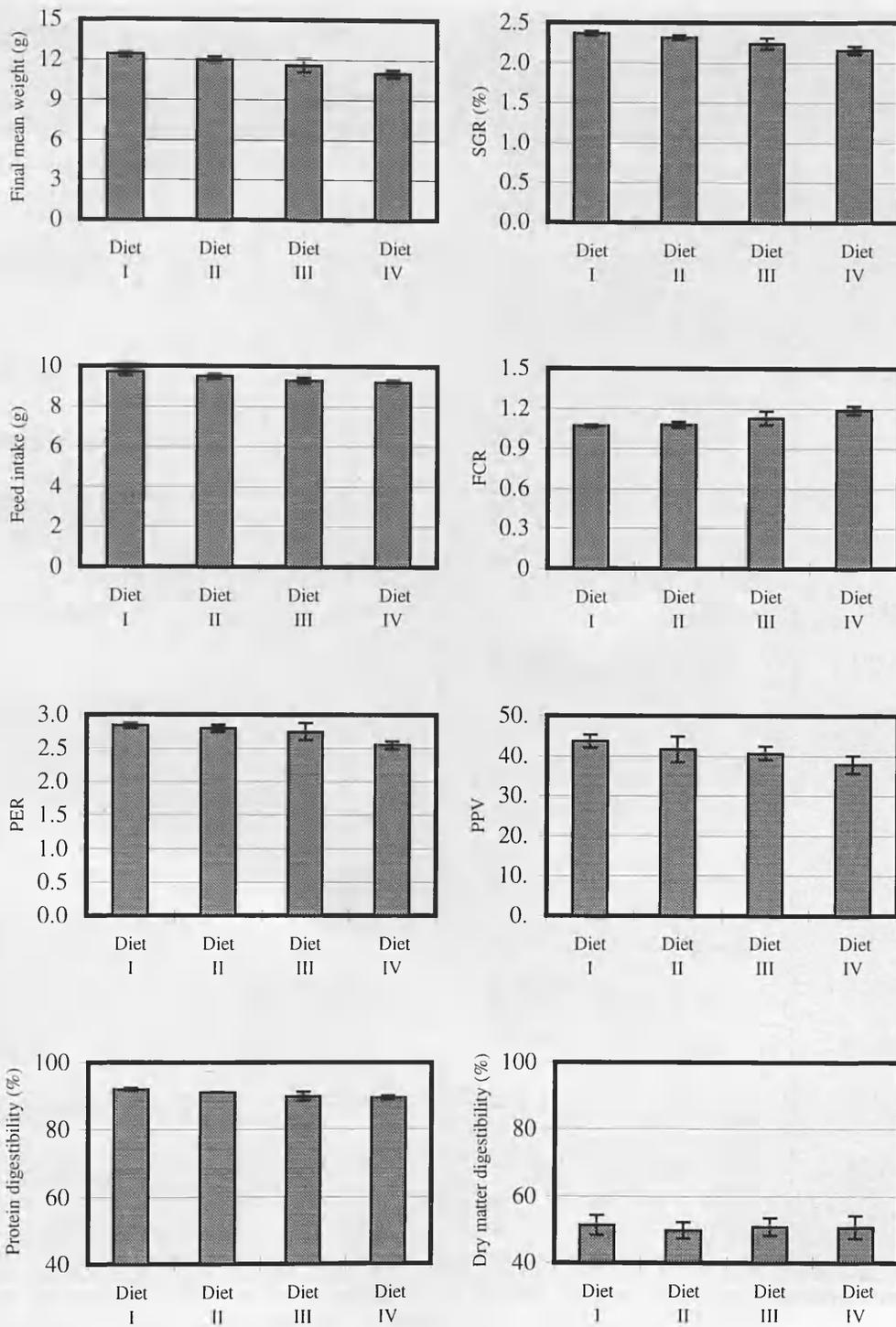
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.06,0.16,0.73 and 1.66% catechin respectively.

Table 5.3 Growth and feed performance of *Clarias gariepinus* (B.) fed graded levels of condensed tannin.

Parameters	Diet I (0.06%)	Diet II (0.16%)	Diet III (0.73%)	Diet IV (1.66%)
Initial mean wt (g)	5.0[a] ± 0.02	5.0[a] ± 0.02	5.0[a] ± 0.01[a]	5.0[a] ± 0.03
Final mean wt (g)	19.6[a] ± 0.07	16.8[b] ± 0.95	16.1[b] ± 0.18	14.0[c] ± 0.50
Feed intake (g/fish/experiment)	13.0[a] ± 0.10	11.5[b] ± 0.35	11.1[b] ± 0.40	9.4[c] ± 0.29
Weight gain (%)	293.78[a] ± 1.00	236.29[b] ± 17.97	223.37[b] ± 3.69	182.12[c] ± 10.29
SGR (%/d)	2.45[a] ± 0.01	2.16[b] ± 0.09	2.09[b] ± 0.02	1.85[c] ± 0.07
FCR	0.89[a] ± 0.01	0.98[b] ± 0.04	0.99[b] ± 0.03	1.03[b] ± 0.03
PER	3.43[a] ± 0.04	3.10[b] ± 0.15	3.12[b] ± 0.11	2.92[b] ± 0.08
PPV	55.86[a] ± 0.96	51.47[a] ± 3.24	52.79[a] ± 2.24	50.93[a] ± 4.44
Protein digestibility (%)	95.45[a] ± 0.40	94.95[a] ± 0.13	94.81[a] ± 0.91	91.31[b] ± 1.13
Dry matter digestibility (%)	60.77[a] ± 2.81	60.77[a] ± 2.35	59.27[a] ± 1.02	59.68[a] ± 1.20

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Fig. 5.4 Growth and feed performance of *Clarias gariepinus* (B.) fed graded levels of condensed tannin.
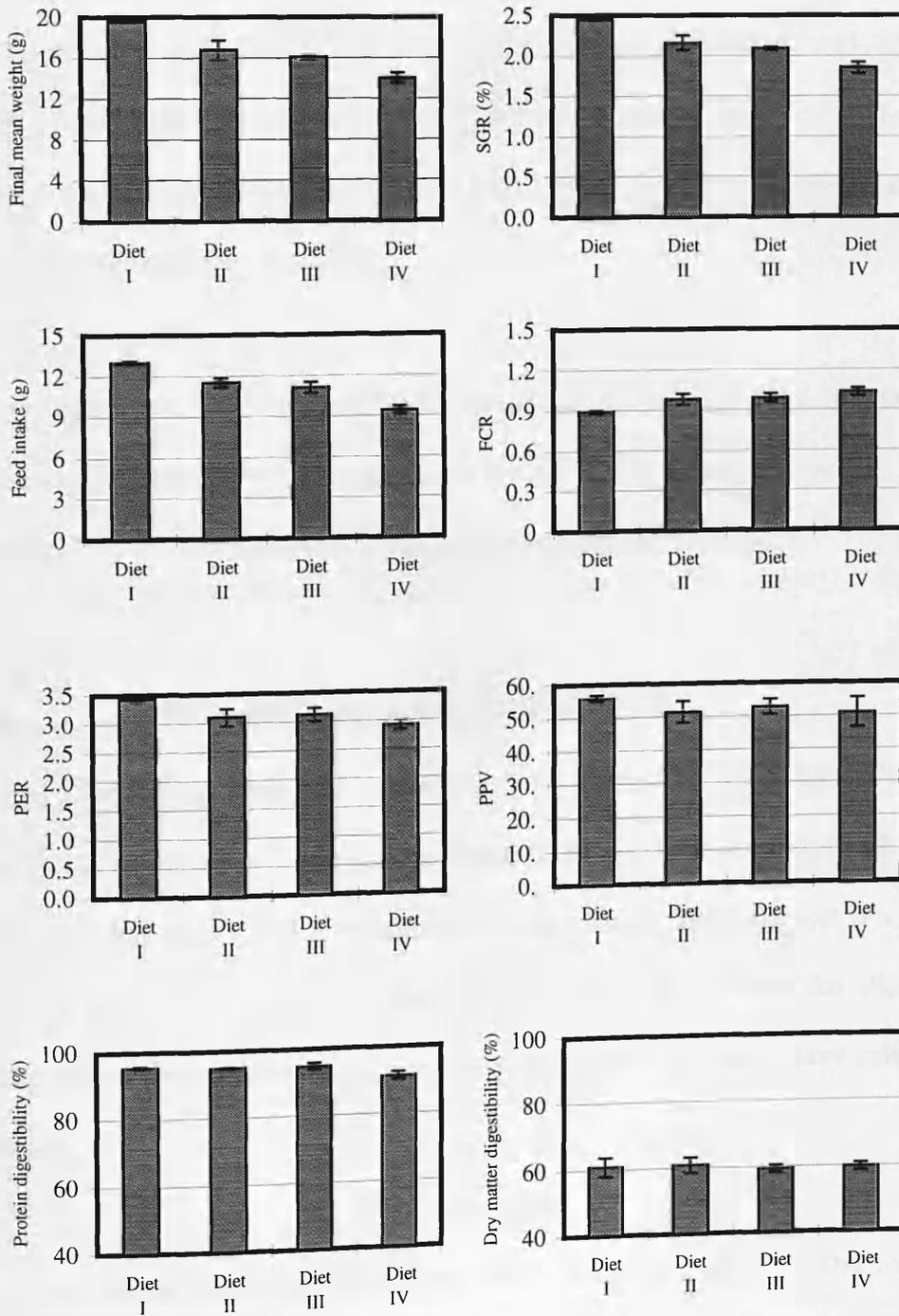
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.06, 0.16, 0.73 and 1.66% catechin respectively.

tended to increase (sometimes significantly $F = 1.10$, d.f.$= 11-3$ and $F = 12.84$, d.f.$= 11-3$, $P < 0.05$) as the level of dietary catechin increased.

PER ranged from 2.55 to 2.84 and from 2.92 to 3.43 for Nile tilapia and African catfish respectively being highest ($F = 1.64$, d.f.$= 11-3$ and $F = 13.08$, d.f.$= 11-3$, $P < 0.05$) for diet I and lowest for diet IV. PER values tended to decrease as the level of dietary catechin increased.

PPV ranged from 37.88 to 43.78 and from 50.93 to 55.86 for Nile tilapia and African catfish respectively being highest for diet I and lowest for diet IV. PPV values tended to decrease as the level of dietary catechin increased.

5.3.2   Protein and Dry matter Digestibility

Protein digestibility data are summarised in Tables 5.2 and 5.3. Protein digestibility values were high, ranging from 89.59 to 92.02% and from 91.31 to 95.45% for Nile tilapia and African catfish respectively being highest ($F = 6.68$, d.f.$= 11-3$ and $F = 19$, d.f.$= 11-3$, $P < 0.05$) for diet I and lowest for diet IV. Protein digestibility values tended to decrease as the level of dietary catechin increased.

Dry matter digestibility data are summarised in Tables 5.2 and 5.3. Dry matter digestibility for all diets was similar and there were no significant differences ($F = 0.15$, d.f.$= 11-3$ and $F = 0.44$, d.f.$= 11-3$, $P < 0.05$) between diets.

5.3.3   Body Composition

Body composition data are summarised in Tables 5.4 and 5.5. There were no significant (F= 0.44, d.f.= 11-3, F= 0.32, d.f.= 11-3, F= 0.65, d.f.= 11-3 and F= 0.25, d.f.= 11-3 for Nile tilapia and F= 0.84, d.f.= 11-3, F= 0.69, d.f.= 11-3, F= 0.51, d.f.= 11-3 and F= 2.09, d.f.= 11-3 for African catfish P< 0.05) differences between the four diets in terms of body moisture, protein level, lipid contents and ash contents of either species.

5.3.4 Histological Examination

Histological examination of intestines and livers of fish fed the experimental diets showed that catechin at all levels had no effect on the examined tissues. In all tested fish intestine was well developed with mucosal epithelium folding and no apparent degeneration or vacuolation. Cells were full of cytoplasm and there was no sign of hypertrophy. Cells were columnar in shape and the nuclei were in the base of cells as usual. Striated borders were well defined and goblet cells were scattered between the epithelial cells. The structure of the *lamina propria* was normal and there was no sign of infiltration.

Sections of livers also showed no differences between treatments. Hepatic cells were well defined in shape and nuclei were near to the cell wall. Cells were well organised and there was no sign of shrinkage or cell wall breakage.

Table 5.4 Body composition of *Oreochromis niloticus* (L.) fed graded levels of condensed tannin.

%	Initial fish	Diet I (0.06%)	Diet II (0.16%)	Diet III (0.73%)	Diet IV (1.66%)
Moisture	70.96	73.24[a] ± 1.13	73.83[a] ± 1.60	74.28[a] ± 0.82	73.91[a] ± 0.75
Crude protein	15.11	15.35[a] ± 0.27	15.00[a] ± 0.90	14.94[a] ± 0.56	14.98[a] ± 0.38
Crude lipid	9.51	7.33[a] ± 0.87	7.03[a] ± 0.60	6.67[a] ± 0.18	6.97[a] ± 0.46
Ash	4.41	4.08[a] ± 0.08	4.15[a] ± 0.15	4.11[a] ± 0.13	4.14[a] ± 0.06

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

Table 4.5 Body composition of *Clarias gariepinus* (B.) fed graded levels of condensed tannin.

%	Initial fish	Diet I (0.06%)	Diet II (0.16%)	Diet III (0.73%)	Diet IV (1.66%)
Moisture	75.99	71.84[a] ± 0.20	71.92[a] ± 0.96	71.42[a] ± 0.21	71.22[a] ± 0.81
Crude protein	14.20	15.77[a] ± 0.10	15.91[a] ± 0.54	16.08[a] ± 0.30	16.33[a] ± 0.79
Crude lipid	7.25	8.86[a] ± 0.28	8.64[a] ± 0.38	8.92[a] ± 0.50	8.63[a] ± 0.24
Ash	2.56	3.52[a] ± 0.05	3.53[a] ± 0.20	3.58[a] ± 0.10	3.83[a] ± 0.27

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($p < 0.05$).

5.4   Discussion

5.4.1   Growth and Feed Performance

Performance of both species fed the experimental diets supplemented with various levels of condensed tannin was generally inferior to that of the fish fed the control diet. Fish generally demonstrated decreasing rates of growth and poorer feed utilisation efficiency as a result of increasing levels of catechin in the experimental diets. African catfish was apparently more affected by feeding of catechin than Nile tilapia. It would appear that catechin may affect growth of fish in two ways: it's astringent taste may affect palatability and decrease feed consumption and it may affect protein utilisation by decreasing protein digestibility.

African catfish fed diet IV consumed only 72% as much as the fish fed diet I and grew significantly less (F= 71.20, d.f.= 11-3, P < 0.05). It is possible that the bitter taste of catechin was the reason behind this as it has been reported that condensed tannins are strongly astringent (Salunkhe et al., 1990). However, Nile tilapia were not as affected by the taste of catechin; although fish fed diet IV consumed only 95% as much as fish fed diet I and thereafter grew significantly (F= 7.75, d.f.= 11-3, P < 0.05) less. It is believed that the growth reduction in Nile tilapia fed the experimental diets was not due to reduced feed intake as a result of feeding unpalatable diets, but may have been associated with poor feed utilisation as all offered feed was consumed completely. However, the growth reduction in African catfish could be a result of both reduced feed intake and poor feed utilisation (Table 5.3). Therefore, it would

appear that the effect of condensed tannin on the palatability of the diet is species dependent.

The literature contains many contradictory reports on the palatability of condensed tannins. A comparative study was performed by (Elkin and Rogler, 1990) to test the effect of low (0.01%) and high (5.6%) sorghum grain condensed tannin on feed intake of ducks, chicks and rats. These authors found that feed intake was depressed in chicks and rats fed high levels of sorghum grain tannin whereas feed intake of ducks was not affected. Moreover, weight gain was significantly greater in ducks fed high tannin as compared with ducks fed low tannin. Ravindran and Sivakanesan (1996) found that condensed tannin of mango seed kernels at level of 0.56% was responsible for feed intake depression in chicks. However, a level of 0.67% of faba bean condensed tannin was not enough to depress feed intake in chicks (Campbell and Marquardt, 1977). Results of the present study showed that 0.73% of catechin was enough to affect feed intake in African catfish but not in Nile tilapia. It seems that different sources of condensed tannins produce variable effects in different species of animals and even the same source may result in species dependent responses.

Based on the growth performance presented in Tables 5.2 and 5.3 it is noted that even 0.73% catechin in the diet depresses growth of both species significantly while 0.16% catechin depressed growth of African catfish alone. Similar growth depressing effects of feeding 0.8% faba bean condensed tannin were observed in chicks (Ortiz et al., 1994). However, Jansman and Longstaff

(1993) reported that tannins do not cause negative effects on animal performance when the dietary content does not exceed 0.3% catechin equivalent. This suggestion was based on their observation of lack of effect on growth performance of chicks fed diets with either 300 g/kg of low or high tannin faba beans.

Mortality was not observed during the period of this study as result of feeding dietary catechin. This result is in disagreement with Ortiz et al. (1994) who found a high mortality rate in one day old chicks fed diets containing 8 and 16 g/kg faba bean condensed tannin. Several reasons might explain such discrepancy: firstly, fish and chicks are different species related to different animal classes which, in turn, might have different metabolic functions; secondly, they are at different ages and weights and the most important reason that each species consumed different type of condensed tannins.

The effect of isolated dietary condensed tannin has not previously been tested in fish. However, products or by-products of plant proteins, in which tannins are present at different levels, are used commonly in fish diets. Hossain and Jauncey (1989) evaluated mustard oil cake as a fish meal substitute in diets for common carp *Cyprinus carpio* at inclusion rates of 305 and 610 g/kg. They observed low growth rates and poorer feed conversion ratios in fish fed these diets compared to those of fish fed a control diet. They concluded that the presence of tannins in mustard oil cake may have partially contributed to the poor growth responses. Similar results have been reported in *Catla catla, Labeo*

rohita, *Cirrhinus mrigala, Cyprinus carpio* and *Puntius gonionotus* fed diets containing mustard oil cake at a rate of 438 g/kg (Mazid et al., 1997). Capper et al. (1982) also found that mustard meal, included at 20% in feed for common carp *Cyprinus carpio* fingerlings, resulted in reduced weight gain and poor feed conversion.

In studies with common carp *Cyprinus carpio* Hasan et al. (1997) found that levels of 19.72% and above of sesame meal in the diet resulted in depressed growth and feed utilisation. Similar results have been also been reported by Hossain and Jauncey (1989). Both of these studies suggested phytic acid as the cause of the reduction in growth and feed utilisation. However, phytic acid was not the sole antinutrient factor present in sesame meal; condensed tannin is another factor involved (Odumodu, 1992) which may partially be responsible for such depressed growth and feed utilisation. According to Odumodu (1992), sesame meal contains 8.25 mg/g condensed tannin. Based on such levels the results of this study are in agreement with those of Hossain and Jauncey (1989) and Hasan et al. (1997). At a level of 19.72% sesame meal with a condensed tannin content of 8.25 mg/g the diet would contain 1.63 g/kg condensed tannin, similar to diet II in this study.

Previous studies (Rowan and Lawrence, 1986a,b) suggested that rapeseed meal gave poorer growth in pigs than soy bean meal on account of differences in availability of amino acids. However, Jackson et al. (1982) reported that rapeseed meal has a relatively a good amino acid profile, comparable to that of

soy bean meal. It is notable that rapeseed meal contains (0.59%) higher concentrations of tannins than soy bean (0.1%) (Terrill et al., 1992).

5.4.2   Protein and Dry matter Digestibility

Protein digestibility appeared to be inversely related to the level of catechin in the diet (Tables 5.2 and 5.4). This negative interaction might be explained either by a direct binding of condensed tannin to protein (dietary and/or enzymic in origin) (Longstaff and McNab, 1991) and/or by increased secretion of endogenous proteins (Marquardt, 1989).

Hagerman et al. (1992) suggested that the chemical structure of condensed tannins, flavan-3-ols, has a substantial effect on the activity of tannins in which condensed tannins would decrease protein and dry matter digestibility. Condensed tannins interact with proteins, either enzymes or substrate, and form strong insoluble complexes (Griffiths, 1991). Such complexes have large sizes, high resistance to hydrolysis and are unlikely to be absorbed from the intestines (Hagerman et al., 1992; MacCoubrey et al., 1997). In this study, addition of catechin to the experimental diets reduced protein digestibility in both species suggesting that a reaction between catechin and protein may have occurred. The reduction of protein digestibility in Nile tilapia was more pronounced than that in African catfish.

A number of *in vitro* protein digestibility studies are in general agreement with the results of the present study. Addition of condensed tannin, extracted from

cowpea, to casein solution at rate of 0.4 mg/ml decreased protein digestibility by 5.1% (Laurena et al., 1984). Similar results have been reported when isolated condensed mung bean tannin was added to aqueous protein (6.25 mg/ml) at rate of 0.4 mg/ml (Barroga et al., 1985). Moreover, a diet containing qubracho (condensed tannin) at a level of 2% has been shown to reduce protein digestibility in prairie voles (Lindroth and Batzli, 1984) and singing voles (Robbins et al., 1991).

Several studies have reported that inclusion of plant protein sources in fish diets results in reduced protein and dry matter digestibility. A proportional reduction in protein and dry matter digestibility in common carp *Cyprinus carpio* was noted as a result of feeding different levels of mustard oil cake and sesame meal (Hossain and Jauncey, 1989). Similar results have been reported (Hilton and Slinger, 1986) with rainbow trout *Salmo gairdneri* fed different levels of canola meal. It is possible that the presence of condensed tannins in such sources is, in part, the reason behind such reductions in digestibility.

However, contradictory results were reported by Hasan et al. (1997) where common carp *Cyprinus carpio* fed different levels of sesame meal showed increased protein digestibility with increased sesame meal inclusion.

It has been widely reported that condensed tannins increase excretion of endogenous proteins in terrestrial animals (Marquardt, 1989; Shahkhalili et al., 1990; Jansman and Longstaff, 1993). These endogenous proteins may come

from increased mucosal cells or from proline-rich salivary proteins that are produced by the parotid glands as a mechanism of defence against the ingestion of tannins (Bravo et al., 1994). Tannin will react with these proteins and form indigestible complexes which in turn pass through the intestine and are excreted in the faeces (Alzueta et al., 1992). Such a reaction will cause hypersecretion of epithelial cells and removal of mucus which in turn causes irritation and tissue breakdown in the alimentary canal (Salunkhe et al., 1990).

In this study there was probably no loss of endogenous proteins through the mechanisms reported in terrestrial animals for two reasons: fish do not have salivary glands in their oral cavity (Steffens, 1989); therefore, it is unlikely there was an interaction between condensed tannin and salivary proteins; in addition, there were no abnormalities observed during histological examination of the gut. Striated borders and the structure of goblet cells (mucus producers) in all fish fed the experimental diets were normal which gives an indication that there was no loss of endogenous glycoproteins. Therefore, it is suggested that the negative effect of catechin on protein digestibility of fish was mainly due to either forming insoluble complexes and/or inhibition of enzymes activity.

Dry matter digestibility in both species (Tables 5.2 and 5.3) was not affected by the addition of catechin; although dry matter digestibility in African catfish was higher than in Nile tilapia. It is reported that fibre content in the diet is a limiting factor in dry matter digestibility and that a high content of fibre (> 8%)

will decrease the digestibility of dry matter (Hilton et al., 1983; Hilton and Slinger, 1986; McGoogan and Reigh, 1996). Crude fibre contents of all diets in this study were similar (10%) and higher than that recommended. However, the results obtained from this study were comparable to those reported for sorghum grain in red drum *Sciaenops ocellatus* (McGoogan and Reigh, 1996), mustard oil cake and sesame meal in common carp *Cyprinus carpio* (Hossain and Jauncey, 1989), lupin seed meal, pea seed meal and faba bean meal in rainbow trout *Oncorhynchus mykiss* (Gomes et al., 1995) and canola meal in rainbow trout *Salmo gairdneri* (Hilton and Slinger, 1986) in which crude fibre contents were in the range of 4.6 -7.8%.

5.4.3   Body Composition and Histological Examination

The results of the present study indicate that diets containing catechin at different levels did not have any effect in terms of dry matter, crude protein, ether extract and ash content of carcasses. No published data are available on the effect of feeding condensed tannins on body composition of fish. However, results obtained from this study are comparable to those reported by Gatlin et al. (1992) for channel catfish *Ictalurus punctatus* and Wee and Ngamsnae (1987) for carp tawes *Puntius gonionotus* in which fish were fed casein-gelatin and casein-fishmeal based diets respectively. Keembiyehetty and de Silva (1993) fed juvenile *Oreochromis niloticus* cowpea and black gram at rates of 50 and 56% of the diet respectively. They reported carcass composition of fish similar to the

results of the present study in terms of moisture and protein. However, lipid content of fish was higher than that reported here. It would appear that the dietary lipid level (12.2 and 14.5%), which reported by Keembiyehetty and de Silva (1993) compared to 10% reported here, is the reason for such a difference. NRC (1983) found that as dietary lipid levels increased, total body lipid also increased. Commonly, carcass composition of the fish is influenced by the composition of the diet (Buentello and Gatlin, 1997). Diets in this study were formulated to be isoenergetic and isoproteic; therefore, similar carcass compositions were likely.

The results of histological examination of intestine and liver sections of all treatments showed no differences. It seems that condensed tannin in the form of catechin does not cuase any alteration or abnormality in intestines or livers of Nile tilapia or African catfish at the levels tested under this experimental conditions. However, such observations are in disagreement with Ortiz et al. (1994) who observed structural abnormalities in the intestine and liver of chicks fed extracted faba bean condensed tannin at rate of 8 and 16 g/kg. The intestinal mucosal surface of the chicks showed atrophy and villi shortening, distortion of their architecture and hypertrophy of goblet cells. Liver showed an extensive vacuolisation of hepatocytes. Similar observations to that of Ortiz et al. (1994) have been reported in chicks fed raw faba bean at a rate of 50% of their diet (Rubio et al., 1990).

CHAPTER VI

THE EFFECT OF DIETARY HYDROLYSABLE AND CONDENSED TANNINS ON DIGESTIVE ENZYMES OF *OREOCHROMIS NILOTICUS* (L.) AND *CLARIAS GARIEPINUS* (B.) FINGERLINGS

6.1   Introduction

It has been reported that the presence of tannin in sorghum grains, field beans and carob contributes considerably to a decrease in the digestibility of dietary protein (Chang and Fuller, 1964; Tamir and Alumot, 1970; Rostagno et al., 1973; Ramachandra et al., 1977; Marquardt, 1989; Longstaff and McNab, 1991) and starch (Howard and Yudkin, 1963; Hibberd et al., 1985; Longstaff and McNab, 1991). The most likely explanation for this effect has been claimed to be a reduction in the activities of trypsin and α-amylase in the digestive tract (Howard and Yudkin, 1963; Griffiths and Moseley, 1980; Horigome et al., 1988; Griffiths, 1991).

However, contradictory results have been reported concerning the effect of tannin on lipase activity. Marquardt et al. (1977) reported that fat retention increased when young chicks were fed on a diet containing extract of condensed tannin from faba bean hull. Griffiths and Moseley (1980) and Horigome et al. (1988) reported that the activity of lipase increased in rats fed a diet containing high tannin field bean hulls and condensed tannin isolates from the leaves of fodder plants respectively.

Results from Chapter IV and V have shown that growth performance of fish was decreased when tannic acid (hydrolysable tannin) or catechin (condensed tannin) were incorporated at levels ≥ 0.08 or 0.16% in diets of Nile tilapia and African catfish respectively. It has been suggested (Chapter IV and V) that the decrease is a consequence of low protein digestibility in fish. It is hypothesised

in this study that the poor growth performance and low protein digestibility found in Chapter IV and V are, in part, a result of inhibition of digestive enzymes. To investigate this hypothesis, four experiments were conducted to evaluate the influence of tannin (hydrolysable and condensed) on the digestive enzymes of Nile tilapia and African catfish.

6.2   Materials and Methods

6.2.1   Experimental System

The experimental system described in section 2.1 was used to conduct two separate experiments to evaluate the effect of hydrolysable tannin on digestive enzymes of fish.

6.2.2   Experimental Fish

Oreochromis niloticus and *Clarias gariepinus* as described in section 2.2 were used as models in this study.

6.2.3   Experimental Diets

The composition of the experimental diets and their proximate analysis are shown in Tables 4.1 and 5.1. Diet formulation and preparation were as described in sections 2.3.1 and 2.3.2.

6.2.4   Experimental Practice

Fish acclimation to casein and gelatin based diets and the feeding regime were as described in sections 2.4.1 and 2.4.3.

6.2.5   Experimental Analyses

Proximate analysis (moisture, protein, lipid, fibre, ash and energy) of diets was as described in sections 2.5.1.1, 2.5.1.2, 2.5.1.3, 2.5.1.4, 2.5.1.5 and 2.5.1.7. Hydrolysable tannin and condensed tannin were determined according to the methods described in sections 2.5.1.9 and 2.5.1.10. Collection of digestive tracts, enzyme extraction and enzyme assays were as described in section 2.7.

6.2.7   Statistical Analysis

Statistical analyses were carried out as described in section 2.8.

6.3   Results

6.3.1   Hydrolysable tannin (tannic acid)

The activities of pepsin, protease, trypsin, lipase and α-amylase of the intestine of fish fed the experimental diets are presented in Tables 6.1 and 6.2. Tannic acid had no effect on the activity of lipase, while it considerably, but not significantly (F= 1.03, d.f.= 11-3, F= 1.46, d.f.= 11-3 and F= 1.95, d.f.= 11-3, P< 0.05), lowered the activities of pepsin, protease and trypsin and slightly and significantly (F= 7.25, d.f.= 11-3, P< 0.05) lowered α-amylase activity in Nile tilapia. In African catfish, tannic acid had no significant (F= 1.93, d.f.= 11-3, P<

0.05) effect on α-amylase activity, while it significantly (F= 9.37, d.f.= 11-3, F= 3.97, d.f.= 11-3, F= 9.37, d.f.= 11-3 and F= 7.92, d.f.= 11-3, P< 0.05) lowered the activities of pepsin, protease, trypsin and lipase.

6.3.2   Condensed tannin (catechin)

The activities of pepsin, protease, trypsin, lipase and α-amylase in the intestine of fish fed the experimental diets are presented in Tables 6.3 and 6.4. Catechin had no effect on lipase activity while it significantly (F= 5.64, d.f.= 11-3, F= 8.25, d.f.= 11-3, F= 13.35, d.f.= 11-3 and F= 8.69, d.f.= 11-3, P< 0.05) lowered the activities of pepsin, protease, trypsin and α-amylase in Nile tilapia. In African catfish, catechin lowered the activities of pepsin and trypsin, but not significantly (F= 1.14, d.f.= 11-3 and F= 1.55, d.f.= 11-3, P< 0.05), and it lowered the activities of protease, lipase and α-amylase significantly (F= 13.02, d.f.= 11-3, F= 4.75, d.f.= 11-3 and F= 5.64, d.f.= 11-3, P< 0.05).

6.4   Discussion

It would appear that the presence of tannin (in the form of tannic acid or catechin) in the diets of Nile tilapia and African catfish affects digestive enzyme activity. Results from the present study are partly in agreement with studies in rats (Griffiths and Moseley, 1980; Horigome et al., 1988; Lizardo et al., 1995) where condensed tannins from field bean, sorghum grains and leaves in fodder plants were shown to inhibit the activities of trypsin and α-amylase, but they differ in their effect on lipase. Longstaff and McNab (1991) reported results comparable to the results of the present study in which a decrease in

Table 6.1. Digestive enzyme activities in *Oreochromis niloticus* fed graded levels of dietary hydrolysable tannin (tannic acid)

Enzymes	Diet I 0.05%	Diet II 0.08%	Diet III 0.27%	Diet IV 0.71%
Pepsin (EC 3.4.23.1)[1]	115.96[a] ±17.27	104.48[a] ±40.94	82.78[a] ±23.29	78.95[a] ±33.38
Protease[2]	40.22[a] ±1.26	38.37[a] ±17.99	37.31[a] ±0.62	23.86[a] ±10.83
Trypsin (EC 3.4.21.4)[3]	1438.4[a] ±70.7	1415.6[a] ±7.7	1387.3[a] ±44.3	1360.0[a] ±11.5
Lipase (EC 3.1.1.3)[4]	6.33[a] ±0.79	6.04[a] ±0.25	5.91[a] ±0.28	5.99[a] ±0.08
αAmylase (EC 3.2.1.1)[5]	30.19[a] ±1.25	28.74[ab] ±0.97	27.97[ab] ±0.36	26.98[b] ±0.64

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$).

1- Pepsin activity was expressed as the amount of tyrosine (ηg) liberated by 200 µl of enzyme solution at pH 2 per min at 30 ^{0}C.

2- Protease activity was expressed as the amount of protein (µg) digested by 0.5 ml of enzyme solution at pH 7.6 per min at 30 ^{0}C.

3- Trypsin activity was expressed as the amount of *p*-nitroaniline (ηg) liberated by 0.5 ml of enzyme solution at pH 8.2 per min at 30 ^{0}C.

4- Lipase activity was expressed as the amount of fatty acids (Sigma/Tietz unit/L) liberated by 1 ml of extracted enzyme solution per min at 30 ^{0}C.

5- α-amylase activity was expressed as the amount of maltose (µg) liberated by 200 µl of enzyme solution at pH 7.0 per min at 30 ^{0}C.

Fig. 6.1　Digestive enzyme activities in *Oreochromis niloticus* fed graded levels of dietary hydrolysable tannin (tannic acid)

Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.05, 0.08, 0.27 and 0.71% tannic acid.

Table 6.2. Digestive enzyme activities in *Clarias gariepinus* fed graded levels of dietary hydrolysable tannin (tannic acid).

Enzymes	Diet I 0.05%	Diet II 0.08%	Diet III 0.27%	Diet IV 0.71%
Pepsin (EC 3.4.23.1)[1]	137.66[a] ± 13.45	101.92[b] ± 10.13	99.37[b] ± 25.50	71.29[b] ± 3.83
Protease[2]	101.20[a] ± 1.59	90.18[ab] ± 12.85	89.36[ab] ± 10.16	78.01[b] ± 0.72
Trypsin (EC 3.4.21.4)[3]	1733.3[a] ± 34.6	1541.6[ab] ± 213.8	1283.8[b] ± 210.8	1222.7[b] ± 48.5
Lipase (EC 3.1.1.3)[4]	2.02[a] ± 0.28	1.48[b] ± 0.36	1.30[b] ± 0.12	1.11[b] ± 0.12
αAmylase (EC 3.2.1.1)[5]	22.00[a] ± 2.49	21.86[a] ± 2.79	19.54[a] ± 1.63	18.23[a] ± 2.08

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$).

1- Pepsin activity was expressed as the amount of tyrosine (ηg) liberated by 200 µl of enzyme solution at pH 2 per min at 30 °C.

2- Protease activity was expressed as the amount of protein (µg) digested by 0.5 ml of enzyme solution at pH 7.6 per min at 30 °C.

3- Trypsin activity was expressed as the amount of *p*-nitroaniline (ηg) liberated by 0.5 ml of enzyme solution at pH 8.2 per min at 30 °C.

4- Lipase activity was expressed as the amount of fatty acids (Sigma/Tietz unit/L) liberated by 1 ml of extracted enzyme solution per min at 30 °C.

5- α-amylase activity was expressed as the amount of maltose (µg) liberated by 200 µl of enzyme solution at pH 7.0 per min at 30 °C.

Fig. 6.2. Digestive enzyme activities in *Clarias gariepinus* fed graded levels of dietary hydrolysable tannin (tannic acid).
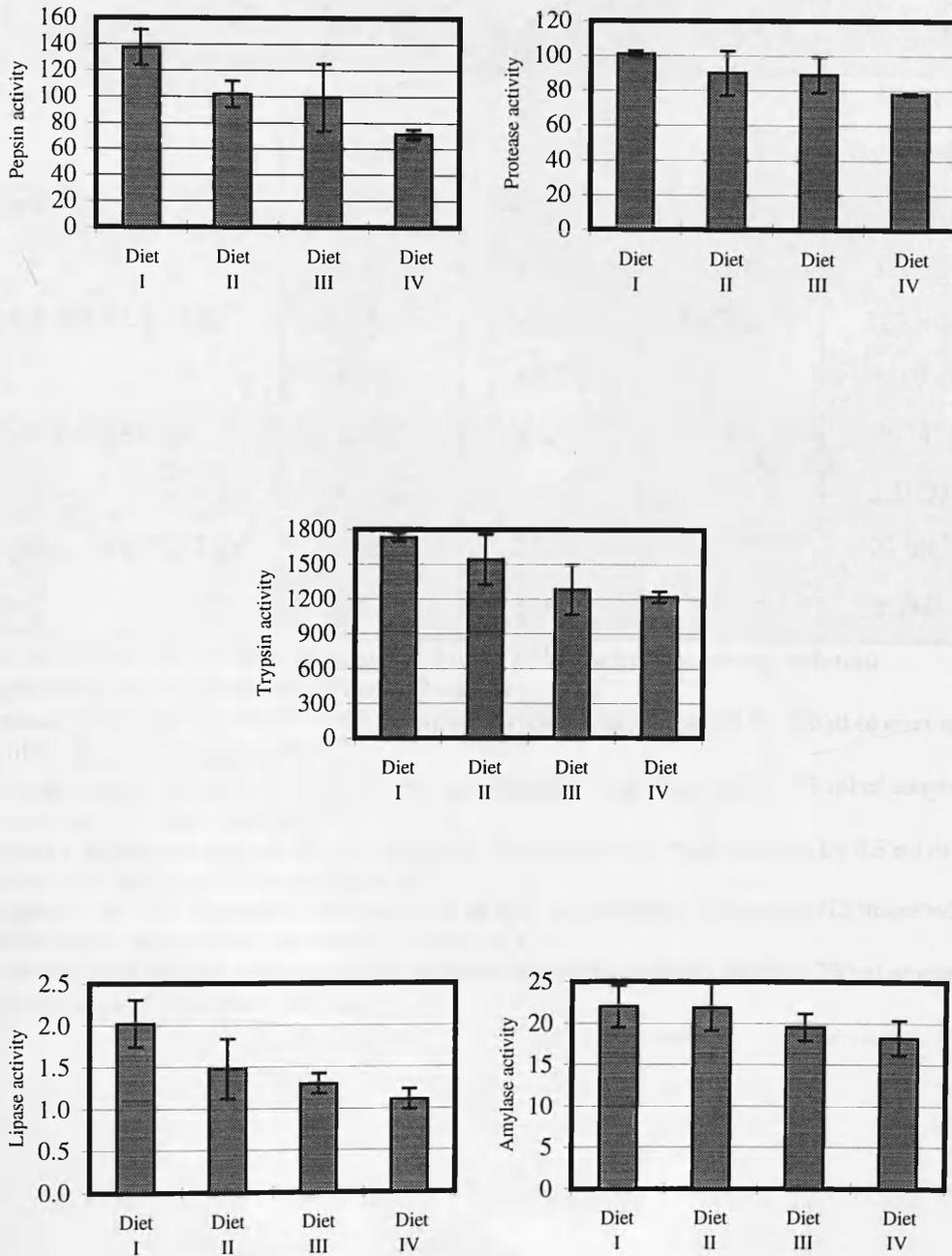
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.05, 0.08, 0.27 and 0.71% tannic acid.

Table 6.3   Digestive enzyme activities in *Oreochromis niloticus* fed graded levels of dietary condensed tannin (catechin)

Enzymes	Diet I 0.06%	Diet II 0.16%	Diet III 0.73%	Diet IV 1.66%
Pepsin (EC 3.4.23.1)[1]	114.69[a]	86.61[ab]	85.33[b]	47.04[b]
	± 18.09	± 31.34	± 4.42a	± 17.68
Protease[2]	44.38[a]	41.87[ab]	40.47[b]	39.94[b]
	± 1.52	± 0.80	± 0.62	± 1.55
Trypsin (EC 3.4.21.4)[3]	1390.2[a]	1320.0[a]	1258.7[bc]	1236.0[c]
	± 12.4	± 61.1	± 4.8	± 19.6
Lipase (EC 3.1.1.3)[4]	6.03[a]	6.25[a]	6.15[a]	6.04[a]
	± 0.21	± 0.05	± 0.13	± 0.20
αAmylase (EC 3.2.1.1)[5]	25.03[a]	24.10[a]	23.57[ab]	21.94[b]
	± 1.01	± 0.78	± 0.55	± 062

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$).

1- Pepsin activity was expressed as the amount of tyrosine (ηg) liberated by 200 μl of enzyme solution at pH 2 per min at 30 °C.

2- Protease activity was expressed as the amount of protein (μg) digested by 0.5 ml of enzyme solution at pH 7.6 per min at 30 °C.

3- Trypsin activity was expressed as the amount of *p*-nitroaniline (ηg) liberated by 0.5 ml of enzyme solution at pH 8.2 per min at 30 °C.

4- Lipase activity was expressed as the amount of fatty acids (Sigma/Tietz unit/L) liberated by 1 ml of extracted enzyme solution per min at 30 °C.

5- α-amylase activity was expressed as the amount of maltose (μg) liberated by 200 μl of enzyme solution at pH 7.0 per min at 30 °C.

Fig. 6.3 Digestive enzyme activities in *Oreochromis niloticus* fed graded levels of dietary condensed tannin (catechin).

Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.06, 0.16, 0.73 and 1.66% catechin.

Table 6.4   Digestive enzyme activities in *Clarias gariepinus* fed graded levels of dietary condensed tannin (catechin).

Enzymes	Diet I 0.06%	Diet II 0.16%	Diet III 0.73%	Diet IV 1.66%
Pepsin (EC 3.4.23.1)[1]	140.21 [a] ± 57.94	115.96 [a] ± 7.97	108.30 [a] ± 27.70	85.33 [a] ± 34.74
Protease[2]	102.44 [a] ± 1.54	93.93 [a] ± 6.55	77.56 [b] ± 11.22	67.90 [b] ± 7.30
Trypsin (EC 3.4.21.4)[3]	1459.6 [a] ± 253.3	1158.9 [a] ± 23.3	1115.8 [a] ± 123.9	1110.0 [a] ± 369.2
Lipase (EC 3.1.1.3)[4]	1.92 [a] ± 0.24	1.74 [ab] ± 0.23	1.58 [ab] ± 0.12	1.37 [b] ± 0.12
αAmylase (EC 3.2.1.1)[5]	21.38 [a] ± 2.86	20.31 [a] ± 0.59	20.43 [a] ± 0.26	16.64 [b] ± 085

Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$).

1- Pepsin activity was expressed as the amount of tyrosine (ηg) liberated by 200 µl of enzyme solution at pH 2 per min at 30 °C.

2- Protease activity was expressed as the amount of protein (µg) digested by 0.5 ml of enzyme solution at pH 7.6 per min at 30 °C.

3- Trypsin activity was expressed as the amount of *p*-nitroaniline (ηg) liberated by 0.5 ml of enzyme solution at pH 8.2 per min at 30 °C.

4- Lipase activity was expressed as the amount of fatty acids (Sigma/Tietz unit/L) liberated by 1 ml of extracted enzyme solution per min at 30 °C.

5- α-amylase activity was expressed as the amount of maltose (µg) liberated by 200 µl of enzyme solution at pH 7.0 per min at 30 °C.

Fig. 6.4 Digestive enzyme activities in *Clarias gariepinus* fed graded levels of dietary condensed tannin (catechin).
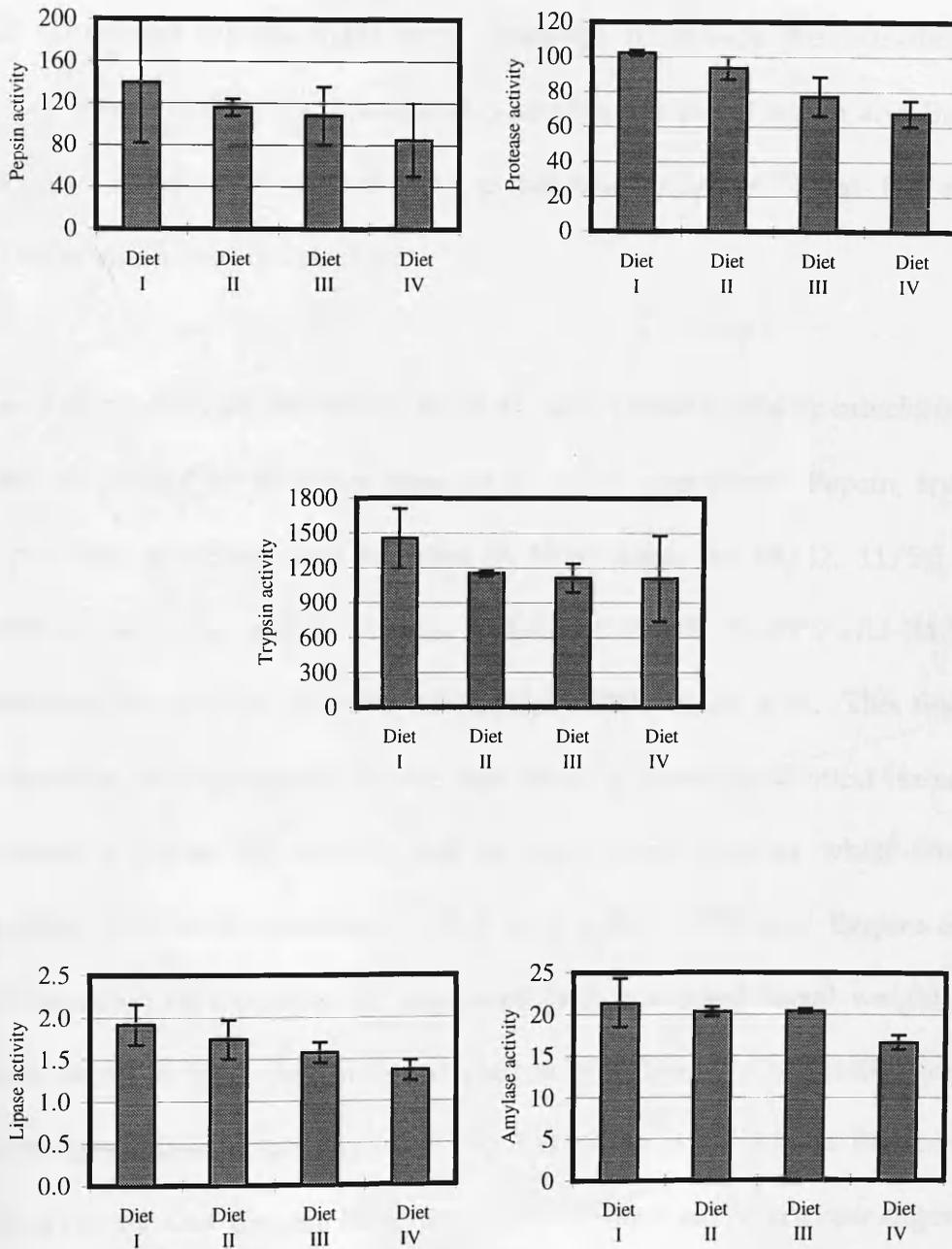
Bars are means ± SD of three replications. Diets I, II, III and IV contained 0.06, 0.16, 0.73 and 1.66% catechin.

the activities of trypsin, lipase and α-amylase were observed in chicks fed faba bean condensed tannin. Similar results to those observed in the present study were also reported by Tamir and Alumot (1969) in which hydrolysable tannins (from carob and digallic acid) were observed to reduce the activities of digestive enzymes (trypsin, lipase and α-amylase) in rat. Nyman and Bjorck (1989) also observed a reduction in proteolytic enzymes in rat fed diets containing tannic acid or catechin.

In the present study the inhibitory effect of tannin (tannic acid or catechin) was greater on proteolytic enzymes than on lipase or α-amylase. Pepsin, trypsin and protease activities were reduced in Nile tilapia by 59/32, 11/5.5 and 10/40% respectively, and in African catfish by 39/48, 24/29.5 and 34/23% respectively by addition of 1.66% catechin or 0.71% tannic acid. This finding may provide an explanation for the high level of protein excretion observed previously (Chapter IV and V) and in many other species when tannin-containing diets were consumed. Glick and Joslyn (1970) and Eggum et al. (1983) reported that tannins are associated with increased faecal weight and protein excretion and that such increase is correlated with a reduction in protein digestibility in rats. Similarly, Shahkhalili et al. (1990) and Bravo et al. (1994) reported that tannins have the ability to bind and inactivate digestive enzymes, an effect that induces a reduction of the intestinal digestion of dietary protein and of other nutrients in rats.

While neither tannic acid nor catechin greatly affected lipase activity in Nile tilapia, they both reduced lipase activity in African catfish. in African catfish the effect of tannic acid was slightly greater than that of catechin. These results are in disagreement with Griffiths and Moseley (1980) and Horigome et al. (1988) observed an increase in lipase activity in rats fed a diet containing tannin rich field bean hulls and condensed tannin extracts from the leaves of fodder plants. However, Nyman and Bjorck (1989) found decreased lipid digestibility as well as decreased lipase activity in rats fed diets containing tannic acid. It would appear that differences in lipase activity between species, as a result of feeding tannin containing diets, reflect differences in feeding habit.

α-amylase activity in both Nile tilapia and African catfish was inhibited by tannic acid and catechin and African catfish was more affected than Nile tilapia. Nitsan (1971) observed a reduction in α-amylase activity in rat fed whole faba bean compared with those fed dehulled bean. Similar results were reported by Nyman and Bjorck (1989) when they fed rats a diet containing tannic acid or catechin. Yuste et al. (1992) also reported that α-amylase activity and starch digestibility in chicks was negatively affected by proanthocyanidin (condensed tannin) extracted from faba bean.

It has been reported that the inhibitory effect on the activities of digestive enzymes is more marked with condensed tannin than with hydrolysable tannin (Tamir and Alumot, 1969). According to the results of the present study (Tables 6.1, 6.2, 6.3 and 6.4), the activities of pepsin, trypsin and α-amylase were more

affected by condensed tannin (catechin) than by hydrolysable tannin (tannic acid) in Nile tilapia, while the opposite was observed in African catfish in which pepsin, trypsin and lipase activities were more affected by hydrolysable tannin than by condensed tannin. There is no general trend in the present study agrees with the results of Tamir and Alumot (1969). It would appear that there is a difference in the tendency of tannic acid and catechin to inhibit different enzymes. Lipase activity in Nile tilapia was not affected either by tannic acid nor by catechin, while the opposite was observed in African catfish. The inhibitory effect probably depends on the affinity of specific tannin to inhibit specific enzymes from specific species.

It appears from the results of the present study (Tables 6.1, 6.2, 6.3 and 6.4) that pepsin activity in Nile tilapia is less efficient compared to pepsin activity of African catfish. This finding might be due differences in gastrointestinal physiology between the species. It is known that African catfish have a true stomach with two sphincters which separate the stomach from the oesophagus and intestine (Lovell, 1989; Steffens, 1989). This structure gives African catfish the advantage of holding feed in the stomach until sufficient pepsin with low pH (1.4-2) is secreted for complete hydrolysis and then material is released into intestine (Lovell, 1989). However, Nile tilapia posses a sac-like stomach (Jauncey and Ross, 1982) without sphincters (Lovell, 1989) in which feed may diverted to the intestine without complete hydrolysis by pepsin and in turn low nutrient digestibility is expected compared to African catfish.

In general, proteolytic activity (Tables 6.1, 6.2, 6.3 and 6.4) in African catfish is higher than in Nile tilapia, which may give an indication that African catfish has the ability to deal with diets containing high levels of protein more easily. The natural diet of African catfish consist of fish, crustaceans and molluscs (Weerd, 1995), whereas Nile tilapia depend on plant matter or detritus of plant origin (Jauncey and Ross, 1982). Therefore, it is possible that African catfish (and other carnivorous fish) are enzymaticly adapted to the high protein levels present in their natural diets. The results of the present study are in agreement with Hofer and Schiemer (1981) and Jonas et al. (1983) who reported that proteolytic activity is higher in carnivorous than in herbivorous fish.

In contrast to proteolytic enzymes, α-amylase activity in Nile tilapia is higher than in African catfish. This may indicate that Nile tilapia is more adapted to use carbohydrate more than African catfish. Bergot (1979) reported that carnivorous fish are ill equipped to utilise starch as an energy source in their diet. Cowey and Walton (1989) reported that the ability of fish to utilise starch as an energy source differs among species and appears to be rather limited among carnivores. Therefore, it is not surprising that common carp *Cyprinus carpio* can digest up to 85% of the starch in a high starch diet (Chiou and Ogino, 1985), whereas rainbow trout is capable of digesting only 38-55% of dietary starch (Bergot and Breque, 1983) and that in rainbow trout starch digestibility declines rapidly with increasing inclusion level.

Lipase activity in the present study is also higher in Nile tilapia than in African catfish. There appears to be no information in literature that either agrees or disagrees with this finding in the present study. However, the natural diet of carnivores contains high levels of protein and lipid and very little carbohydrate (Smith, 1989). Thus it might be expected that carnivorous fish would have high lipase activity compared to herbivorous fish since enzymes activities are correlated with the food habits of the species (Olatunde and Ogunbiyi, 1977). It has also been reported that fish, in general, utilise dietary lipid effectively as an energy source (Sargent et al., 1989). More research is needed in this area particularly to clarify this point.

CHAPTER VII

CONCLUSION

7.1   Conclusion

According to the available literature concerning the effect of dietary saponin and tannin, it is clear that a considerable amount of research has been conducted on terrestrial animals, such as rats, mice, small mammals and poultry. However, research on fish is unavailable. Hence, Nile tilapia and African catfish have been adopted to establish the effect of dietary saponin and tannin (hydrolysable and condensed) on fish.

The present thesis is designed to elucidate the influence of dietary saponin and tannin on growth and feed performance, digestive enzymes and histology of intestine and liver of Nile tilapia and African catfish. All experiments have conducted in controlled closed system using casein/gelatin based diets.

Based on the results obtained from the present thesis, it is clear that neither saponin nor tannin acted as a positive factor. They both worked as neutral or negative factors with respect to feed intake, protein digestibility, growth performance, digestive enzymes or intestine and liver histology.

The results from the first study (Chapter III) showed that addition of saponin to the diets resulted in a significant reduction in fish growth performance. Even inclusion of 0.08 and 0.09% saponin for Nile tilapia and African catfish respectively resulted in some decrease in growth and feed performance. Increasing the saponin content from 0.08 to 0.42% and from 0.09 to 0.47% for Nile tilapia and African catfish respectively resulted in a very large decline in

growth and feed performance. It is likely that growth depression observed in this study could be largely attributed to the bitterness of saponin resulting in reduced feed intake. Attempts to increase the palatability of the diet (not reported in this thesis) by adding 1 or 2% of a commercial stimulant, Finnstim, failed. Fish exhibited similar feeding behaviour to that reported earlier (Chapter III) when diet IV with Finnstim was offered for one week. It is possible that the selected levels of Finnstim were insufficient to alleviate the astringent taste of saponin.

The results of Chapters IV and V showed that tannin in the form of tannic acid or catechin also affected growth and feed performance of fish at a dietary level of 0.08 and 0.16% and more of tannic acid and catechin respectively. However, growth depression observed in these two Chapters was not a result of restricted feed intake as much as a result of low feed utilisation, except for catechin which acted as a feeding depressant only for African catfish at a level of 1.6%.

Low protein digestibility (Chapters III, IV and V) was the second factor involved in growth depression observed in fish fed diets containing dietary saponin or tannin. Lowered protein digestibility was significant at a dietary level of ≥ 0.42, ≥ 0.08 or 1.16 of saponin, tannic acid or catechin respectively. Three possible explanations have been suggested for the low protein digestibility observed in the present studies. Firstly, saponin or tannin may react with protein in the digestive system and form complexes of high molecular weight which are unlikely to be absorbed from the intestine.

Secondly, saponin or tannin may cause a high loss of endogenous protein as a result of membranolysis or irritation. Thirdly, saponin or tannin may react with digestive enzymes and inhibit their action. While the last two theories were supported by the results of Chapter III and VI, the first theory was supported by results reported elsewhere (Section 5.4.2).

Tannin in both forms (tannic acid and catechin) had no effect on body composition of fish fed the experimental diets (Sections 4.3.3 and 5.3.3). However, saponin caused changes in moisture, lipid and ash contents and sometimes in protein content as a result of feeding 0.42% saponin or more (Section 3.3.3).

Histological examination of intestines and livers of both species showed no abnormalities (Sections 4.3.4 and 5.3.4) when fish were fed tannin-containing diets. However, the opposite was reported (Section 3.3.4) when fish were fed diets containing saponin at level of 0.42% and more in which cytoplasmic vacoulation of intestinal cells, disruption of straited borders, infiltration of *lamina propria* and degeneration of hepatic cells were observed.

The results from Chapter VI showed that tannin in both forms had inhibitory effects on digestive enzyme activities. This effect was more pronounced on proteolytic activity followed by amylolytic activity in both species. However, the results presented in Chapter VI support the view taken by many researchers (Section 6.4) that the relative activities of digestive enzymes present

in fish can be correlated with the nature of the food normally taken. The results also showed that neither tannic acid nor catechin had any greater effect on digestive enzyme in any species more than the other. There were differences in the tendency of tannic acid and catechin to inhibit different enzymes.

The results presented in Chapter III, IV and V show that the deleterious effects of saponin, tannic acid or catechin on growth and feed performance are more marked in African catfish than Nile tilapia. It is suggested that feeding behaviour of fish in nature is the reason behind this difference where Nile tilapia depends on diets of plant origin in which saponin or tannin may constitute a part. Whereas African catfish depends on diets largely of animal origin which are known to be effectively saponin or tannin-free (Sections 1.6.1 and 1.6.2).

It appears that the inhibitory effect on growth and feed performance is more marked with hydrolysable tannin than with condensed tannin. Hydrolysable tannin (in the form of tannic acid) at rate of 0.71% caused 21 and 25.5% reduction in growth compared to 8.8 and 24% for 0.73% condensed tannin (in the form of catechin) in Nile tilapia and African catfish respectively. It seems that the negative effect of a single tannin depends on its molecular weight (Singleton, 1981) in which high molecular weight will be more effective than low molecular weight of tannin.

In general, growth and feed performance in African catfish was much better than Nile tilapia. This might be due to greater stomach volume and higher proteolytic activity that characterised African catfish over Nile tilapia.

7.2   Suggestions and Recommendations

The effects of saponin and tannin in the present study have been tested on small fish (3.5-5 grams) and for a specific period (8 weeks). Therefore, a long term study with larger fish is suggested, in order to observe the differences between the two sizes in term of acceptability and digestibility of feed.

In view of shortage of faeces, especially in African catfish, only protein digestibility in the present study was performed; therefore, a study to determine lipid and ash digestibility under the effect of saponin or tannin is recommended. Moreover, the determination of amino acid digestibility individually is strongly recommended since there is a tendency of tannin to react with proteins that have a high proportion of hydrophobic amino acids and a high proline content (Marquardt, 1989).

In the present study, commercial saponin and tannin were used to carry out the experiments; therefore, it is preferable in the future to use extracted saponin or tannin from their respective sources.

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