Institute for Animal Production in Tropics and Subtropics

Department of Aquaculture Systems and Animal Nutrition

Evaluation of suitability of non-toxic and detoxified *Jatropha curcas* L. meal as feed for fingerling common carp, *Cyprinus carpio* L.: with reference to phytase application

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Dedicated to my mother, father (†19.03.2012) and all those who made this work possible

Erklärung

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List of Abbreviations

Abbreviations used in text

ABM	average body mass
AMR	apparent metabolical rate
AOAC	Association of agricultural chemists
AUE	Apparent unmetabolised energy
BMG	body mass gain
CA	crude ash
CP	crude protein
CE	crude energy
CF	condition factor
DM	dry matter
DO	dissolved oxygen
EE	energy expenditure
EFA	essential fatty acid
EAA	essential amino acid
ER	energy retention
FAA	free amino acid
FAO	food and agricultural organisation
FBM	final body mass
FCR	feed conversion efficiency
FFSBM	full-fat soybean meal
FI	feed intake
FM	fish meal
FTU	phytase unit
GE	gross energy

HSI	hepatosomatic index
IBM	initial body mass
JM	Jatropha meal
ME	metabolisable energy
MJ	mega joule
ND	not detected
NFE	nitrogen free extract
NSP	non-starch polysaccharide
PE	protein efficiency
PER	protein efficiency ratio
pH	hydrogen ion activity
PPV	protein productive value
PVPP	polyvinyl polypyrrolidone
RIL	relative intestinal length
RP	relative profile
SGR	specific growth rate
TJM	toxic Jatropha meal
TI	trypsin inhibitor
VI	viscerosomatic index
WB	wet basis

Chemical terms

BAPNA	benzoyl-Arg p-nitroanilide
HCl	hydrochloric acid
NaCl	sodium chloride
NaOH	sodium hydroxide
MeOH	methanol

KH_2PO_4	potassium Phosphate

Minerals

Fe	iron
Zn	zinc
Mn	Manganese
Mg	Magnesium
Na	sodium
K	potassium
Ca	Calcium
P	phosphorus

Units

^{o}C	degree Celsius
d	day
g	gramme
kg^{-1}	per kilogramme
kJ	kilojoule
M	molar
min	minute
mg	milligramme
mg/ml	milligramme per millilitre
ml	millilitre
nm	nanometer
rpm	revolutions per minute
g	gravity
µ	micro

Statistical terms

ANOVA	Analysis of variance
SD	Standard deviation
SE	Standard error
P-value	probability level

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1. General introduction

1.1 Current and future state of world aquaculture

Global consumption of fish has doubled since 1973, and the developing countries have been responsible for nearly all of this growth (Delgado et al., 2003). The driving force behind the enormous surge in the consumption of animal products including fish is a combination of population growth, rising incomes and increasing urbanisation. Dietary diversification is expected to create additional demand and to continue to shift the composition of food consumption towards a growing share of animal products in developing countries (Delgado et al., 2003).

Historically, the oceans were considered to shelter enough fish to feed a steadily rising human population, particularly in developing countries. However, the demands of ever-increasing populations strip the sustainable yield of the seas by far. At the same time fishing has become more industrialised, and wild stocks increasingly depleted, so that aquaculture production has grown rapidly to address the shortfalls in capture fisheries (Tacon and Metian, 2008). The contribution of aquaculture to global supplies of fish, crustaceans, molluscs and other aquatic animals increased from 3.9% of the total production by weight in 1970 to 36.0 percent in 2006 with an increasing per capita supply from 0.7 kg in 1970 to 7.8 kg in 2006 (FAO, 2008a). Of total food fish from world fisheries production (about 110 million tonnes providing an apparent per capita supply of 16.7 kg), aquaculture accounted for 47% and offset the effect of the stagnating capture fisheries production (FAO, 2008a). During this period aquaculture went through an annual growth of 6.9%, representing a greater increase in production than any other animal food-producing sector (FAO, 2008a). It has been estimated that aquaculture growth will continue over the coming decades as the demand and consumption of aquaculture products increases (FAO, 2008b). By 2050, aquaculture will need

to produce nearly 80 million tonnes of fish per year to maintain current per capita consumption levels (FAO, 2008a).

In developing countries aquaculture has emerged as a sector of economic importance in terms of its contribution to food security and nutrition, foreign exchange and employment generation, as well as poverty alleviation in the rural communities. One of the reason for the phenomenal growth of aquaculture is progressive intensification of many production systems and the key driver for such an intensification is use of feed inputs/formulated diets that meet nutritional requirements of target species. Feed inputs may include the use of industrially made compound aquafeeds, or the use of natural food organisms of high nutrient value such as forage/trash fish and natural/cultivated invertebrate food organisms (Tacon and Metian, 2008). Feeds and feeding usually represent the largest operating cost item of most fish and crustacean farming operations (FAO, 2006; Tacon and Metian, 2008). For most carnivorous and omnivorous species the protein and lipid source of choice for formulated diets has been fish meal. The preference for fish meal is because of its high palatability, well-balanced essential amino acids profile, fatty acid composition, digestible energy, vitamins and minerals (Tacon, 1993). The estimated fish meal use within aquafeeds increased two-fold from 1882 thousands tonnes in 1995 to a maximum of 4300 thousands tonnes in 2005, thereafter decreasing by 13.4% to 3724 thousands tonnes in 2006 (Tacon and Metian, 2008). If the finfish and crustaceans production of aquaculture is to sustain its current growth rate of 8.5% per year (FAO, 2006), then it follows that the supply of feed inputs will also have to grow at similar rates so as to meet the demand. This supply has now become more critical because of the current dependency of the export oriented fish and crustaceans aquaculture sector upon capture fisheries as a source of feed inputs, including fish meal and fish oil (Tacon and Metian, 2008).

On the other hand, despite increases in the total global consumption of fish meal and fish oil by the aquaculture sector, the average dietary fish meal and fish oil inclusion levels

within compound aquafeeds for some species have steadily declined, such as shrimp from 28 to 20%, marine fish from 50 to 32%, salmon from 45 to 30%, carp from 10 to 5% (Tacon and Metian, 2008). This trend of long term decline of fish meal and fish oil use by the aquaculture sector is due to a variety of factors, including:

- stagnating or declining global supplies of wild forage fish which resulted in the reduction of fish meal and fish oil supplies. (In recent decades, fish meal production has been remarkably stable at about 6 million tonnes, fluctuating between 5 million and 7 million tonnes depending on the catch levels of anchovy off South America (FAO, 2008a; Tacon and Metian, 2008)).
- increasing market price of small pelagic forage fish in the long term due to increasing fishing costs and rising demand for forage fish for direct human or animal consumption (Zertuche-González, 2008)
- increasing global energy, processing, shipping and transport costs (FAO, 2008b; Tacon and Metian, 2008)
- as a result of the aforementioned global trends, increasing price of fish meal and fish oil in the long term and consequent pressure on feed manufactures for dietary substitution so as to remain profitable (Tacon and Metian, 2008).

As an alternative to fish meal and fish oils, a wide range of ingredients has been evaluated over the past decades. These ingredients can generally be classified into those being either of plant origin or of terrestrial animal origin.

Despite the promise of animal by-products such as meat and bone meals, poultry by-product meals, feather meals and blood meals in fish diets there is still much public concern especially in Europe due to the recent BSE and prion risks attributed to such materials arising within the animal and consumer food chain. Consequently the use of plant proteins (such as

grain legumes, pulses and cereals) to replace fish meal has become more acceptable in recent years.

1.2 Constraints to the use of plant proteins as an alternative to fish meal in practical fish feeding

Many plant protein sources have reasonable protein or energy digestibility but imbalances in the proportion of some essential amino acids, presence of a wide variety of antinutrients and/or high levels of non-digestible material like oligosaccharides and non-starch poly-saccharides, which implies that they are not viable alternatives for fish meal unless some measures have been taken to eliminate the problems.

Firstly, the amino acid compositions of many plant proteins differ significantly from that of fish meal and feeding such diets may induce essential amino acid deficiencies that would restrict growth and protein utilisation unless supplemented. In order to maximise protein growth in cultured fish, the composition and proportion of the ten essential amino acids in the feed should meet the requirement of the fish. Since no single plant protein has a suitable amino acid composition (Kaushik, 1990), a supplementation with deficient essential amino acids (normally lysine, methionine, tryptophan and threonine) is often required to improve the nutritive value of plant protein meals (Rodehutscord et al., 1995). On the other hand, stomachless fish (e.g. common carp) have been shown to utilise synthetic amino acids less efficiently (Murai et al, 1981; Plakas et al, 1981, Becker, 1984) compared to fish with stomachs (e.g. rainbow trout). In carp, individual free amino acids (FAA) appear to be absorbed at varying rates from the intestinal tract and consequently peak plasma concentrations of individual amino acids do not occur simultaneously (Plakas et al., 1981). It is widely accepted that the apparent reduction in the utilisation of FAAs is related to their rapid absorption, which may result in excessive amino acid catabolism and reduced utilisation

efficiency (Lovell, 1991). In addition, differences in the ability of free amino acids and intact protein to stimulate specific digestive enzymes in the gut have also been suggested as a factor affecting utilisation efficiency (Chiji et al., 1990).

The second problem in the utilisation of plant protein sources is the presence of many endogenous antinutritional factors (e.g. tannins, saponins, phytates, trypsin inhibitors, lectins, glucosinolates, alkaloids, etc.) at varying levels that may interfere with the palatability and digestive physiology, reducing the digestibility and utilisation of nutritional components in the diet and affecting animal health in general (Francis et al., 2001). According to Francis et al. (2001) these components could generally be divided into four groups:

- factors affecting protein utilisation and digestion, such as tannins, saponins, phytates, trypsin inhibitors and lectins,
- factors affecting mineral utilisation which include phytates, gossypol, pigments, oxalate, glucosinolates,
- antivitamins,
- miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents, phytoestrogens.

Because of the detrimental effect of these substances on fish health, it is important that these compounds are removed or inactivated before inclusion into feed.

Plant materials used as alternative sources of proteins to fish meal will inevitably contain a significant amount of complex carbohydrates, predominantly in the form of oligosaccharides, non-starch polysaccharides (NSPs) and starch. The effects of oligosaccharides and NSPs have been extensively explored in the nutrition of terrestrial animals; however, their impacts in fish nutrition have only in few cases been well defined. Omnivores and herbivores have been shown to digest starch more efficiently than carnivorous fish (Hepher, 1988) and can tolerate diets containing high proportions of digestible carbohydrates. However, dietary oligosaccharides and NSPs can have numerous effects on

digestive tract morphology, rate of passage, digestive efficiency and microbial activity besides interacting with limiting macro- and micro-nutrients (Bach Knudsen, 2001; Wenk, 2001). The NSPs (including cellulose, hemi-cellulose and pectins) can be divided into water-soluble and water-insoluble fractions. Water-insoluble NSPs are indigestible and can decrease the gut passage time and diet digestibility. Water-soluble NSPs are known to possess anti-nutritional properties by either encapsulating nutrients and/or depressing overall nutrient digestibility through gastro-intestinal tract modifications (Storebakken and Austreng, 1987).

Since plant protein sources are normally much cheaper than fish meal, there is considerable scope to process the potential alternatives and produce economical products with increased nutritive value especially when dealing with aquaculture diets, which generally require very high protein levels. Therefore, certain technological processes largely based on thermal treatment (for inactivation of protease inhibitors and lectin) or solvent extraction (for removal of phenolics, saponins and oligosaccharides and soluble NSPs), pre-enzyme treatment (e.g. phytase to reduce phytic acids) or using protein concentrates following extractions of non-starch polysaccharides has resulted in a new generation of products applicable in fish formulations (Drew et al., 2007; Gatlin et al., 2007).

1.3 Common Carp, current and future culture

The common carp, *Cyprinus carpio*, is a stomachless fish belonging to the family Cyprinidae. This species is one of the oldest domesticated fish for food. Carp culture in China dates back to at least the 5th century BC. The natural habitat of carp is in the middle or lower reaches of a river with slow currents, or in marches with muddy bottom where there is abundant vegetation to provide food and shelter. Carp fry feed on zooplankton such as rotifers and copepods, but as they grow up they become benthic feeders, feeding on animals or other organic materials.

Cyprinid culture is very important to the world aquaculture industry, outweighing all the other species groups in its contribution to world aquaculture production (De Silva, 2003). According to FAO statistics 2004, production of farmed common carp was about 13% (3.387,918 tonnes) of the total global freshwater aquaculture production. Common carp production increased at an average global rate of 9.5% per year between 1985 (less than 0.5 billion tonnes in 1980) and 2004 (about 2.8 billion tonnes). A large percentage of this is from the Asian countries, particularly China which claimed about 70% of the world production in 2005.

The supplementary feeds used in carp culture are diverse. Most of these feeds are simple mixes of agricultural by-products which are readily available at relatively low costs. The most common of these feeds are brans of rice and wheat, often mixed with cakes or meals of various oil seeds such as mustard, canola and soybean. The quantity of feed as well as the amounts of the individual ingredients used in the feed mixes can vary greatly (De Silva, 2003). This trend is indicative of a potential constraint to the expansion of culture activities, namely the increasing competing demands for the same food ingredients from other animal husbandry activities and from other users (livestock or human) (Veerina et al.,1999). Furthermore, due to the expansion and intensification of carp culture, the traditional crude feeds for carp prepared on site from local ingredients have given way to commercial diets such as fish meal. Despite the aforementioned facts, little effort has been put into investigating alternative protein sources in common carp diet.

1.3.1 Aspects of nutrient requirements of common carp

Of commonly cultured fish the nutrient requirements of common carp are best known (NRC, 1993). This is mainly due to the fact that this fish is one of the earliest species cultured and experimented with. The nutrient requirement of common carp is discussed below.

1.3.1.1 Protein and amino acids

Generally, the protein requirements of fish species are higher than those of terrestrial livestock, ranging from 30% for tilapia to 42% for rainbow trout (NRC, 1993). In most fish species, protein is utilised as an energy source. However, once this requirement has been fulfilled the remainder can be utilised for growth and protein accretion (NRC, 1993). Investigations on the optimal requirement of common carp have demonstrated that crude protein levels ranging from 30 to 38% appear to satisfy the fish (Jauncey, 1982; Watanabe, 1988). Generally this level has been determined using semipurified diets containing a single high quality protein source such as casein, whole egg protein or fish meal. If sufficient digestible energy is contained in the diet, the optimal protein level can be efficiently kept at 30-35% (Watanabe, 1982).

The same ten essential amino acids (EAAs) described for most fish are indispensable for carp growth as well. The quantitative requirement for amino acids was established through different studies and is shown in Table B. It should be mentioned that there may be minor changes in the requirement of individual amino acids, depending on growth stage (Baloguma, 1995). The lysine requirement at the fingerling stage is 2.25% of the diet (6% of protein) and decreases to 1.75% of the diet (5.4% of protein) at the juvenile stage.

The protein, lipid and essential fatty acid and carbohydrate requirements of common carp are presented in Table A.

Table A: Macronutrient requirements of common carp, *Cyprinus carpio*

Nutrient	requirement	reference
Protein	30-38 g $100g^{-1}$	*Watanabe, 1988*
Lipid	5-15 g $100g^{-1}$(related to energy)	*Takeuchi et al., 1979a*
Essential fatty acid		
Linoleate	1 g $100g^{-1}$	*Takeuchi and watanabe,1977*
Linolenate	1 g $100g^{-1}$	*Takeuchi and watanabe,1977*
Digestible energy	13-15 MJ k g^{-1}	*Takeuchi et al., 1979a*
Carbohydrate (as starch)	30-40g $100g^{-1}$	*Murai et al., 1983b*

Table B: Essential amino acid requirements of common carp, NRC 1993; data from Nose et al., (1974)

	% of dietary protein (at 38.5% of diet)	% of dry diet
Arginine	4.2	1.6
Histidine	2.1	0.8
Isoleucine	2.3	0.9
Leucine	3.4	1.3
Lysine	5.7	2.2
Methionine	3.1	1.2
Phenylalanine	6.5	2.5
Threonine	3.9	1.5
Tryptophan	0.8	0.3
Valine	3.6	1.4

1.3.1.2 Energy

There is little information on the energy requirement of carp, compared with the volume of data on other aspects of their nutrition. Protein and lipid requirements are related to digestible energy. A dietary energy budget was provided by Ohta and Watanabe (1996) for carp fed a practical diet containing 25% fish meal, 4% meat meal, 10% soybean meal and 8% maize-gluten meal as the main protein source. The various fractions of gross energy intake (100%) at the level required for optimum growth were 29.9% lost as faecal energy, 1.5% lost as non-faecal energy, 31.9% as heat increment and 36.7% as net energy (including 12.6% for maintenance and activity and 24.1% as productive energy). The authors also reported that the digestible energy requirements for maximum growth were 285 kJ kg^{-}1 body weight day^{-1} (at feeding rate 1.83 of body weight day^{-}1), 548 kJ kg^{-}1 body weight day^{-1} (at feeding rate 3.6 of body weight day^{-}1) and 721 kJ kg^{-}1 body weight day^{-1} (at feeding rate 5.17 of body weight day^{-}1), these figures being influenced by both diet and fish size.

1.3.1.3 Lipids and essential fatty acids (EFAs)

The common carp is an omnivorous fish and can efficiently utilise both lipids and carbohydrates as dietary energy sources. Therefore the digestible energy content is more important than the lipid content in the diet. It has been shown that the enrichment of the digestible energy content from 13 to 15 MJ kg^{-1} diet by the addition of lipid at levels of 5-15% to diets did not result in an improvement of either growth performance or net protein utilisation; however, body lipid deposition increased dramatically (Murai et al., 1985).

As far as essential fatty acids (EFAs) are concerned, common carp require both n-6 and n-3 fatty acids (Table A). The deficiency symptoms related to EFAs do not easily show

up in common carp, however, poor growth, high mortality and skin depigmentation have been reported (Takeuchi et al., 1992).

1.3.1.4 Carbohydrates

The amylase activity in digestive tract and the digestibility of starch in fish are generally lower than those of terrestrial animals. Among fish, the intestinal activity of amylase is higher in omnivorous fish including common carp than in carnivorous fish. Murai et al. (1983b) studied the effect of various dietary carbohydrates and frequency of feeding on patterns of feed utilisation by carp. While the starch diet produced the highest weight gain and feed efficiency at two daily feedings, glucose and maltose were as efficiently utilised as starch when fed at least four times daily. The optimum levels of dietary carbohydrate are presented in Table A.

1.3.1.5 Vitamins

The quantitative vitamin requirement of common carp to prevent signs of deficiency are presented in Table C. Although thiamine, folic acids, Vitamins D, B_{12}, C and K are required but they have not been investigated quantitatively for common carp.

Vitamin requirements of carp may be affected by various factors, such as fish size, water temperature and diet composition. For example, juvenile or adult common carp do not require vitamin C because they can synthesise ascorbic acid from D-glucose. However, common carp fry do show vitamin C deficiency sign, such as caudal fin erosion and deformed gill arches (Dabrowski et al., 1988).

Table C: Vitamins requirements of common carp to prevent deficiency signs (NRC, 1993)

Vitamin	requirement (mg kg^{-1} diet)	reference
Riboflavin	7.0	Takeuchi et al., 1980
Pyridoxine	5-6	Ogino, 1965
Pantothenic acid	30-50	Ogino, 1967
Nicotinic acid	28	Aoe et al., 1967b
Biotin	1	Ogino et al., 1970a
Choline	4000	Ogino et al., 1970b
Inositol	440	Aoe and Masuda, 1967
Vitamin A	10,000 IU	Aoe et al., 1968
Vitamin E	200-300	Watanabe et al., 1977

1.3.1.6 Minerals

Mineral requirements and their deficiency signs are summarised in Table D. Common carp lack an acid-secreting stomach essential for digesting and dissolving various compounds containing both calcium and phosphorus; thus the availability of phosphorus depends on the water solubility of the salt and ingredients (Satoh et al., 1992, 1997). Phosphorus from tricalcium phosphate or fish meal (FM) is less available to fish than that from the more soluble mono- and dicalcium phosphate. Supplementation of monobasic phosphate to FM-based diets resulted in an increase in growth response of common carp (Satoh et al., 1992, 1997). It should be mentioned that an excess amount of tricalcium phosphate may inhibit the availability of trace elements, such as zinc and manganese (Satoh et al., 1989).

Table D: Mineral requirements of common carp and deficiency symptoms (Ogino and Takeda. 1976; Satoh et al., 1992; NRC, 1993; Kim et al., 1998)

Mineral	requirement DM fed	deficiency symptoms
Phosphorus	6-8 g kg^{-1}	poor growth, skeletal abnormality, low feed efficiency, low ash in whole body and vertebrae, increased visceral fat
Calcium	<0.30 g kg^{-1}	poor growth, bone deformation
Magnesium	0.4-0.5 g kg^{-1}	poor growth, anorexia, high mortality, cataracts, high mortality, high calcium in bone, sluggishness
Iron	150 mg kg^{-1}	low specific gravity, low haemoglobin and haematocrit values, abnormal mean corpuscular diameter
Zinc	15-30 mg kg^{-1}	poor growth, high mortality, erosion of fins and skin, low zinc content in bone
Manganese	13 mg kg^{-1}	poor growth, dwarfism, skeletal abnormality, high mortality, low calcium, magnesium, phosphorus, zinc and manganese in bone
Copper	3 mg kg^{-1}	poor growth
Cobalt	0.1 mg kg^{-1}	poor growth

1.4 Background to experimental feeding common carp with plant protein sources

To date, the main consumers of fish meal in aquaculture are carnivorous fish such as rainbow trout, salmon and others. In addition, as carp culture continues to expand, more and more of the culture operations will become intensive, necessitating the development of advanced diet formulation to address issues of environmental concerns.

Although common carp is an omnivorous fish and it seems they could make a more efficient use of high levels of vegetable protein, considerable variations were observed in the ability to utilise different plant protein sources. Studies have shown that the replacement of high quality fish meal with vegetable proteins causes a reduction in growth or feed efficiency, the magnitude of which depends on the protein source and level of replacement.

1.4.1 Soybean meal and soybean products

Soybean meal with its relatively high protein content and complementary mixture of amino acids has traditionally been evaluated in comparison to fish meal, which is generally considered the most nutritious protein feed stuff for aquatic animals. However, results on utilisation of soybean meal and the effects of crystalline amino acid supplementation are contradictory.

Early studies with common carp indicated that the reduction of fish meal from 15 to 5% in low protein diets (25%) while increasing soybean meal from 15 to 35% caused growth retardation which could not be altered by additional supplementation of methionine and lipids (Viola, 1975). However, subsequent experiments (Viola et al., 1981, 1981/1982) demonstrated that total replacement of fish meal with soybean meal could be achieved by supplementation of lipid (up to 10%), methionine (0.4%) and lysine (0.4-0.5%) to the soybean meal based diet.

Similar results with common carp were obtained in another study in which high-protein (37%) diets were evaluated (Pongmaneerat et al., 1993). In that study, a control diet containing 45% brown fish meal was compared to diets in which the fish meal was replaced progressively with a combination of soybean meal and either gluten corn meal or meat meal. Reducing fish meal from 45% in control diet to 22% along with 25% soybean meal and 10% corn gluten meal resulted in similar weight gain, feed efficiency and protein efficiency ratio (PER) of common carp. Further replacement of fish meal with the other ingredient combination did impair the measured responses, although supplementation of lysine, methionine and threonine in a diet containing 5% meat meal, 15% corn gluten meal and 40% soybean meal and no fish meal improved weight gain and feed efficiency to 90% of the levels found in fish fed the control diet.

A similar result was obtained in a second experiment in which an amino acid mixture was added to diets containing 5% meat meal, 14% corn gluten meal and 38% soybean meal. This supplementation resulted in weight gain, feed efficiency and PER responses approximately 90% of those achieved by fish fed the control diet with 45% brown fish meal (Pongmaneerat et al., 1993). Combining soybean meal with other feed stuffs rich in amino acids, such as crustacean squilla (*Oratosquilla nepa*) meal also has been reported to support rapid growth in common carp (Nandeesha et al., 1989).

The nutritional value of properly heated full-fat soybean meal (FFSBM) for various warm water species is somewhat variable, based on very limited number of publications. In common carp, the value of FFSBM was reported to be equivalent to commercial soybean meal or soyprotein concentrate reconstituted with soybean oil (Viola et al., 1983). In contrast Abel et al. (1984) reported that a diet containing hydrothermically treated FFSBM (at 50% replacement of fish meal with FFSBM) supported growth of common carp at only 60-65% of those fish fed control diet (fishmeal base diet). This was attributed to an inferior amino acid balance in the test diet.

Murai et al. (1986) studied the effects of crystalline amino acid supplementation and methanol treatment on utilisation of soy flour by fingerling carp. Adding essential amino acids to the diets in which 75% of the fish meal was replaced by methanol treated or untreated soy flour significantly improved carp growth to a level about 90% of weight gain of fish fed the fish meal base diet. However, methanol treatment failed to show any effect on growth performance of carp. Earlier Arai et al. (1983) reported that utilisation of soybean meal by rainbow trout, *Salmo gairdneri*, is improved by treating it with alcohol. Therefore, it was concluded that common carp might be less sensitive to alcohol soluble components in soybean products than other fish species.

In another study, Escaffré et al. (1997) evaluated the nutritional value of soy protein concentrate (SPC) for common carp larvae. Incorporation of SPC up to 40% in the diet did not adversely affect survival of carp larvae when compared to fish fed a control diet containing 85% of a beef liver-yeast mixture and 2% fish oil. However, higher inclusion of SPC at levels 60 or 70%, depressed growth performance of fish and even sulphur amino acid supplementation did not improved the performance of common carp.

1.4.2 Other oilseed meals and leaf meal

The assessment of protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp indicated that fish meal had significantly higher nutrient digestibility coefficients than the listed plant ingredients (Hossain and Jauncey, 1989a). However, among the plant proteins, sesame meal resulted in lower protein and amino acid digestibility than mustard oil cake and linseed meal. In a subsequent study, the aforementioned plant proteins were used to replace fish meal at 25-75% in diets containing 40% crude protein (Hossain and Jauncey, 1989b). Growth performance of common carp was significantly affected by type and inclusion level of oilseed proteins. Fish fed the fish meal

diet had significantly higher growth rates and protein utilisation than other fish fed the experimental diets. However, of the oilseed proteins tested, fish fed 25% mustard oil cake and linseed meal showed better growth performance than those given the higher inclusion levels tested (Hossain and Jauncey, 1989b). At the same time, fish fed 50% mustard protein showed histological abnormalities in liver and thyroid tissues. Later, Hossain and Jauncey (1990) observed that detoxification of linseed and sesame meal either by aqueous extraction or autoclaving at 120^{o}C for 2 hours resulted in better food utilisation of those meals (at 25% of fish meal replacement) for common carp compared to fish fed untreated meals. Nevertheless, the performance of fish was not at the same level as that of fish fed the fish meal based control diet.

In a similar study Hasan et al. (1997) reported that the growth and feed utilisation of common carp was significantly affected by the inclusion of sesame oil cake (at 25, 50 and 75% replacement of fish meal), mustard (at 25 and 50% replacement of fish meal) and linseed meal (at 50% replacement of fish meal), groundnut oil cake (at 75% replacement of fish meal), copra and Leucaena (at 25% replacement of fish meal). However, based on growth performance, protein efficiency ratio and protein productive value, fish fed diets containing 25% linseed meal and 25% groundnut showed no significant differences to those fish fed a fish meal based diet. Meanwhile, the liver of fish fed a diet containing mustard cake showed severe intracellular fat deposition. Moreover, deformation of the body was observed for fish fed a 75% groundnut diet.

1.4.3 Pea seed meal

Recently, the effectiveness of pea seed meal treated in different ways (autoclaved and dry cooked) was evaluated for common carp (Davies and Gouveia, 2008). The treated diets replaced solvent extracted soybean meal in a fish meal/soybean meal control diet. At the end

of the experimental period, significant differences were found in BMG and SGR of fish fed the control diet and that with untreated pea seed meal. However, BMG and SGR of fish fed either autoclaved or dry cooked pea seed meal were not deemed to be significantly different from those fish fed a control diet. The apparent nutrient and energy digestibility were highest for fish fed the control diet. Moreover, the apparent nutrient and energy digestibility of autoclaved and dry cooked pea seed meal significantly improved when these were compared to those of fish fed the untreated diet. It was concluded that these two thermal treatments improved the quality of pea seed meal, however, slightly better results were observed when the diet was dry cooked.

1.4.4 Mucuna pruriens *Var.* utilis

The *Mucuna pruriens* is a tropical legume with nutritional quality comparable to soybean and other conventional foodstuffs as it contains similar proportions of protein, lipid and minerals (Siddhuraju et al., 2000). Common carp fed diets containing 13% untreated or autoclaved Mucuna seed meal (at replacement of about 10% of total dietary protein) showed no significant differences in growth rate, feed and energy utilisation when compared to fish fed a control diet (fish meal base). Higher inclusion of Mucuna seed meal was shown to have detrimental effects on the growth performance of fish (Siddhuraju and Becker, 2001). This experiment also indicated that hydro-thermal treatment of Mucuna did not improve the nutritional quality although it reduced the levels of most of the heat labile antinutrients such as trypsin inhibitors. The presence of L-DOPA by-products, L-DOPA metabolites and non-starch polysaccharides in Mucuna were attributed to cause the reduced growth performance of common carp fed higher level of Mucuna meal (Siddhuraju and Becker., 2001).

1.4.5 Sesbania aculeata

Sesbania aculeata is a legume crop widely available in many tropical countries of Asia and Africa. This plant can grow in poor and degraded soils. Sesbania contains 30-36% crude protein and has been used as fodder for livestock and green manure to improve the fertility of the land (Hossain et al., 2001a). The nutritional value of Sesbania was evaluated in a series of experiments on common carp (Hossain et al., 2001a, 2001b, 2001c, 2001d). Hossain et al. (2001a) reported that replacement of fish meal with untreated Sesbania at 20%, 30% and 40% total crude protein level significantly reduced growth rate, protein efficiency ratio and protein and energy retention of common carp when compared to fish fed a control diet (fish meal). In a subsequent study, the reduction of antinutrients of Sesbania by soaking (to remove water soluble components such as total phenolics, tannins and phytic acids) and soaking plus autoclaving (to destroy heat labile trypsin inhibitors and lectins) significantly improved the growth performance and feed utilisation of common carp when compared to that of untreated seeds but this was not at the level of performance obtained with a fish meal based diet (Hossain et al., 2001b). The non-starch polysaccharide galactomannan was later found to be the component primarily responsible for retardation of growth of common carp (Hossain et al., 2001d).

1.4.6 Growth performance and tolerance levels of fish to plant secondary metabolites

Becker and Makkar (1999) studied the effects of 2% dietary tannic acid (hydrolysable tannin) and quebracho tannin (condensed tannin) on the growth performance and metabolic rates of common carp. They found that 2% quebracho tannin did not affect feed intake, body weight gain, average metabolic growth rate and oxygen consumption during the 84 days experimental period when compared to the control group. However, tannic acid caused

adverse effects on fish performance and the test diet was rejected after 28 days. They concluded that protein sources of plant origin which contain high amounts of tannins, in particular hydrolysable tannins should be used with caution as a fish meal substitute in carp diets.

Francis et al. (2001, 2002) showed that 150 mg kg^{-1} Quillaja saponin in the diet of common carp and 300 mg kg^{-1} Quillaja saponins in the diet of Nile tilapia significantly increased the body weight gain of fish when compared to the control group. They concluded that Quillaja saponins at these levels can act as a growth stimulant for fish.

Siddhuraju and Becker (2002) evaluated the effect of the phenolic non-protein amino acid L-DOPA, present in the tropical grain legume *Mucuna pruriens*, on growth performance of common carp. They showed that more than 7 g kg^{-1} L-DOPA had antinutritional activity and could significantly depress the growth performance of common carp.

The effect of purified alcohol extract (Saponins) from *Sesbania aculeata* seed on growth and feed utilisation in common carp was reported by Hossain et al. (2001c). Test diets progressively received 0.3 to 2.4 g/kg alcohol extract from Sesbania seed meal. Fish fed 2.4 g/kg alcohol extract from Sesbania seed meal had significantly lower growth rate and feed utilisation parameters than fish fed a fish meal based diet or other test diets. On the other hand, fish fed 1.2 and 2.4 g/kg alcohol extract from Sesbania seed had significantly lower muscle and plasma cholesterol levels than other experimental fish. It was concluded that purified alcohol extract (Saponin) from Sesbania had a detrimental effect on growth performance of common carp when this exceeded 2.4 g/kg inclusion level.

The effect of diets containing purified and semi-purified phytic acids (at 0.5 or 1%) on common carp was investigated by Hossain and Jauncey (1991). Irrespective of the source of dietary phytic acid, this component depressed growth rate, nutrient utilisation and protein digestibility. Dietary treatment significantly affected plasma levels of calcium, zinc and iron.

Zinc levels in liver, kidney and whole carcass were significantly reduced by high dietary phytic acid. Hossain and Jauncey (1991) demonstrated that common carp fed purified diets containing 1% phytic acid showed hypertrophy and vacuolisation of the cytoplasm of the intestinal epithelium.

1.4.7 Jatropha curcas *meal as feed for common carp*

Makkar and Becker (1999) indicated that 15 minutes moist heat treatment of non-toxic Jatropha meal reduced the trypsin and lectin activity significantly. Common carp fed 15 min. heat treated (at 121^{o}C and 66% moisture) non-toxic Jatropha meal had higher weight gain, protein efficiency ratio and protein productive value than those fish fed untreated Jatropha meal. However, these parameters were significantly lower in the aforementioned trial when compared to fish fed a fish meal based diet (Makkar and Becker, 1999).

Becker and Makkar (1998) also demonstrated that Phorbolesters, the major toxic component in toxic variety of Jatropha, have a detrimental effect on common carp. In this experiment extracted phorbolesters from toxic Jatropha were added at various levels (3.75, 7.5, 15, 31, 62.5, 125, 250, 500 and 1000 μg/g feed) to a standard diet (fish meal based diet with 40% crude protein). Common carp fed diets containing phorbolesters at 15 μg/g feed or higher showed significant decreases in body mass gain. Moreover, production of mucus and rejection of feed started at 31 μg phorbolesters per gram feed. Vitamin C addition (as an antioxidant) did not alleviate the detrimental effect of phorbolesters in common carp.

1.4.8 Phytase supplementation in the diet of common carp

Studies on the effect of microbial phytase in the diet of common carp are very scant. Schäfer et al. (1995) reported the impact of the addition of monocalcium phosphate (MCP) in

comparison with microbial phytase on growth, body mineralisation, phosphorus (P) retention and phosphorus excretion on common carp. They observed that weight gain and crude ash were enhanced by increasing dietary P content and phytase supplementation. Moreover, phytase addition improved the utilisation of native P and thereby reduced P excretion.

Sardar et al., 2007 investigated the effect of dietary supplementation of microbial phytase on growth performance and haematological values in common carp. The aim of their experiment was to evaluate if the level of dicalcium phosphate (DCP) trace-mineral premixes and lysine and methionine supplementation levels could be reduced in a basal diet (composed of 60% soybean meal, 28% maize, 4.5% rice bran, 2% wheat gluten meal and 2% wheat flour) if microbial phytase was supplemented. The results indicated that phytase (at 500 FTU kg^{-1} level) was effective in releasing most of the phytate bound proteins, amino acids and minerals for optimum utilisation and performance of common carp.

Nwanna and Schwarz (2007) studied the effect of phytase supplementation (at 1000FTU, 2000FTU and 4000FTU levels) on common carp in diets containing a plant mixture (isolated soy protein 26%, maize gluten meal 30%, wheat meal 18.6% and maize 10.5%). They observed that addition of phytase regardless of level of supplementation marginally improved the growth performance and P digestibility in comparison to fish fed diet without treatment. They concluded that the higher levels of phytase are required to promote significant growth performance and mineral absorption by fish. In subsequent study, the effect of incubation on the diets supplemented with phytase was investigated (Nwanna et al., 2008). The result has shown that common carp fed the diets containing a plant mixture was either supplemented with 3g P/kg (Diet CP0) or supplemented with 3g P/kg and incubated (Diet CPI) or supplemented with 4000 U phytase/kg and incubated (Diet PhytI) had significantly higher mean weight gain than the fish fed diets either without pre-incubation or additional inorganic P. Moreover, the better growth performance of fish with aforementioned treatments was accompanied with increasing level of bone P and Ca. They concluded that the result of

this experiment suggesting phytase pre-treatment is the most effective method to increase mineral bioavailability for common carp.

1.5 **Jatropha curcas** *Nutrients and Antinutrients*

1.5.1 Nutrients

Jatropha curcas is a shrub or small tree belonging to the family Euphorbiaceae. This plant is widely cultivated in the tropics and subtropics and it can thrive on degraded lands (Makkar and Becker, 1998). Jatropha has been investigated mainly as a potential source of oil for fuel, since seeds contain about 300-350g kg^{-1} oil. There has been large-scale plantation of Jatropha in India, China, Madagascar, Myanmar, Egypt and many other developing countries in order to use the oil (in transesterified form) as biodiesel (Francis et al., 2005). Jatropha plants are also used in medicine, soap and cosmetics in various tropical countries (Martinez-Herrera et al., 2006).

Moreover, Jatropha kernels have high nutritional value. Defatted Jatropha kernel meal contains high levels of protein (56-63%), higher than Soybean meals (40-45%) (Table E). The levels of essential amino acids, except lysine, are higher than in the FAO reference protein for growing children (Makkar et al., 2008) (Table F).

Table E: Proximate composition of defatted kernel meal of non-toxic and toxic Jatropha (According to Makkar 2007**)**

Proximate composition (% DM)	non-toxic Jatropha	toxic Jatropha
Crude protein	63.8	56.4
Crude lipid	1.0	1.5
Crude ash	9.8	9.6
NDF	9.1	9.0
Gross energy (kJ g^{-1})	18.2	18.0

Table F: Essential amino acids of defatted kernel meal of non-toxic and toxic Jatropha (According to Makkar 2007**)**

Essential amino acids (g kg^{-1})	non-toxic Jatropha	toxic Jatropha
Arginine	12.9	11.8
Histidine	3.08	3.30
Isoleucine	4.85	4.53
Leucine	7.50	6.94
Lysine	3.40	4.28
Phenylalanine	4.89	4.34
Methionine	1.76	1.91
Threonine	3.59	3.96
Tryptophan	0.80*	1.31
Valine	5.30	5.19

*Data from experiment 1 in this thesis

1.5.2 Antinutrients

Despite its nutritive value, Jatropha is toxic to rats, mice and ruminants (Becker and Makkar, 1998). It has been reported that in humans, consumption of Jatropha seeds caused symptoms of giddiness, vomiting and diarrhoea (Becker and Makkar, 1998). The main substance in Jatropha seeds responsible for toxicity is phorbolester (Makkar and Becker, 1998). Furthermore, Jatropha meal contains high levels of heat labile antinutrients such as trypsin inhibitors and lectins as well as heat stable ones such as phytic acids (Makkar and Becker, 1998). Removal of toxic agents by solvent extraction and destruction of heat labile factors through heat treatment of Jatropha kernel meals was found to make this non-toxic to rats (Makkar and Becker, 1998). In addition to the more common toxic varieties, a non-toxic

variety has been reported from the Papanla region of the State of Veracruz in Mexico (Makkar and Becker, 1999). The moist-heat treated defatted seed meal of non-toxic variety has been shown to have no toxicity to rats (Makkar and Becker, 1999).

In order to obtain a better insight into the nature and mode of action of antinutrients present in Jatropha meal (Table G), a summary of the most important known antinutrients is provided.

Table G: Major antinutrients present in non-toxic and toxic Jatropha meal (According to Makkar 2007)

Component	non-toxic	Toxic
Total phenolics (% tannic acid equivalent)	0.22	0.36
Tannins (% tannic acid equivalent)	0.02	0.04
Phytates (%DM)	8.90	9.40
Saponins (% diosgenin equivalent)	3.40	2.60
Phorbolesters (mg/g kernel)	ND to 0.11	2.79
Trypsin inhibitor (mg trypsin inhibited per g sample)	26.5	21.3
Lectins (1/mg of meal that produced haemagglutination per ml of assay medium)	51	102

1.5.2.1 Trypsin inhibitors (TIs)

Trypsin inhibitors (TIs) are protein based substances widely distributed throughout the higher plants including the seeds of most cultivated legumes and cereals. These have the ability to inhibit the activity of proteolitic enzymes within the gastrointestinal tract of animals.

In soybean for instance, TIs can be divided into two main types. The heat labile Kunitz inhibitors with a molecular weight of 20,000-25,000 possess relatively few disulphide bonds and have a specificity directed mainly towards trypsin. The more heat-stable Bowmann-Birk inhibitors have a molecular weight of 6000-10,000 and a high proportion of disulphide bonds. Bowmann-Birk inhibitors are capable of inhibiting trypsin and chymotrypsin at independent sites (Liener, 1980, 1989).

Makkar and Becker (1999) indicated that 15 minutes moist heat treatment of non-toxic Jatropha meal reduced the trypsin and lectin activity significantly. They also reported that carp fed diets containing non-toxic Jatropha seed meal with 24.8 mg TI/g and heat-treated meal with 1.3 to 8.3 mg TI/g showed no differences in growth performance, implying that the fish were able to tolerate the high level of TI.

However, for many plant feed stuffs containing high level of TIs, moist heat treatment (autoclaving for 15-30 min., Norton, 1991) is recommended.

1.5.2.2 Lectins

Lectins or phytohaemagglutinins are found in most legume seeds. These are capable of bounding reversibly to carbohydrate moieties of complex glycoconjugates present in cell membranes (Francis et al., 2001). Although these components are proteins, they are at least partially resistant to proteolitic degradation in the intestine. It has been known that their biological effects are the disruption of the small intestine metabolism and morphological damage to the villi (Grant, 1991).

Lectins can be removed by aqueous heat treatment (100^{o}C for 10 min.) or autoclaving. Aregheore et al. (1998) reported that the lectin content in Jatropha seed meal can be reduced from 102 to 1.17 haematogglutination units by moist heating at 100^{o}C for 10 minutes.

Irritation caused by lectins to the intestinal membrane resulting in over secretion of mucus may impair the enzymatic and absorptive capacity of the intestine wall.

Makkar and Becker (1999) reported that common carp fed diets containing high (51 haematogglutination units) or low lectin (<1.2 haematogglutination units) activity had similar growth. However the detrimental effect of lectins may be more potent when present with other antinutrients.

1.5.2.3 Phytic acids

One of the most problematic antinutrients in Jatropha meal are the phytates, which are present at high levels. Phytate bound phosphorus is poorly utilized by monogastrics and consequently excreted via the faeces. Besides being an indigestible constituent, phytate bound phosphorus tends to act as an antinutritional factor in the diet in that it possesses strong chelating properties, thereby markedly reducing the bioavailability of several multivalent cations, mainly Ca^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}, by forming insoluble, phytate-metal complexes (Francis et al., 2001). Moreover, phytates also decrease protein digestibility through the formation of indigestible protein-phytate complexes and strongly inhibit the activity of amylase (Francis et al., 2001). Many potential alternative plant protein sources, namely cereals, legumes and oil seed meals, have been shown to have 3-8% phytic acid. Salmonids seem to tolerate dietary levels of phytate around 5-6 g/kg, while carp appear to be sensitive to these levels (Francis et al., 2001). Several studies on poultry, swine and fish have shown that dephosphorylation of phytic acid (dephytinisation) can be accomplished successfully by phytase pretreatment of plant proteins, that is through the addition of the appropriate dosage of phytase to the diet (Rodehutscord and Pfeffer, 1995 Schäfer et al., 1995).

1.5.2.4 Saponins

Saponins can generally contribute to a bitter taste, foaming in aqueous solution and ability to haemolyse red blood cells (Francis et al., 2001). Saponins can reduce the protein digestibility by forming indigestible saponin-protein complexes (Shimoyamada et al., 1998). Saponin containing feeds have been shown to have adverse effects on feed intake, growth and histology of fish (Kaushik et al., 1995; Olvera Novoa et al., 1990; Bureau et al., 1998; Krogdahl et al., 1995). Although saponins are soluble in water, they can be carried over with the protein during the extraction, most probably as a result of their surface activity (Ireland et al., 1986; Bureau et al., 1998). Since alcohol is a bond-breaker, it helps to remove saponins from proteins (Bureau et al., 1998).

1.5.2.5 Tannins

Tannins are a diverse group of polyphenolic compounds which are widely distributed in nature, food crops and legumes. These compounds are divided into hydrolysable and condensed tannins (Francis et al., 2001). Their antinutritional effects include interference with the digestive process either by binding to the enzymes or by binding to feed components like feed protein and minerals (Liener, 1989). Tannins can also reduce the absorption of Vitamin B_{12}. The effect of tannins on common carp is discussed in Section 1.4.6.

Recommended methods for the removal of condensed tannins include dehulling the seed to remove the tannin rich outer layer, autoclaving or treatment with alkali (Griffiths, 1991).

1.5.2.6 Phorbolesters

Phorbolesters are toxic substances found in *Jatropha curcas* which act as a co-carcinogen and posses a wide range of adverse biochemical and cellular effects in animals (Francis et al., 2001). Common carp was found to be extremely sensitive to these compounds (Becker and Makkar, 1998). At a level of 15 ppm in the feed, they induce a depression in feed intake and growth and the production of faecal mucus.

1.6 Objectives and experimental design

An overview of the research work undertaken on important aspects of fish nutrition during the last three decades has revealed that a wealth of valuable information has been gained from these studies; however, most of the work has concentrated on carnivorous fish. Fish meal free diets have been used with success in some studies for rainbow trout (Kaushik et al., 1995). The possibility of high incorporation levels of plant protein in feeds for salmonids has been demonstrated (Gomes et al., 1995; Burel et al., 2000; Refsti et al., 2000). On the other hand, the application of these results to herbivorous or omnivorous fish may not give comparable results because of anatomical and physiological differences in the gastrointestinal tracts. In view of the fact that carp culture continues to expand, more and more of the culture operations will become intensive, necessitating the development of advanced diet formulations to address issues of environmental concerns. It seems strange that, despite the importance of this fish in aquaculture world, so little effort has been made to investigate alternative plant protein sources in its diet.

It has to be kept in mind that the human population is increasing rapidly and agricultural land is decreasing due to soil degradation, urbanisation and industrialisation. Production of grain in developing countries is mostly for human consumption. For sustainable

development of animal production, it is essential to ensure sufficient supplies of balanced feeds from resources which do not compete with human food. Moreover, it is advisable to enhance the efficiency of utilisation of locally available feed resources such as agroindustrial by-products and lesser known and new plants adapted to harsh conditions and capable of growing in poor, marginal and degraded soils.

Feed-processing methods have been used for many years to improve the physical characteristics and nutritional quality of these protein sources since the plant proteins are not maximally utilised by the fish when compared with animal protein sources. Pre-processing refers to treatment of specific ingredients before mixing the ingredients into a complete feed. 70% of total phosphorus in plants is present as phytate, the bioavailability of which is very limited to fish. As is mentioned earlier (Section 1.5.2.3), because of the high density of negatively charged phosphorous groups, phytate chelates with mineral cations (Sardar et al., 2007) and this reduces the mineral bioavailability. Phytate also forms complexes with proteins and amino acids, thus decreasing the digestibility of proteins (Sardar et al., 2007). Phytase is an enzyme from fungus *Aspergillus niger*, which is specific to hydrolysis of phytates. Before, 1990s addition of phytase has been mainly reported to improve the utilisation of plant P in poultry and swine (Han et al., 1997). After mid 1990s, more and more studies have been started to evaluate the efficiency of microbial phytase in fish nutrition (Oliva-Teles et al., 1998; Schäfer et al., 1995; Rodehutscord et al., 1995; Vielma et al., 1998, Forster et al., 1999; Jackson et al., 1996; Liebert and Portz, 2005; Yoo et al., 2005). Although the role of phytase supplementation has been well proved in pigs (Han et al., 1997), its use in stomachless fish such as common carp is still in early stage and many fundamental issues relating to phytate and phytase remain to be clarified.

Although the nutritional characteristic of Jatropha kernel meal as an alternative feed for common carp is promising, so far high levels of replacement of fish meal with Jatropha meal either from non-toxic or detoxified toxic varieties has not been successful for common

carp. Consequently, the current investigation was undertaken to evaluate the effect of further treatment of non-toxic and detoxified *Jatropha curcas* meal at high levels of inclusion into common carp diets in the following experiments:

Experiment 1:

Growth performance and mineral utilisation of common carp in response to differential treatment of Jatropha kernel meal (addition of lysine, phytase supplementation and alcohol extraction)

- Treatment 1: one reason for reduced growth of common carp fed Jatropha meal might be the high level of requirement of common carp for essential amino acids, especially lysine, their requirement for which is the highest among fish after rainbow trout (NRC, 1993). Furthermore, heat treatment of Jatropha meal might have resulted in amino acid loss or reduced bioavailability due to the Millard reaction (Makkar and Becker, 1999). Therefore, in this experiment the effect of supplementation of L-lysine into the diets containing non-toxic Jatropha meal will be evaluated.
- Treatment 2: As described in Section 1.5.2.3, phytic acids present in Jatropha seed meal might have negative effects on the feed utilisation of fish when this is not reduced or eliminated. Thus, in this experiment the influence of adding 500 FTU phytase to Jatropha meal will be assessed. The mineral utilisation of fish fed a diet supplemented with phytase is expected to provide additional information on the effect of phytase supplementation.
- Treatment 3: Previous studies have shown that the removal of alcohol soluble components from soybean meal and lupin meal had beneficial nutritional implications for salmonoids and yellowtail (Arai et al., 1983; Bureau et al., 1998; Glencross et all., 2003). The aim of this treatment is not to assess which component/s (saponins, soluble sugars, soluble

oligosaccharides and non-starch polysaccharides (NSPs)) in the alcohol fraction might have a negative effect on growth performance of fish fed untreated-diet, the emphasis is rather on the effect of the treatment itself on the nutritional quality of Jatropha meal.

Experiment 2:

Effect of phytase in diets containing Jatropha meal or soybean meal sufficiently supplemented with P: Effect of high levels of dietary Jatropha meal on liver and fore-gut histology

Additional information will be provided by histological analyses which can be regarded as a good indicator of the adverse effects of plant ingredients on fish health and welfare.

Experiment 3:

Experiment 3-I: Energy metabolism response of common carp to the diets containing high level of Jatropha kernel meal (at 75% replacement with fish meal) with and without phytase in diets without P supplementation.

The aim of this experiment is to assess to what extent phytase supplementation can facilitate the utilisation of endogenous P for common carp. Moreover, bioenergetics assessment concerns the rates of energy expenditure, the losses and gains, and the efficiency of energy transformation, as functional relations of whole organism, not only provides a framework for the study of the feeding regime and growth rate of fish, but also provide some insights into the root causes of these relationships based upon the partitioning of energetic resources within the organism. These evaluations will be carried out in a computer operated

respiration chamber system where the oxygen consumption will be measured automatically. Furthermore, together with the chemical analysis of diets, this may allow a more detailed estimation of the nutritive value of a particular protein source in a complete diet for fish.

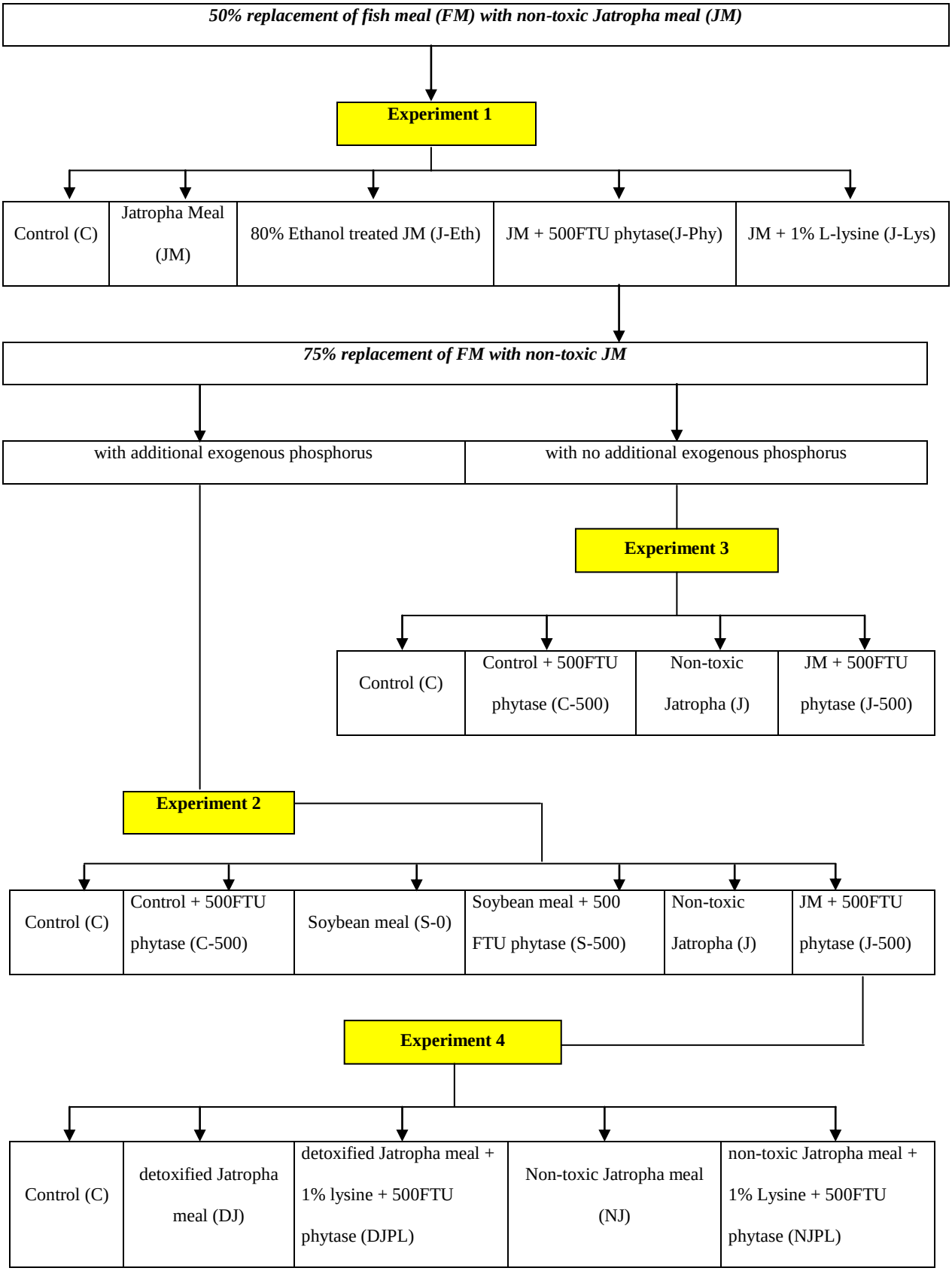
Experiment 3-II:

This study will replicate Experiment I in which the fish will be kept in an aquaria system with groups of four fish in each aquaria. Since common carp normally live in groups, separating them in respiration chambers could cause some stress. Therefore, it will be important to do a parallel experiment in order be able to assess any discrepancies due to this cause.

Experiment 4: Interactive effect of lysine and phytase supplementation of diets containing high level of detoxified Jatropha or non-toxic Jatropha kernel meal (at 75% replacement with fish meal).

In Experiments 1,2 and 3 with non-toxic variety of Jatropha meal (in which phorbolesters are not detected), we expect to be able to clarify the problems which affect the nutritional value of the aforementioned diets. In this experiment a detoxified Jatropha kernel meal receiving appropriated treatment will also be compared with non-toxic Jatropha meal.

Figure 1: Flow chart of experiments



2. Material and methods

2.1 Fish meal, wheat meal and preparation of Jatropha seed meal (detoxified and non-toxic)

Fish meal (65% CP, Normtyp) was obtained from Württembergische Zentralgenossenschaft (Germany). Whole wheat meal was purchased from local market.

Non-toxic Jatropha seeds were obtained from the Papantala region of Veracruz state in Mexico. Non-toxic Jatropha seeds were shelled and ground with a domestic coffee grinder. The ground meal was defatted with petroleum ether (boiling point 40-60 ^{o}C) for 8 h. The residues were air dried, ground and extracted with petroleum ether for another 8 h. At the end, Jatropha seed meal was autoclaved at 121^{o}C for 15 min. with 66% moisture (to inactivate trypsin inhibitor and lectin, Makkar and Becker, 1999) and then lyophilised.

Toxic variety of Jatropha seeds were obtained from India. They were shelled and ground and the pulverised meal defatted with petroleum ether (boiling point 40-60 ^{o}C) for 8 h using a Soxhlet apparatus. The residues were air dried, ground again and extracted with petroleum ether for another 8 h. Toxic Jatropha meal (TJM) was extracted with organic solvent (80% ethanol) to remove phorbolesters by using a Soxhlet apparatus for 16 hours. Finally, the Jatropha seed meal was autoclaved at 121^{o}C for 15 min. at 66% moisture (Makkar and Becker, 1999) and then lyophilised.

2.2 Diet Formulation

Before inclusion of ingredients to the diets the chemical proximate analysis (DM, CP, CL, CA and GE), antinutrients (trypsin inhibitor activity, lectin, phytic acids, tannins,

saponins and phorbolesters), non-starch polysaccharides and mineral analysis were performed.

In diets preparation, all the ingredients were thoroughly mixed before adding water and oil. The resulting dough was passed through a 2mm pellet disc. The moist pellets (feeds) were placed in an oven to be dried at 35^{o}C. The feeds were sealed in polyethylene packets and stored at –18^{o}C.

2.2.1 Experiment 1

Before inclusion of Jatropha meal (JM) into the diets, a portion of JM was extracted with 80% aqueous ethanol (1:2 w/v) which was vigorously mixed using an Ultra-Turrax T25 for 15 min. After centrifugation (3000 rpm for 15 min.) and removal of the supernatant, the residue was extracted again with 80% aqueous ethanol for another 15 min. To evaporate the residual ethanol, the sample was first air dried for 24 h and then transferred to an oven adjusted at 35^{o}C for another 24 h.

Five diets were formulated to be isonitrogenous (37% CP) and isoenergetic (19.7 ± 0.2 kJ g^{-1}) (Table 1.2). The basic diet (Control) had only fish meal as the main source of protein. Fish meal was replaced at 50% of total crude protein by either Jatropha meal (Diet Jat) or ethanol extracted Jatropha meal (Diet J-Eth). Diets J-Lys and J-Phy had the same composition as diet Jat but they were supplemented either with 1% L-lysine or 500 FTU kg^{-1} phytase (5000G, Natuphos®; BASF Ludwigshafen, Germany), respectively. One FTU is defined as the amount of enzyme that liberates 1 µmol of inorganic phosphorus/min from 5.1mmol/*l* of sodium phosphate at 37^{o}C and 5.5 pH (Engelen et al., 1994).

2.2.2 Experiment 2

Six diets were formulated to be isonitrogenous (about 37.4 ± 0.5 CP) and isoenergetic (about 20.0 ± 0.2 kJ g^{-1}) (Table 2.2). Diet Control (Diet C-0) had only fish meal as the main source of protein. Fish meal was replaced at 75% of total crude protein by either Jatropha meal (Diet J-0) or Soybean meal (Diet S-0). Diets C-500, S-500 and J-500 had the same composition as diets C-0, S-0 and J-0 respectively but they were supplemented with 500 FTU kg^{-1} phytase (5000G, Natuphos®; BASF Ludwigshafen, Germany).

2.2.3 Experiment 3 (respiration system (3-I) and aquaria (3-II))

Four diets were formulated (Table 3.2) to be isonitrogenous (37.8 ± 0.7% CP) and isoenergetic (19.8 ± 0.2 kJ g^{-1}). The base diet (Diet C-0) contained fish meal as the main source of protein. Fish meal was replaced at 75% of total crude protein by non-toxic Jatropha meal (Diet J-0). Diets containing fish meal (C-0 and C-500) received sub-optimum level of phosphorus at about 0.3% of dry matter of diets whereas diets containing Jatropha were not supplemented with exogenous phosphorus. Diets C-500 and J-500 had the same composition as diets C-0 and J-0 but they were supplemented with 500 FTU kg^{-1} phytase (5000G, Natuphos®; BASF Ludwigshafen, Germany), respectively.

2.2.4 Experiment 4

Five diets were formulated to be isonitrogenous (about 38.1 ± 0.7 % CP) and isoenergetic (about 20.1 ± 0.3kJ) (Table 4.2). The basic diet (Diet C) had only fish meal as the main source of protein. Fish meal was replaced at 75% of total crude protein by either non-toxic Jatropha meal (Diet NJ) or detoxified Jatropha meal (Diet DJ). Diets NJPL and DJPL

had the same composition as diet NJ and DJ , respectively, but were supplemented with 1% L-lysine and 500 FTU kg^{-1} phytase (5000G, Natuphos®; BASF Ludwigshafen, Germany). One FTU is defined as the amount of enzyme that liberates 1 µmol of inorganic phosphorus/min from 5.1mmol/*l* of sodium phosphate at 37°C and 5.5 pH (Engelen et al., 1994).

2.3 Experimental set up

A group of 1000 common carp (*Cyprinus carpio* L.) fingerlings (about 1 g) reared at Institute for Coastal and Inland Fisheries (Ahrensburg, Germany) were transferred to Hohenheim University, and kept in 200 *l* tanks and maintained at 23 ± 1°C. They were fed with Hohenheim standard feed (40% CP, 10% CL, 10% CA and GE 20kJ g^{-1}) until they had reached a body weight suitable for each experiment.

The aquaria system was part of a recirculating system and was subjected to a 12 h light: 12 h dark photoperiod.

In each experiment fish were weighed individually at the beginning and end of the experiment, but in groups at weekly intervals to adjust the feeding level for subsequent week. No feed was given on the weighing days. Before starting the experiment, ten fish of the same population were killed and analysed for initial body composition. The fish were autoclaved at 107°C for 30 min, homogenised and freeze-dried for the proximate analysis and mineral composition of whole body.

2.3.1 Fish and feeding

2.3.1.1 Experiment 1

Ninety fish were randomly selected and divided into fifteen groups of six fish (6.3 ± 0.3 g), which were stocked in ten 45 *l* aquaria at 25 ± 1 ^{o}C. Water quality was monitored during the experiment and dissolved oxygen and pH fluctuated around 6.8 ± 1.2 mg l^{-1} and 7.4 ± 1.1 respectively. The ten groups of fish were randomly assigned to five treatments. The fish were starved for 2 days before the start of experiment, while during the experimental period, they were fed at levels of (7 x 3.2 x body mass (kg) $^{-0.8}$) per day, equivalent to about seven times maintenance, provided in seven equal instalments (fed at 8^{00}, 9^{30}, 11^{30}, 13^{00}, 14^{30}, 16^{30} and 18^{00}) using an automatic feeder.

2.3.1.2 Experiment 2

Ninety six fish were randomly selected and divided into twenty four groups of four fish (6.3 ± 0.2 g), each of which were stocked in ten 45 *l* aquaria at 23 ± 2 ^{o}C. The twenty four groups of fish were randomly assigned to six treatments. During the experimental period, they were fed at levels calculated to supply five times maintenance requirement (5 x 3.2 x (body mass (kg) $^{-0.8}$) per day in five equal instalments (fed at 8^{00}, 10^{30}, 13^{00}, 15^{30} and 18^{00}) using an automatic feeder.

2.3.1.3 Experiment 3

2.3.1.3.1 3-I:In respiration chambers: A computer-controlled system for the continuous determination of metabolic rates of fish

An automatic respiration system linked to a desktop computer for the continuous measurement of oxygen consumption in fish was used in this experiment. This system accommodates 15 chambers (17 cm x 17 cm x 39 cm; volume 11.3 *l*), and measures the

oxygen concentration in the in- and outflow water as well as the flow rate of each aquarium once every 45 minutes (Focken et al. 1994). The consumption is calculated as the difference between in- and outflow and is later converted to an energy basis by means of an oxyenergetic equivalent ((14.85 x kJ ($g\ O_2$)$^{-1}$, Huisman, 1976a).

Twelve fish from the same stock as experiment (A-II) (with a mean body mass 20.2 ± 2.3 g) were randomly selected and divided up between twelve respiration chamber at 23 (±0.5)^{o}C and a photoperiod of 12 h light: 12 h dark. Before the start of experiment, a 45 days acclimatisation period was carried out and during this period fish were fed the standard Hohenheim diet (crude protein 40%, lipid 10%, ash 10% and energy 20 kJg^{-1}) at a level to provide the maintenance requirement (3.2 g kg$^{-0.8}$ day^{-1}). Water flow through the respirometer chambers was controlled at 0.5-0.6 l min^{-1}. During the first four weeks of experiment, once a week, about two third of water was replaced and the oxygen electrode calibrated. However, water quality parameters fluctuated higher in the last four weeks of experiment, thus, about one third of water was replaced every day. During the experimental period the water quality parameters were monitored, pH: 7.0 ± 0.8, DO: 7.1 ± 1.0 mg l^{-1}, nitrite: 0.1 ± 0.3, nitrate: 1.8 ± 0.3 and NH_3-NH^{4+} 0.16 ± 0.6. The fish were starved for 2 days before the start of experiment, while during the experimental period, they were fed at levels calculated to supply five times maintenance (5 x 3.2 x (body mass (kg) $^{-0.8}$) per day in five equal instalments (fed at 8^{00}, 10^{30}, 13^{00}, 15^{30} and 18^{00}) using an automatic feeder. Fish were weighed at weekly intervals to adjust the feeding level for subsequent week. No feed was given on the weighing days.

2.3.1.3.2 3-II: In aquaria system

Thirty two fish were randomly selected and divided into 16 groups of 2 fish (21.8 ± 0.8 g), each of which were stocked in ten 45 *l* aquaria at 23±1.0 ^{o}C. The sixteen groups of fish were

randomly assigned to four treatments. The fish were starved for 2 days before the start of experiment, while during the experimental period, they were fed at levels calculated to supply five times maintenance (5 x 3.2 x (body mass (kg) $^{-0.8}$) per day in five equal instalments (fed at 8^{00}, 10^{30}, 13^{00}, 15^{30} and 18^{00}) using an automatic feeder.

2.3.1.4 Experiment 4

Eighty fish were randomly selected and divided into twenty groups of four fish (4.0 ± 0.1 g), which were stocked in ten 45 *l* aquaria at 28 ± 3 ^{o}C. To keep the water quality parameters above standard one third of water was daily changed with fresh water. The twenty groups of fish were randomly assigned to five treatments. The fish were starved for 2 days before the start of experiment, while during the experimental period, they were fed at levels of (5 x 3.2 x (body mass (kg) $^{-0.8}$) per day in five equal instalments (fed at 8^{00}, 10^{30}, 13^{00}, 15^{30} and 18^{00}) using an automatic feeder.

2.4 Analysis of antinutrients

All the analysis was performed in duplicates.

2.4.1 Trypsin inhibitors (TIs)

Trypsin inhibitor activity was measured by the enzymatic method of Smith et al. (1980) and some modifications as suggested by Liu and Markakis (1989). Briefly, 12.5 ml of 0.01 M NaOH was added to the 0.25 g dry matter fat-free sample. Sample was Ultra-turraxed for 30 sec. pH was adjusted to 9.4-9.6 with 1 M NaOH or 1 M HCl and ultra-turraxed for 5 min. Then, sample was centrifuged (3600 rpm) for 15 min. The supernatant was slowly

collected by pipetting this between the residue and the fatty layer on the top of supernatant. The solution was used for the assay. From solution a sample blank (0.25 *ml* Distilled water, 0.25 *ml* sample, 1.25 *ml* BAPNA) and a sample (0.25 *ml* Distilled water, 0.25 *ml* sample, 1.25 *ml* BAPNA) was prepared. These were kept at 37^{o}C for 10 min. Then sample was received 0.5 *ml* trypsin and exactly was kept for another 10 min. in 37^{o}C. Immediately 0.25 *ml* 30% acetic acid was added to the samples. The content of tubes were transferred in eppendorf tubes and centrifuged at 15000 rpm for 10 min. The absorbance was read against a blank at 410 nm using a photometer.

2.4.2 Lectin

Lectin was determined using the haemagglutination method as described by Aregheore et al. (1998). Briefly, to 1.0 g fat-free sample 20 *ml* phosphate buffer saline (PBS) was added and extracted under stirring condition using a magnetic stirrer at approximately 1-4^{o}C for 16 hours. Then the content was centrifuged at 4000 g for 20 min. Twofold serial dilutions of the samples were prepared with PBS in a microtiter plate and these were mixed with equal volume of 1% erythrocyte suspension. The sedimentation of 1% erythrocytes suspensions were read after 3 hours incubation at room temperature. Minimum amount of the sample required to show the agglutination after two fold dilution in 1 ml of final assay .

2.4.3 Phorbolesters (PEs)

Extraction of phorbolesters (PEs) and HPLC estimation (using reverse phase C18, LiChrospher 100, endcapped 5 µm column) was done according to the procedure described by Makkar et al. (1997). Briefly, 0.5 g of Jatropha meal was extracted four times with methanol.

A suitable aliquot was loaded on HPLC column. The column was projected with a head column containing the same material. The separation was performed at room temperature (23^{o}C) and the flow rate was 1.3 ml/min using a gradient elution (Makkar, 2007). The four-PEs peaks were detected at 280 nm and appeared between 25.5 and 30.5 min. The results were expressed as equivalent to a standard, phorbol-12-myristate 13-acetate. Detection limit of PEs is 3µg/g meal. PEs in Jatropha meal was measured after 30 minutes, 2 hours, 6 hours, 8 hours and 16 hours of extraction with 80% ethanol.

2.4.4 Phytic acids

Phytic acid estimation was carried out by the modified spectrophotometric procedure of Vaintraub and Lepteva (1988). Briefly, 5 g plant materials in form of powder was extracted with 100 ml of 3.5% HCl for 1 h at room temperature using a magnetic stirrer. The content was centrifuged at 3000 g for 10 min. at room temperature. The supernatant was removed. A suitable amount of aliquot (between 1 to 5 ml) was diluted with distilled water to a final volume of 25 ml. 10 ml of solution was passed through a 200-400 mesh AGI X8 chloride anion exchange column (0.5g). The solution was eluted with 15 ml of 0.1 M NaCl and subsequently the phytate was eluted with 15 ml of 0.7 M NaCl. 3 ml of above eluted sample was taken in a test tube and 1 ml of Wade reagent was added. The solution was vortexed and then centrifuged at 3000 x g for 10 min. The absorbency was read photometrically at 500 nm against a reagent blank.

2.4.5 Phenolics and Tannins

The total phenolics and tannins were determined by the spectrophotometric method described by Makkar (1993). Briefly, 0.5 g plant samples was taken and transferred to a 25 ml

beaker. 10 ml of aqueous acetone (70%) was added to the sample and beaker was suspended in a water bath and subjected to ultrasonic treatment for 20 min. at room temperature. The content of beaker was centrifuged at 3000 x g for 10 min and 4^{o}C. Supernatant was collected. A suitable aliquot was taken in a test tube and the volume was made to 500 µl with distilled water. then to the solution 250 ml of the folin-Ciocalteu reagent and 1.25 ml of the sodium carbonate was added. The solution was vortexed and the absorbance was read at 725 nm after incubation period of 40 min. This value shows the total phenolics content of sample.

For tannins, to 100 mg PVPP in a test tube, 1.0 ml distilled water and 1.0 of supernatant prepared for phenolics was added. The mixture was vortexed at 4oC and centrifuged at 3000 x g for 10 min. The supernatant was collected. This supernatant contains only simple phenolics and tannins were precipitate by PVPP). Again the phenolic content of the sample was measured as described above and tannin content was expressed as difference of total phenolics and non-tannin phenolics.

2.4.6 Saponins

Total saponin content was measured by the method of Hiai et al. (1976). Briefly, 10 g of fat free plant sample was transferred in to a 250 ml flask and 100 ml of 50% methanol was added. The sample was stirred overnight. The content was centrifuged at 3000 x g for 10 min. at 4^{o}C and the supernatant was collected. The extraction was repeated with the same solvent and supernatant was collected and was added to the first extracted. Then the methanol was evaporated from the solution under a *in vacuo* condition. Then the aqueous phase was centrifuged at 3000 x g for 10 min. at 4^{o}C. The aqueous solution was extracted with equal volume of chloroform to remove pigments (3 times). Finally concentrated saponins in the aqueous solution were extracted with equal volume of *n*-butanol (2 times). The solvent *n*-butanol was evaporated under a *in vacuo* condition. The dried saponin was dissolved with 5-

10 ml distilled water and solution was transferred into a separate pre-weighed container. After freeze-drying the fraction the percentage of recovery was calculated.

2.5 Amino acid and non-starch poly-saccharide analysis

An automated amino acid analyser (LKB 4151, Alpha-plus, LKB Biochrome, UK) was used to determine the amino acid composition of feed ingredients. The samples were hydrolysed for 24 h with 6 N HCl at 110^{o}C and the sulphur-containing amino acids were oxidised using performic acid before acid hydrolysis.

The analysis of non-starch polysaccharide was carried out in Englyst Carbohydrates Ltd. laboratory, Southampton, S016, Uk.

2.6 Mineral analysis

2.6.1 Phosphorous analysis

Phosphorous determination was performed based on an ammonium-vanadate-molybdate method developed by Gerike and Kurmies (1952). Briefly, 500 mg of samples were weighed in porcelains and they were left over night at 500^{o}C in an oven. After cooling down 5 ml HNO_3 was added to the samples. After all vaporised 5 ml HCl was added to the samples to dissolved ash. With distilled water all samples were reached to 50 ml volume. Then 50µl of each sample was added to cuvettes. The volumes were made to 1.75 ml by adding HCL. To each sample 0.75 vanadate-molybdate was added. The samples left for 2 hours and they were read at 436 nm in a photometer.

2.6.2 Experiment 1

Mineral composition of whole body of fish was determined using a Siemens SRS 200 X-Ray Fluorescence Spectrometer with the aid of the software Spectra 3000 Ver. 3.3 in the Institute for Soil Science, University of Hohenheim.

2.6.3 Experiment 4

Mineral composition of whole body of fish, ingredients and diets (except phosphorus) was analysed in Gesellschaft für Labor- and Ingenieurdienstleistung Prignitz, Wittenberg.

2.7 Biochemical analysis of ingredients, diets and whole body of fish

The proximate analysis of ingredients, diets and whole bodies of fish (dry matter, DM; crude protein, CP; crude ash, CA) were based on the procedures of the AOAC (1990) standard methods. A modified Smedes method (1999) was used for the measurement of the lipid content of ingredients, experimental diets and whole body of fish (Schlechtriem et al., 2003). Moisture content was measured by putting samples in an oven for 24 h at 105^{o}C. Gross energy was measured using a bomb calorimeter (IKA-calorimeter C7000) with benzoic acid as a standard.

2.8 Calculation of growth parameters

Growth performance and diet nutrient utilisation were analysed in terms of percentage of body mass gain (BMG), feed conversion ratio (FCR), specific growth rate (SGR), protein

efficiency ratio (PER), protein productive value (PPV), energy retention (ER) and nutrient gain (mg/100g average body mass (ABM) per day). The following formulae were used:

BMG = final body mass – initial body mass

FCR= dry matter feed intake / fresh body mass gain

SGR (%) =100 x [ln (FBM) – ln (IBM)]/no. of days

PER = 100 x (wet weight gain/ crude protein intake)

PPV (%) =100 x (protein $_{Final}$ –protein$_{Initial}$)/crude protein intake

ER (%) = 100 x (energy $_{Final}$ –energy$_{Initial}$)/gross energy intake

Intake (mg/100g ABM/day) = 100 x total component intake (mg) / $((\sum_{i=1}^{i=n} BM_i)/n)$ /duration

Gain (mg/100g ABM/day) = 100 x (FBM component (mg) – IBM component (mg)/ $((\sum_{i=1}^{i=n} BM_i)/n)$/duration

where n is number of weekly weighing, FBM is final body mass and IBM is initial body mass, Component regarded was protein, lipid, energy, ash, and minerals.

2.9 Calculation of oxygen consumption and energy budget

The total oxygen consumption was calculated based on the data gathered from the respirometric system. Gross energy (GE) of fish (initial (GE_{ini}) and final (GE_{fin})), total GE fed (GE_{fed}), energy retention (ER), energy expenditure (EE), metabolisable energy (ME); efficiency of energy retention (ER/EE); apparent unmetabolisable energy (AUE) were calculated as below:

The oxygen consumption was calculated based on data gathered daily from respirometric system.

GE_{ini} (kJ) = initial dry weight of fish (g) x GE (kJ^{-1}) of dried fish

GE_{fin} (kJ) = final dry weight of fish (g) x GE (kJ^{-1}) of dried fish

GE_{fed} (kJ) = total feed intake x GE (kJ) of feed

ER (% of GE fed) = 100 x (GE_{fin} – GE_{ini})/ GE_{fed}

EE (% of GE fed) = 100 x (total oxygen consumption (g) x Q_{oxy}^{1})/ GE_{fed}

ME (% of diet) = EE (kJ) + ER (kJ)

EE/CP retained (kJ g^{-1}) = total EE (kJ)/ total protein retained in body (g)

AUE (% of GE_{fed}) = GE_{fed} (kJ) – EE (% of GE fed) – ER (% of GE fed)

1, Q_{oxy}, the oxyenergetic equivalent for growth, was 14.85 kJ for each gram of oxygen consumed by the fish (Huisman 1976a).

2.10 Blood cholesterol estimation

Blood was collected from the caudal vein with a heparinised syringe and was immediately centrifuged at 10000 rpm for 2 min. Plasma was transferred to a new vial and kept at 4^{o}C for plasma cholesterol analysis using an enzymatic kit from Roche/Hitachi (Mannheim, Germany).

2.11 Body morphological traits

The calculation of body morphological traits was performed for each fish individually. These parameters included condition factors (CF), relative profile (RP), viscerosomatic index (VSI), relative intestinal index (RIL) and hepatosomatic index and were calculated as below.

CF (%) =100 x fish weight (g) / (fish length)^{3}

RP = fish height/fish length

VSI (%) = 100 x viscera weight (g)/ fish weight (g)

RIL = Intestine length / fish length

HSI (%) = 100 x liver weight (g)/ fish weight (g)

2.12 Statistical analysis

2.12.1 Experiment 1 and 4

All results were subjected to one-way analyses of variance (ANOVA) and the significance of the differences between means was tested using Duncan's multiple range test. Differences were deemed to be significant at $\rho < 0.05$. The software used was Statistica® Version 5.1 (Statsoft, Tulsa, USA). Values are expressed as mean ± standard deviation.

2.12.2 Experiment 2

All results were subjected to 3 X 2 factorial analyses of variance (ANOVA) to assess the significance of feed and phytase effects as well as that of their interaction. Effects were deemed to be significant at $\rho < 0.05$. Whenever the ANOVA showed effects to be significant, Duncan's multiple range test was used to analyse the differences in the mean values of the four factor combinations. The software used was Statistica® Version 5.1 (Statsoft, Tulsa, USA). Values are expressed as means and pooled standard error; where significant effects were found, the results of the Duncan test are shown by subscript letter codes.

2.12.3 Experiment 3 (3-I and 3-II)

All results were subjected to 2 X 2 factorial analyses of variance (ANOVA) to assess the significance of feed and phytase effects as well as that of their interaction. Effects were deemed to be significant at $\rho < 0.05$. Whenever the ANOVA showed effects to be significant,

Duncan's multiple range test was used to analyse the differences in the mean values of the four factor combinations. The software used was Statistica® Version 5.1 (Statsoft, Tulsa, USA). Values are expressed as means and pooled standard error; where significant effects were found, the results of the Duncan test are shown by subscript letter codes.

3. Results

3.1 Experiment 1

3.1.1 Composition of experimental diets

Table 1.3a shows the proximate composition of the experimental diets. Diets did not vary with respect to crude protein, lipid, and gross energy. Crude ash was higher in diet Control (about 13%) than diets Jat, J-Eth, J-Lys and J-phy (about 11.2%, 10.6, 10.7 and 11.1% respectively).

All the essential amino acids in diets exceeded the requirement of common carp (NRC, 1993); however, lysine in diets Jat, J-Eth and J-Phy is marginally adequate (1.8%). pH was about 6.3 in control and 6.4 in other diets.

Table 1.3b shows the antinutrient and non-starch polysaccharide levels in Jatropha meal. Total phenolics, tannins and saponins were not detected in diet J-Eth. Phytic acid was recorded at high levels in all experimental diets (about 2.4%) except for control. Phorbolesters were not detected in any test diet.

3.1.2 Growth performance

Fish in all experimental groups fed actively on the test diets (calculated 7 times maintenance per day) and no mortality was observed during the study period. Table 1.4 presents feed utilisation and growth performance of fish during experimental period. At the end of the feeding period (52 days), fish fed diets Jat and J-Eth showed lower BMG (%), SGR and higher FCR when these were compared to those of the control group. Moreover, BMG (%), SGR of fish fed diets J-Lys and J-Phy did not differ significantly either from those of

fish fed the control diet nor those of fish fed diets Jat and J-Eth. Feed intake and protein efficiency among the experimental groups was not significantly different ($p < 0.05$).

3.1.3 Chemical composition of whole body

Chemical composition of whole body of fish at the beginning and at the end of experiment is given in Table 1.5. Replacement of fish meal with J-Eth meal significantly decreased and increased whole body moisture and lipid respectively. Fish fed diets Control and J-Phy had higher crude ash than fish fed other experimental diets ($p > 0.05$). There was no difference in crude protein of fish fed the experimental diets. However, gross energy was significantly higher in fish fed J-Lys.

3.1.4 Retention and gain

Protein productive value (expressed as percentage of intake) and energy retention (Table 1.5) were not affected by the different dietary treatments. However, Protein gain (expressed as mg/100g average body mass/day) was significantly lower in fish fed diet Jat compared to those fish which received the control diet. Fat gain by fish fed the control diet was significantly lower than in fish fed diets Jat, J-Eth, J-Lys and J-phy. Phytase addition significantly improved ash gain to a level comparable to the fish in the control group ($p < 0.05$).

3.1.5 Mineral retention and gain (mg/100g average body mass/day)

Replacement of fish meal with Jatropha meal significantly reduced whole body P, Mg, K and Fe levels, whereas addition of phytase increased P, Mg, K and Fe levels to those of fish fed the control diet (Table 1.6).

Na, Mg, P and Ca gain (mg/100g average body mass/day) was significantly higher in fish fed the control diet than in other experimental fish. However, fish fed the diet containing phytase indicated significantly higher Fe gain (mg/100g average body mass/day) than fish fed either control diet or other experimental diets (Table 1.6).

Table 1.1 a: Proximate composition, amino acids composition, and mineral content of ingredients

	Fish meal	Wheat meal	Jatropha meal	Ethanol extracted Jatropha
Proximate composition (%DM)				
Dry matter	90.4	88.0	93.5	93.0
Crude protein	71.3	14.5	70.6	73.5
Crude lipid	8.6	1.9	0.6	0.2
Crude ash	20.1	1.7	11.1	12.7
Nitrogen free extract	-	81.9	17.7	13.6
Crude fibre	-	0.1	9.5	10.5
Gross energy (kJ g^{-1})	21.1	19.0	19.2	19.5
Essential amino acids (% DM)				
Arg	3.8	0.6	8.5	9.0
His	2.3	1.0	2.1	2.1
Ile	2.7	0.4	2.7	2.7
Leu	5.1	3.3	4.4	4.5
Lys	5.2	0.4	2.3	2.3
Met + Cys	2.4	0.6	2.1	2.3
Phe + Tyr	4.9	0.9	4.1	4.3
Thr	2.9	0.4	2.3	2.4
Trp	0.8	0.2	0.8	0.9
Val	3.0	0.6	2.9	3.0
Minerals (% DM)				
Na	1.8	-	0.2	0.2
Mg	0.5	-	0.9	0.9
P	2.9	-	2.3	2.3
K	1.1	-	2.0	2.1
Ca	1.03	-	0.4	0.4
Fe	0.22	-	0.04	0.05

Values are means of duplicate determination.

Table 1.1 b: Antinutrients and non-starch polysaccharides level of ingredients

	Fish meal	Wheat meal	Jatropha meal	Ethanol extracted Jatropha
Antinutrients				
Total phenolics[a] (% DM)	-	-	0.4	ND
Tannins[a] (% DM)	-	-	0.15	ND
Saponins[b] (% DM)	-	-	2.2	ND
Trypsin inhibitor, TI (mg/g)[c]	-	-	6.4	6.9
Phytic acid (% DM)	-	-	8.6	9.0
Lectin (mg/mL)[d]	-	-	0.34	0.36
Phorbolesters (mg/g)	-	-	ND	ND
Non-starch polysaccharides (NSP, % DM)				
Rhamnose	-	-	0.2	0.1
Fucose	-	-	0.1	0.1
Arabinose	-	-	2.7	2.3
Xylose	-	-	1.4	1.4
Mannose	-	-	0.3	0.3
Galactose	-	-	1.2	1.1
Glucose	-	-	4.7	3.0
Glucuronic acid	-	-	0.0	0.0
Galacturonic acid	-	-	3.0	3.0
Total NSP	-	-	13.4	11.2

Values are means of duplicate determination. a, as tannic acid equivalents; b, as diosgenin equivalents; c, trypsin inhibitor activity, mg of pure trypsin inhibited/g sample; d, minimum amount of the sample required to show the agglutination after two fold dilution in 1 ml of final assay .

Table 1.2: Composition of experimental diets (%DM)

	Control	Jat[3]	J-Eth[4]	J-Lys[5]	J-Phy[6]
Fish meal	42	21	21	21	21
Wheat meal	50	47	49	46	46.99
Defatted non-toxic Jatropha meal	-	23	-	23	23
Ethanol extracted Jatropha meal	-	-	21	-	-
L-lysine	-	-	-	1	-
Phytase (5000G, Natuphos)	-	-	-	-	0.01
Sunflower oil	4	5	5	5	5
Minerals premix[1]	2	2	2	2	2
Vitamin premix[2]	2	2	2	2	2
Total	100	100	100	100	100

Values are means of duplicate determination.

1, Mineral premix (g kg^{-1}): $CaCO_3$ 336g, KH_2PO_4 502g, $MgSO_4.7H_2O$ 162g, NaCl 49.8 g, Fe(II) gluconate 10.9g, $MnSO_4.H_2O$ 3.12g, $CuSO_4.5H_2O$ 0.62g, KI 0.16g, $CoCl_2.6H_2O$ 0.08g, NH_4molybdate 0.06g, $NaSeO_3$ 0.02g..

2, Vitamin premix (mg or IUg^{-1}): retinol palmitate 500.000 IU thiamine 5 mg; riboflavin 5mg; niacin 25 mg; folic acid 1 mg; biotin 0.25 mg; pyridoxine 5 mg; cyanocobalamine 5 mg; ascorbic acid 10 mg; choleocalciferol 50,000 IU; α-tocopherol 2.5 mg; menadione 2 mg; inisitol 25 mg; pantothenic acid 10 mg; choline chloride 100 mg..

3, non-toxic Jatropha meal; 4, ethanol treated jatropha meal; 5, jatropha meal plus 1% lysine; 6, Jatropha meal plus 500 FTU phytase.

Table 1.3 a: Proximate composition, pH, amino acids composition and requirement of common carp (NRC, 1993) and mineral content of experimental diets

	Control	Jat[1]	J-Eth[2]	J-Lys[3]	J-Phy[4]	req. of carp[5]
Proximate composition (% DM) and pH						
Dry matter	93.8	93.1	92.4	93.2	92.5	
Crude protein	37.6	37.1	37.9	38.1	37.3	
Crude lipid	9.4	8.3	8.2	7.6	7.7	
Crude ash	13.6	11.2	10.6	10.7	11.1	
Nitrogen-free extract	39.4	43.4	43.3	43.6	43.9	
Crude fibre	0.8	2.9	2.9	2.9	2.9	
Gross energy (kJ g^{-1})	19.5	19.9	19.9	19.8	19.5	
pH	6.3	6.4	6.4	6.3	6.4	
Essential amino acids (% DM)						
Arg	1.9	3.1	3.2	3.1	3.1	1.3
His	1.5	1.4	1.3	1.4	1.4	0.6
Ile	1.3	1.4	1.5	1.4	1.4	0.8
Leu	3.8	3.6	3.7	3.6	3.6	1.0
Lys	2.4	1.8	1.8	2.8	1.8	1.7
Met + Cys	1.3	1.3	1.6	1.3	1.3	0.9
Phe + Tyr	2.5	2.4	2.7	2.4	2.4	2.0
Thr	1.4	1.3	1.4	1.3	1.3	1.2
Trp	0.4	0.5	0.6	0.5	0.5	0.2
Val	1.6	1.6	1.7	1.6	1.6	1.1
Minerals (% DM)						
Na	0.86	0.53	0.54	0.53	0.53	
Mg	0.50	0.62	0.60	0.62	0.62	
P	2.16	2.08	2.08	2.08	2.08	
K	1.40	1.64	1.67	1.64	1.64	
Ca	1.06	0.93	0.94	0.93	0.93	
Fe	0.11	0.08	0.08	0.08	0.08	

Values are means of duplicate determination.
1, non-toxic Jatropha meal; 2, ethanol treated jatropha meal; 3, jatropha meal plus 1% lysine;
4, Jatropha meal plus 500 FTU phytase ; 5, requirement of common carp (NRC, 1993).

Table 1.3 b: Antinutrients and non-starch polysaccharides level of experimental diets

	Control	Jat[5]	J-Eth[6]	J-Lys[7]	J-Phy[8]
Antinutrients					
Total phenolics[1] (% DM)	-	0.1	ND	0.1	0.1
Tannins[1] (% DM)	-	0.04	ND	0.04	0.04
Saponins[2] (% DM)	-	0.5	ND	0.5	0.5
Trypsin inhibitor, TI (mg/g)[3]	-	1.5	1.7	1.5	1.5
Phytic acid (% DM)	-	2.1	2.5	2.1	2.1
Lectin (mg/mL)[4]	-	0.1	0.1	0.1	0.1
Phorbolesters (mg/g)	-	ND	ND	ND	ND
Non-starch polysaccharides (NSP, % DM)					
Rhamnose	-	0.07	0.02	0.07	0.07
Fucose	-	0.02	0.02	0.02	0.02
Arabinose	-	0.46	0.48	0.46	0.46
Xylose	-	0.23	0.29	0.23	0.23
Mannose	-	0.18	0.06	0.18	0.18
Galactose	-	0.69	0.23	0.69	0.69
Glucose	-	0.90	0.63	0.90	0.90
Glucuronic acid	-	0.00	0.00	0.00	0.00
Galacturonic acid	-	0.37	0.63	0.37	0.37
Total NSP	-	2.85	2.35	2.85	2.85

Values are means of duplicate determination. 1, as tannic acid equivalents;2, as diosgenin equivalents; 3, trypsin inhibitor activity, mg of pure trypsin inhibited/g sample; 4, minimum amount of the sample required to show the agglutination after two fold dilution in 1 ml of final assay .
5, non-toxic Jatropha meal; 6, ethanol treated jatropha meal; 7, jatropha meal plus 1% lysine;
8, Jatropha meal plus 500 FTU phytase .

Table 1.4: Fish growth performance during experimental period (52 days)

	Control	Jat[1]	J-Eth[2]	J-Lys[3]	J-Phy[4]
Initial weight (g)	6.3 ± 0.3	6.3 ± 0.1	6.4 ± 0.1	6.5 ± 0.0	6.4 ± 0.2
Final weight (g)	46.1 ± 0.3^{a}	38.1 ± 1.4^{b}	38.5 ± 0.9^{b}	42.9 ± 0.1^{ab}	43.5 ± 5.1^{ab}
Body weight gain (%)	631 ± 35.9^{a}	503 ± 11.8^{b}	499 ± 1.4^{b}	560 ± 5.6^{ab}	580 ± 56.70^{ab}
Feed intake (g)	34.5 ± 1.18	31.8 ± 0.7	31.6 ± 0.3	34.2 ± 0.3	34.5 ± 2.3
Feed conversion ratio	0.87 ± 0.04^{a}	1.00 ± 0.02^{c}	0.98 ± 0.01^{c}	0.94 ± 0.01^{b}	0.93 ± 0.05^{ab}
Specific growth rate (%)	4.06 ± 0.10^{a}	3.67 ± 0.04^{b}	3.65 ± 0.00^{b}	3.85 ± 0.02^{ab}	3.91 ± 0.17^{ab}
Protein efficiency ratio	3.1 ± 0.04	2.7 ± 0.11	2.7 ± 0.06	2.8 ± 0.01	2.9 ± 0.38

Values in the same row with the same superscripts are not significantly different ($p < 0.05$).
Values are mean (n = 3) ± standard deviation
1, non-toxic Jatropha meal; 2, ethanol treated jatropha meal; 3, jatropha meal plus 1% lysine;
4, Jatropha meal plus 500 FTU phytase

Table 1.5
Initial and final whole body chemical composition, retention and gain of nutrients experimental fish

	Initial fish	Control	Jat[1]	J-Eth[2]	J-Lys[3]	J-Phy[4]
Chemical composition (% wet basis)						
Moisture	80.6	77.4 ± 0.3^{a}	76.6 ± 0.03^{ab}	76.0 ± 0.81^{b}	76.6 ± 0.49^{ab}	76.5 ± 0.23^{ab}
Crude protein	13.1	13.8 ± 0.13	13.7 ± 0.42	13.9 ± 0.08	14.2 ± 0.30	14.1 ± 0.11
Crude lipid	3.2	5.5 ± 0.01^{c}	6.8 ± 0.01^{b}	7.3 ± 0.40^{a}	6.5 ± 0.37^{b}	6.2 ± 0.31^{b}
Crude ash	2.4	2.40 ± 0.08^{a}	2.16 ± 0.02^{b}	2.12 ± 0.07^{b}	2.02 ± 0.09^{b}	2.41 ± 0.04^{a}
Gross energy (kJ g^{-1})	4.4	5.7 ± 0.05^{d}	6.2 ± 0.04^{bc}	6.2 ± 0.08^{ab}	6.3 ± 0.05^{a}	6.1 ± 0.05^{c}
Protein productive value		42.3 ± 0.7	37.0 ± 0.2	37.8 ± 0.7	39.6 ± 0.9	41.1 ± 6.2
Energy retention		34.7 ± 0.3	32.4 ± 1.7	33.9 ± 0.8	35.9 ± 0.1	34.9 ± 4.6
Gain (mg/100g average body mass/day)						
Protein gain		254.2 ± 11.9^{a}	228.9 ± 5.5^{b}	235.3 ± 1.1^{ab}	248.3 ± 3.3^{ab}	244.5 ± 13.3^{ab}
Fat gain		104.4 ± 4.0^{c}	121.8 ± 1.4^{b}	135.6 ± 6.7^{a}	122.6 ± 8.9^{ab}	115.4 ± 1.5^{bc}
Ash gain		44.0 ± 0.6^{a}	35.0 ± 0.9^{b}	34.6 ± 1.0^{b}	33.8 ± 2.1^{b}	40.6 ± 2.4^{a}

Values in the same row with the same superscripts are not significantly different ($p < 0.05$).
Values are mean (n = 3) ± standard deviation
1, non-toxic Jatropha meal; 2, ethanol treated jatropha meal; 3, jatropha meal plus 1% lysine;
4, Jatropha meal plus 500 FTU phytase .

Table 1.6
Initial and final whole body mineral composition (% wet basis) and gain (mg/100g average body mass/day) of experimental fish

	Initial fish	Control	Jat[1]	J-Eth[2]	J-Lys[3]	J-Phy[4]
Na	0.20	0.23 ± 0.05^{a}	0.17 ± 0.00^{ab}	0.17 ± 0.02^{ab}	0.16 ± 0.01^{b}	0.18 ± 0.01^{ab}
Mg	0.04	0.05 ± 0.003^{a}	0.04 ± 0.000^{b}	0.04 ± 0.001^{b}	0.04 ± 0.002^{b}	0.05 ± 0.001^{a}
P	0.50	0.50 ± 0.02^{a}	0.4 ± 0.01^{b}	0.4 ± 0.01^{b}	0.4 ± 0.01^{b}	0.5 ± 0.00^{a}
K	0.30	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}	0.31 ± 0.00^{a}	0.27 ± 0.00^{b}	0.31 ± 0.02^{a}
Ca	0.58	0.62 ± 0.03^{a}	0.52 ± 0.02^{b}	0.51 ± 0.03^{ab}	0.49 ± 0.05^{b}	0.59 ± 0.05^{ab}
Fe	0.007	0.007 ± 0.001^{a}	0.005 ± 0.000^{b}	0.007 ± 0.002^{ab}	0.006 ± 0.001^{ab}	0.008 ± 0.002^{a}
Gain (mg/100g average body mass/day)						
Na		7.7 ± 0.2^{a}	4.7 ± 0.0^{b}	4.6 ± 0.0^{b}	4.7 ± 0.0^{b}	5.2 ± 0.1^{b}
Mg		1.5 ± 0.03^{a}	1.2 ± 0.01^{c}	1.1 ± 0.01^{c}	1.1 ± 0.01^{c}	1.4 ± 0.04^{b}
P		9.2 ± 0.3^{a}	6.3 ± 0.1^{c}	6.4 ± 0.1^{c}	6.6 ± 0.1^{c}	8.6 ± 0.4^{b}
K		4.7 ± 0.2^{a}	4.1 ± 0.1^{bc}	4.3 ± 0.1^{ab}	3.7 ± 0.0^{c}	4.5 ± 0.3^{ab}
Ca		11.5 ± 0.4^{a}	8.5 ± 0.1^{c}	8.3 ± 0.1^{c}	8.2 ± 0.1^{c}	10.2 ± 0.5^{b}
Fe		0.13 ± 0.00^{b}	0.08 ± 0.00^{e}	0.12 ± 0.00^{c}	0.10 ± 0.00^{d}	0.14 ± 0.01^{a}

Values in the same row with the same superscripts are not significantly different ($p < 0.05$).
Values are mean (n = 3) ± standard deviation
1, non-toxic Jatropha meal; 2, ethanol treated jatropha meal; 3, jatropha meal plus 1% lysine;
4, Jatropha meal plus 500 FTU phytase

3.2 Experiment 2

3.2.1 Composition of experimental diets

Table 2.2 shows the proximate composition of experimental diets. Diets did not vary with respect to crude protein, lipid, and gross energy. Crude ash was higher in diet Control (C-0, about 13%) than diets Soybean and Jatropha meal without phytase (S-0, 8.8% and J-0, 9% respectively). Phosphorus (P) was also higher in diet Control (C-0, 3.6%) than Soybean meal and Jatropha meal (S-0, 2.2% and J-0, 2.8% respectively). Phytic acid was found at high levels in diets containing Soybean and Jatropha meal (2.0 and 3.0% respectively). There was no variation in the pH (about 6.3 in Control and 6.4 in other diets) of diets.

3.2.2 Growth performance

As a result of the increasing percentage of Jatropha meal in this experiment, in the preparation phase common carp did not accept 7 times maintenance per day. Therefore, the ration was reduced to 5 times maintenance. The fish in all experimental groups fed actively on the test diets (at 5 times maintenance) and no mortality was observed during the study period. Table 2.3 presents feed utilisation and growth performance of fish during the experimental period. At the end of the eight week feeding period, BMG (%), SGR and FCR of were negatively affected by the replacement of fish meal by plant protein sources. Moreover there was no effect of phytase or interaction of feed and phytase for the aforementioned parameters, nevertheless, fish fed diet J-500 had an improved mean BMG (%), SGR and FCR values when compared to fish fed diet J-0.

3.2.3 Chemical composition of whole body

Whole body composition of fish at the beginning and at the end of the experiment is given in Table 2.4. Replacement of fish meal with Jatropha meal significantly decreased and increased whole body moisture and lipid respectively, whereas fish on the soybean meal diet had similar moisture content but lower lipid than the control group. There was no effect of phytase or interaction of feed and level of phytase on moisture, crude protein, lipid, ash and energy of fish fed the experimental diets ($p > 0.05$).

With respect to whole body phosphorus (P) there was no significant difference between the fish fed either control diet or Jatropha meal or soybean meal. However, addition of phytase significantly improved the level of P in fish fed diets containing phytase, especially in the J-500 group (Jatropha meal with 500 FTU phytase, $p < 0.05$).

Replacement of fish meal with Soybean meal and Jatropha meal significantly affected the level of blood cholesterol level. Fish fed soybean meal indicated significantly lower blood cholesterol than fish fed diets control or Jatropha meal, whereas phytase treatment and interaction of feed and phytase did not affect the serum cholesterol level.

3.2.4 Retention and gain

Replacement of fish meal with plant protein sources negatively affected protein and energy retention of fish whereas neither phytase or the interaction of feed and phytase did affect these parameters (Table 2.5). Feeds and phytase addition significantly affected phosphorous (P) retention. Phytase supplementation significantly improved P retention of fish fed the diet containing Jatropha meal. Moreover, fish fed diet Control (C-0 and C-500) had significantly higher protein and energy gain (expressed as mg/100g ABM per day) than the other groups. Lipid gain was shown to be highest for fish fed Jatropha meal. Meanwhile, P

gain was significantly higher in fish fed diets C-0, C-500 and J-500 than in other experimental fish.

3.2.5 Liver and intestinal histology

The hepatocytes of fish fed diets Control, soybean meal and soybean meal plus phytase (C-0, S-0 and S-500 respectively) showed a regular shape, with moderate cytoplasmic lipid content. The hepatocytes of fish fed Diet Jatropha meal (J-0) showed severe anomalies including small nuclei which were peripherally located. The cytoplasm was mainly composed of lipid. Addition of phytase to the diet containing Jatropha meal (Diet J-500) decreased lipid storage in the cytoplasm and the hepatocytes were of regular shape.

The intestines of fish fed C-0 and S-0 showed an epithelium with normal columnar enterocytes with small nuclei localised in the mid portion of the cells. No accumulation of lipid vacuoles was observed in the cytoplasm. In contrast, fish fed J-0 showed histological changes such as decreased mucosal foldings, supranuclear vacuolisation of the absorptive cells and submucusa enriched by phagocyte cells indicative of inflammation. Fish fed diet J-500 showed a normal mucosa epithelium as fish fed S-0 and C-0.

3.2.6 Body morphological traits

Replacement of fish meal with plant protein sources, addition of phytase or interaction of feed and phytase did not affect condition factor, relative profile and relative intestinal length (Table 2.6). On the other hand, the diet composition significantly affected hepatosomatic index (HSI) and viscerosomatic index (VSI). Fish fed Jatropha meal had significantly higher HSI and VSI than those fish fed either fish meal or soybean meal diets.

Table 2.1: Proximate composition, lysine and phytic acid level of ingredients

	Fish meal	Wheat meal	Soybean meal	Jatropha meal
Proximate composition (%DM)	90.4	90.6	94.4	93.5
Dry matter	90.4	90.6	94.4	93.5
Crude protein	63.5	14.5	50.0	70.6
Crude lipid	8	1.9	1	0.6
Crude ash	20.1	1.7	6.4	11.1
NFE*	8.4	81.9	42.6	17.7
Gross energy (kJ g^{-1})	20.7	16.8	20.5	19.2
Lysine	5.2	0.4	3.2	2.3
Phytic acid (% DM)	-	0.04	3.8	8

Values are means of duplicate determination.
* Nitrogen free extract

Table 2.2: Diet formulation, chemical composition, lysine, phytic acid, phosphorous and pH level of experimental diets

Components	C-0[3]	C-500[4]	S-0[5]	S-500[6]	J-0[7]	J-500[8]
Diet formulation						
Fish meal	47	47	7	7	7	7
Wheat meal	45	44.99	28.81	28.79	43.81	43.79
Soybean meal	-	-	52	52	-	-
Jatropha meal	-	-	-	-	38	38
Fish oil	-	-	1.2	1.2	1.2	1.2
Phytase (5000G, Natuphos)	-	0.01	-	0.01	-	0.01
Sunflower oil	4	4	7	7	6	6
Minerals premix[1]	2	2	2	2	2	2
Vitamin premix[2]	2	2	2	2	2	2
Chemical composition (% DM)						
Dry matter	93.7	94.2	93.3	94.5	92.5	92.9
Crude protein	37.9	37.7	37.0	36.8	37.9	37.2
Crude lipid	12.1	12	11.2	11.3	11.3	11.7
Crude ash	13.0	12.9	9.0	8.6	8.8	8.8
Crude fiber	1.4	1.4	5.7	5.5	2.9	3.0
Gross energy (kJ g^{-1})	20.1	20.4	19.9	20.1	20.2	20.2
Lysine	2.6	2.6	2.1	2.1	1.4	1.4
Phosphorus	3.6	3.6	2.2	2.2	2.8	2.8
Phytic acid	0.02	0.02	2.0	2.0	3.0	3.0
pH	6.3	6.3	6.4	6.4	6.4	6.4

Values are means of duplicate determination.

1**,** Mineral premix (g kg^{-1}): $CaCO_3$ 336g, KH_2PO_4 502g, $MgSO_4.7H_2O$ 162g, NaCl 49.8 g, Fe(II) gluconate 10.9g, $MnSO_4.H_2O$ 3.12g, $CuSO_4.5H_2O$ 0.62g, KI 0.16g, $CoCl_2.6H_2O$ 0.08g, NH_4molybdate 0.06g, $NaSeO_3$ 0.02g. 2**,** Vitamin premix (mg or IUg^{-1}): retinol palmitate 500.000 IU thiamine 5 mg; riboflavin 5mg; niacin 25 mg; folic acid 1 mg; biotin 0.25 mg; pyridoxine 5 mg; cyanocobalamine 5 mg; ascorbic acid 10 mg; choleocalciferol 50,000 IU; α-tocopherol 2.5 mg; menadione 2 mg; inisitol 25 mg; pantothenic acid 10 mg; choline chloride 100 mg. 3**,** Control; 4**,** Control plus phytase; 5**,** Soybean meal; 6**,** Soybean meal plus phytase; 7**,** Jatropha meal; 8**,** Jatropha meal plus phytase.

Table 2.3: Growth performance of common carp fed experimental diets (8 weeks)

Feed[1]	Level of phytase	IBM[2] (g)	FBM[3] (g)	BMG[4] (%)	FI[5] (g fish^{-1})	FCR[6] (g feed/g mass gain)	SGR[7] (%)
C	0	6.4	28.5	348	23.9	1.1	2.8
C	500	6.4	29.7	367	24.2	1.1	2.9
S	0	6.3	18.2	187	19.2	1.6	2.0
S	500	6.2	18.8	202	19.8	1.6	2.0
J	0	6.2	19.4	216	19.5	1.5	2.1
J	500	6.3	23.6	274	21.4	1.3	2.4
Pooled SE		0.04	1.3	16.6	0.5	0.1	0.1
P-value							
Feed		-	<0.00001	<0.001	<0.001	<0.001	0.001
level of phytase		-	0.1	0.09	0.09	0.20	0.09
Feed x level of phytase		-	0.4	0.54	0.46	0.35	0.48

Values are mean (n = 4).

1, C-0, Control; C-500, Control plus phyttase; S-0, Soybean meal; S-500; Soybean meal plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase.

2, initial body mass; 3, final body mass; 4, percentage of body mass gain; 5, feed intake; 6; feed conversion ratio; 7, specific growth rate.

Table 2.4: Initial and final whole body chemical composition of experimental fish

Feed[1]	Level of phytase	Moisture (% WB)	Crude protein (% WB)	Lipid (% WB)	Crude ash (% WB)	Gross energy (kJ g^{-1})	Phosphorus (% WB)	Blood Cholesterol (mg/dl)
Initial fish		80.6	13.1	3.2	2.4	4.4	0.95	-
C	0	78.7	14.4	5.0	2.4	5.2	1.09	202
C	500	79.0	14.1	4.6	2.5	5.0	1.12	214
S	0	80.3	14.1	3.5	2.4	4.6	1.06	158
S	500	79.6	13.8	4.4	2.4	4.8	1.07	172
J	0	77.7	13.8	6.6	2.2	5.5	1.01	182
J	500	77.7	13.4	6.5	2.3	5.6	1.15	189
Pooled SE		0.3	0.1	0.3	0.03	0.1	0.5	6.5
P-value								
Feed		<0.001	0.02	<0.001	0.02	<0.001	0.51	0.02
level of phytase		0.6	0.06	0.8	0.29	0.8	0.04	0.27
Feed x level of phytase		0.6	0.93	0.5	0.71	0.6	0.15	0.95

Values are mean (n = 4).

1, C-0, Control; C-500, Control plus phyttase; S-0, Soybean meal; S-500; Soybean meal plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase.

Table 2.5: Component retention (%) and gain (mg/100g ABM d^{-1}) of experimental fish after 8 weeks

Feed[1]	Level of phytase	*Retention*			*Component gain*			
		Protein (%)	Energy (%)	Phosphorus (%)	Protein (mg/100g ABM d^{-1})	Lipid (mg/100g ABM d^{-1})	Gross energy (kJ/100g ABM d^{-1})	Phosphorus (mg/100g ABM d^{-1})
C	0	35.0	25.0	22.7	320.5	120.8	1.18	24.6[a]
C	500	35.6	24.5	24.4	323.1	111.6	1.17	26.3[a]
S	0	24.4	13.8	26.7	233.0	58.9	0.74	17.9[b]
S	500	24.3	15.6	29.9	230.0	81.4	0.82	18.5[b]
J	0	25.2	20.7	22.9	240.9	139.7	1.04	17.9[b]
J	500	29.1	24.0	30.6	262.5	151.5	1.18	23.8[a]
Pooled SE		1.1	1.2	0.9	8.9	9.1	4.8	0.8
P-value								
Feed		<0 .001	<0.001	0.018	<0.001	0.001	<0.001	<0 .001
level of phytase		0.21	0.35	0.004	0.39	0.56	0.33	0.004
Feed x level of phytase		0.31	0.65	0.147	0.44	0.65	0.71	0.045

Values are mean (n = 4).
1, C-0, Control; C-500, Control plus phyttase; S-0, Soybean meal; S-500; Soybean meal plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase.

Table 2.6: Body morphological traits of experimental fish after 8 weeks

Feed[1]	Level of phytase	Condition factor (%)	Relative profile	Hepatosomatic index (%)	Vicerosomatic index (%)	Relative intestinal length
C	0	3.1	0.39	1.5	6.2	1.23
C	500	3.1	0.35	1.6	6.6	1.17
S	0	3.0	0.36	1.4	6.5	1.20
S	500	3.0	0.37	1.7	6.8	1.19
J	0	3.1	0.37	1.8	7.1	1.29
J	500	3.0	0.38	1.6	7.0	1.27
Pooled SE		0.03	0.004	0.10	0.04	0.02
P-value						
Feed		0.7	0.9	0.005	0.02	0.1
level of phytase		0.6	0.5	0.147	0.18	0.4
Feed x level of phytase		0.9	0.1	0.134	0.75	0.9

Values are mean (n = 4).

1, C-0, Control; C-500, Control plus phytase; S-0, Soybean meal; S-500; Soybean meal plus phytase; J-0, Jatropha meal; J-500 Jatropha meal plus phytase

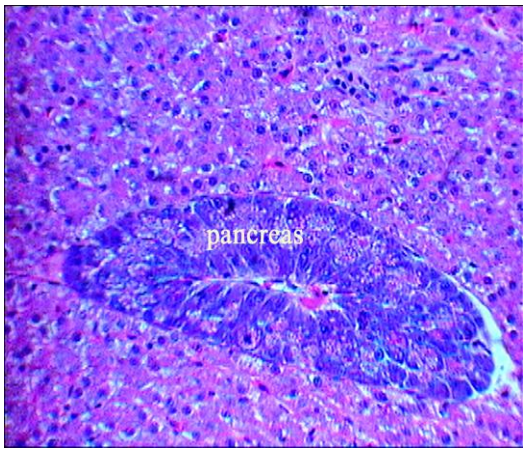


Fig. 2.1. Section of liver from carp fed fish meal diet

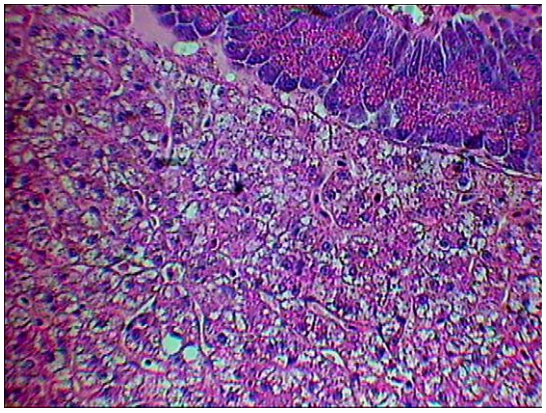


Fig. 2.2. Section of liver from carp fed Jatropha meal diet

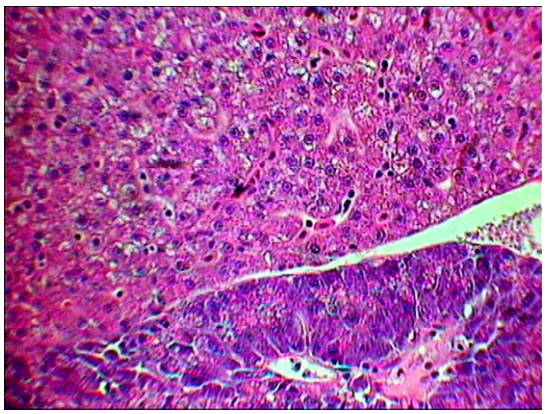


Fig. 2.3. Section of liver from carp fed Jatropha meal plus phytase diet

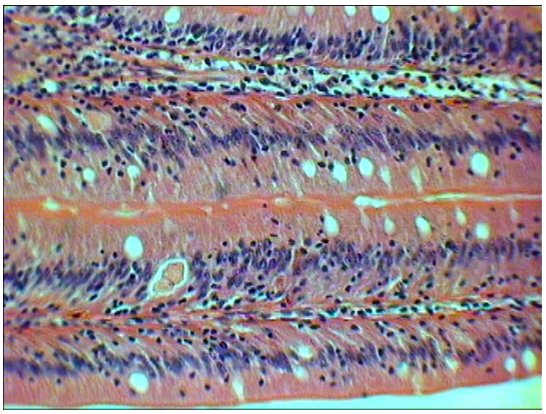
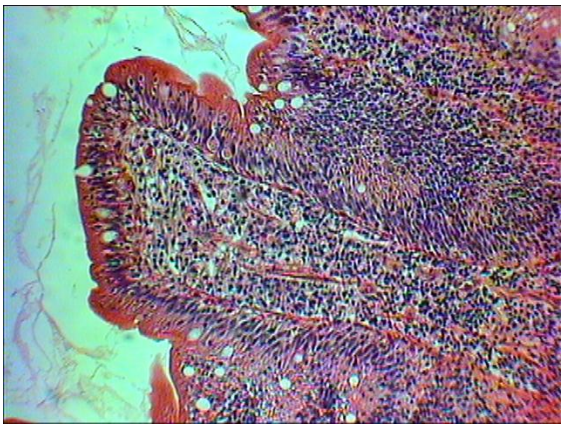


Fig. 2.4.Section of fore-gut from carp fed fish meal diet



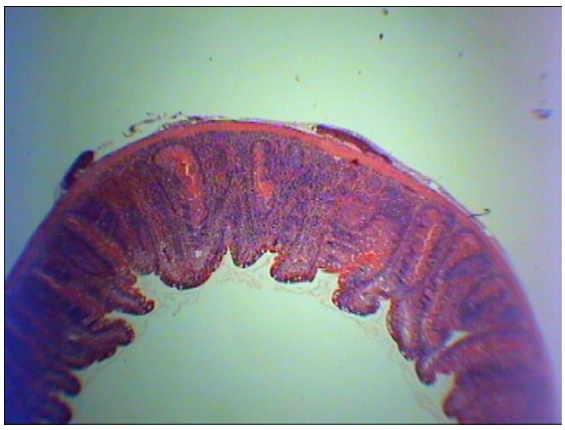
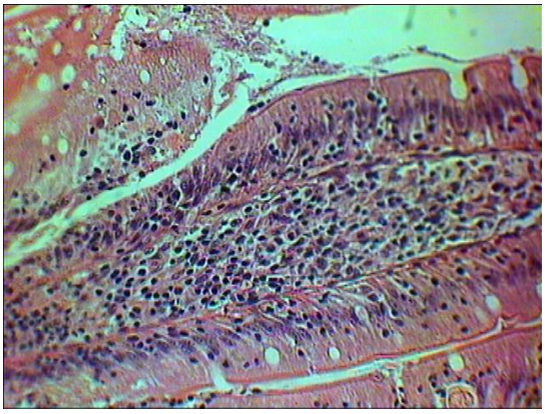


Fig. 2.5. Section of fore-gut from carp fed Jatropha meal



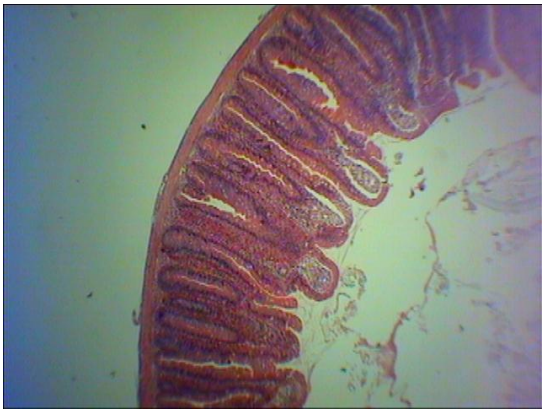


Fig. 2.6. Section of fore-gut from carp fed Jatropha meal plus phytase

3.3 Experiment 3

3.3.1 Composition of experimental diets

Table 3.2 shows the proximate composition of experimental diets. Diets did not vary with respect to crude protein and gross energy. Lipid was slightly higher in diets C-0 and C-500 (about 12%) than diets J-0 and J-500 (about 9.7%). Crude ash and P were higher in diet C-0 (about 11.0% and 1.8 % respectively) than diet J-0 (7.6% and 1.2% respectively). The level of minerals in all test diets was in excess of the known requirements of common carp (NRC, 1993).

3.3.2 Feed utilisation and growth performance of fish

3.3.2.1 Respiration system (3-1)

Generally the fish in the respiration system did not perform as well as fish in the aquaria system (Table 3.3a). Although fish in the aquaria system accepted daily rations very well, fish in the respiration system did not eat all the feed, regardless of the type of treatment. This effect worsened towards the end of experiment. In general, fish in the respiration system were more nervous and showed greater swimming activity than fish in the aquaria system. From the beginning of the experiment, all the respiration chambers were covered with partitioning plates in order to avoid any further disturbance of the fish from outside by activity in the respiration room. As a result of nervousness, the overall performance of fish in the respiration system was inferior to that of fish in aquaria system. Fish fed diets Jatropha meal (J-0) and Jatropha meal plus phytase (J-500) had significantly lower BMG, FI, SGR and higher FCR than fish fed control (C-0) and Control plus phytase (C-500) ($\rho < 0.001$).

Replacement of fish meal with non-toxic Jatropha meal significantly decreased protein productive value and energy retention of fish ($\rho < 0.001$; Table 3.3a).

3.3.2.2 Aquaria system (3-II)

Fish in all experimental groups fed actively on the test diets and no mortality was observed during the study period. Fig. 3.1 shows a weekly specific growth rate (SGR) of the experimental fish. This figure shows that the SGR of fish fed diets Control (C-0) and Control plus phytase (C-500) gradually decreased towards the end of experiment, however, the SGR of fish fed Jatropha meal (J-0) and Jatropha meal plus phytase (J-500) remained fairly stable after week third. Table 3.3b presents the performance of fish at the end of the experimental period. Fish fed diets J-0 and J-500 had significantly lower BMG ($\rho < 0.001$) and SGR and higher FCR than fish fed diets C-0 and C-500 ($\rho < 0.0001$). Phytase supplementation had no effect on BMG, FCR and SGR of the fish. Replacement of fish meal with non-toxic Jatropha meal significantly decreased protein productive value and energy retention of fish ($\rho < 0.001$).

3.3.3 Chemical composition of whole body

Whole body composition of fish at the beginning and at the end of the experiment is given in Tables 3.4a and 3.4b. In the aquaria system (Table 3.4b), replacement of fish meal with Jatropha meal significantly decreased CP and increased lipid in whole body of fish fed Jatropha diets ($\rho < 0.05$). There was no effect of level of phytase or interaction of feed and phytase on moisture, crude protein, lipid and energy content. Phytase and interaction of feed and phytase significantly affected ash level in fish. Crude ash was significantly higher in fish fed J-500 than fish fed other experimental diets ($\rho < 0.001$). Moreover, fish on diet J-500 had significantly higher phosphorus (P) than fish fed diet J-0 ($\rho < 0.05$).

Replacement of fish meal with Jatropha meal significantly decreased serum cholesterol levels of fish ($\rho < 0.01$), whereas phytase treatment and the interaction of feed and level of phytase did not affect the serum cholesterol.

In the respiration system (Table3.4a), however, the data were not consistent with those from the aquaria system. There were no significant differences in moisture, CP, lipid, ash and P levels of the experimental fish. An effect of phytase addition on ash and P level in fish was not apparent either. Fish in the respiration system showed higher fat deposition than fish in the aquaria system.

3.3.4 P gain and retention

Table 3.4b represents the P-gain and P-retention of experimental fish. Replacement of fish meal with Jatropha meal significantly decreased P-gain of fish when compared to fish fed diets Control (C-0) and Control plus phytase (C-500) ($\rho < 0.00001$). Nevertheless, interaction of feed and phytase showed a strong positive influence on P-gain and P-retention of fish fed diets containing Jatropha meal ($\rho < 0.01$).

Table 3.4a also indicates a drastic reduction in P gain and P retention of fish fed Jatropha meal. However, despite increasing in mean value of P-gain and R-retention for fish fed diet Jatropha meal plus phytase (J-500), these were not significantly different with fish fed diet Jatropha meal (J-0).

3.3.5 Energy budget of fish

Total oxygen consumed (g) was higher for fish fed fish meal diets although, this was not significantly different from those fed Jatropha meal (Table 3.5). However, oxygen consumed per (g) body weight gain was significantly higher in fish fed diets containing

Jatropha meal ($\rho < 0.001$). Energy retention was affected significantly by dietary treatments. Fish fed Control (C-0) and Control plus phytase (C-500) showed higher energy retention than fish on Jatropha diets. Apparent unmetabolisable energy was significantly higher in fish fed Jatropha meal. Furthermore, metabolisable energy and efficiency of energy retention were significantly higher for fish fed C-0 and C-500. Energy expenditure per gram protein retained was significantly higher in fish fed Jatropha meal than in fish fed fish meal diets. Meanwhile, energy budget of fish was not affected by phytase or interaction of feed and phytase.

Table 3.1: Proximate composition of ingredients (% DM)

	Dry matter	Crude protein (% DM)	Lipid (% DM)	Crude ash (% DM)	Gross energy (% DM)	Phosphorus (% DM)	Phytic acid (% DM)
Fish meal	90.4	69.0	13.3	16.5	19.5	2.9	-
Non-toxic Jatropha meal	93.5	70.0	1.7	11.1	17.2	2.3	8.00
Wheat meal	89.5	13.5	1.9	1.7	15.5	0.3	0.04

Values are means of duplicate determination.

Table 3.2: Diet formulation and proximate composition of experimental diets (% DM)

	C-0[3]	C-500[4]	J-0[5]	J-500[6]
Fish meal	46	46	7	7
Wheat meal	47.7	47.69	44.5	44.49
Jatropha seed meal	-	-	38	38
Fish oil	-	-	1.2	1.2
Sunflower oil	2	2	5.3	5.3
Phytase (Natuphos)	-	0.01	-	0.01
Mineral premix[1]	2	2	2	2
P as KH_2PO_4	0.3	0.3	-	-
Vitamin premix[2]	2	2	2	2
Proximate composition of experimental diets				
Dry matter	91.4	91.5	90.7	91.1
Crude protein (% DM)	37.5	37.0	38.1	38.8
Lipid (% DM)	12.5	12.0	9.6	9.7
Crude ash (% DM)	11.1	10.9	7.6	7.5
Gross energy (kJ/g sample)	19.7	19.5	20.1	20.0
Nitrogen free extract (% DM)	37.9	38.8	42.7	43.0
Phosphorus (% DM)	1.8	1.8	1.2	1.2
Phytic acid (% DM)	0.02	0.02	3.0	3.0

Values are means of duplicate determination.

1, Mineral premix (g kg-1): CaCO3 336g, MgSO4.7H2O 162g, NaCl 49.8 g, Fe(II) gluconate 10.9g, MnSO4.H2O 3.12g, CuSO4.5H2O 0.62g, KI 0.16g, CoCl2.6H2O 0.08g, NH4molybdate 0.06g, NaSeO3 0.02g.

2, Vitamin premix (mg or IUg-1): retinol palmitate 500.000 IU thiamine 5 mg; riboflavin 5mg; niacin 25 mg; folic acid 1 mg; biotin 0.25 mg; pyridoxine 5 mg; cyanocobalamine 5 mg; ascorbic acid 10 mg; choleocalciferol 50,000 IU; α-tocopherol 2.5 mg; menadione 2 mg; inisitol 25 mg; pantothenic acid 10 mg; choline chloride 100 mg.

3, Control; 4, Control plus phytase; 5, Jatropha meal; 6, Jatropha meal plus phytase.

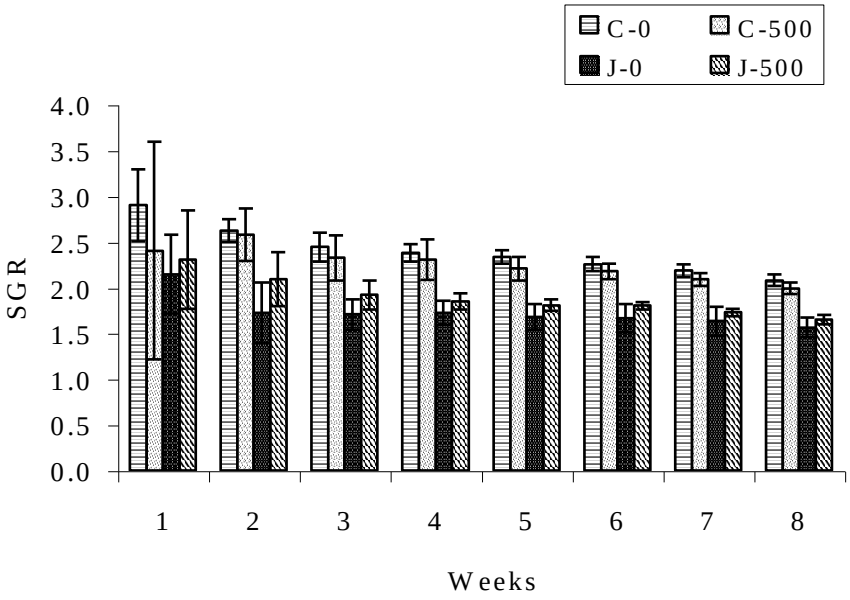


Fig 3.1, Weekly SGR of fish fed experimental diets in aquaria system

Table 3.3a

Experiment 3-I (Respiration system): Growth performance of fish fed experimental diets in respiration system during 8 weeks

Feed	level of phytase	IBM[2] (g)	FBM[3] (g)	BMG[4] (%)	FI[5] (g fish^{-1})	FCR[6] (gFI gBMG^{-1})	SGR[7] (% day^{-1})	PPV[8] (% of CP fed)	P-Gain[9] (mg/100g ABM day^{-1})	P-Retention[10] (%)
Mean of individual treatment										
C	0	21.8	52.4	155.7	51.0	1.7	1.7	28.8	2.2	23.7
C	500	21.6	49.3	136.2	49.2	1.8	1.5	27.6	2.1	22.4
J	0	21.8	31.7	52.3	41.6	4.0	0.7	13.9	0.8	11.6
J	500	21.9	32.8	67.9	41.1	3.3	0.9	19.6	1.2	17.5
Pooled S.E.		0.6	3.7	14.4	2.0	0.3	0.1	3.6	0.2	1.8
P-value										
Feed		-	0.01	0.003	0.05	<0.001	<0.001	<0.001	<0.0001	0.01
level of phytase		-	0.9	0.9	0.8	0.5	0.9	0.5	0.4	0.4
Feed x level of phytase		-	0.7	0.5	0.9	0.3	0.2	0.4	0.2	0.2

Values are mean (n=3).

1, C-0, Control; C-500, Control plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase; 2, initial body mass; 3, final body mass; 4, percentage of body mass gain; 5, feed intake; 6, feed conversion ratio; 7, specific growth rate; 8, protein productive value; 9, phosphorus gain; 10, phosphorus retention

Table 3.3b: Experiment 3-II (Aquaria system): Growth performance of fish fed experimental diets in aquaria system during 8 weeks

Feed[1]	level of phytase	IBM[2] (g)	FBM[3] (g)	BMG[4] (%)	FI[5] (g fish^{-1})	FCR[6] (gFI gBMG^{-1})	SGR[7] (% day^{-1})	PPV[8] (% of CP fed)	ER[9] (% of GE fed)	P-Gain[10] (mg/100g ABM day^{-1})	P-Retention[11] (%)
Mean of individual treatment											
C	0	21.8	70.0	220.6	59.2	1.2	2.1	33.4	31.6	10.4	27.3
C	500	21.6	66.0	205.0	57.4	1.3	2.0	32.0	29.3	10.0	25.8
J	0	21.8	52.3	140.4	51.2	1.7	1.6	22.6	24.6	6.2	23.0
J	500	21.9	55.1	151.7	53.0	1.6	1.7	23.4	27.5	8.1	30.2
Pooled S.E.		0.2	2.0	9.1	0.9	0.1	0.1	1.3	0.9	0.5	0.9
P-value											
Feed		-	<0.001	<0.001	<0.0001	<0.0001	<0.0001	<0.001	0.01	<0.00001	0.94
level of phytase		-	0.7	0.7	1.0	0.8	0.9	0.7	0.8	0.1	0.07
Feed x level of phytase		-	0.04	0.02	0.1	0.1	0.04	0.2	0.1	0.01	0.01

Values are mean (n=4)

1, C-0, Control; C-500, Control plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase; 2, initial body mass; 3, final body mass; 4, percentage of body mass gain; 5, feed intake; 6, feed conversion ratio; 7, specific growth rate; 8, protein productive value; 9, energy retention; 10, phosphorus gain; 11, phosphorus retention.

Table 3.4a: Experiment 3-I (Respiration system): Initial and final whole body chemical composition of experimental fish (% WB)

Feed[1]	level of phytase	Moisture (% WB)	Crude protein (% WB)	Lipid (% WB)	Crude ash (% WB)	Gross energy (kJ g-1)	Phosphorus (% WB)
Initial fish		79.8	14.6	2.5	2.5	3.9	0.89
Mean of individual treatment							
C	0	73.7	15.3	9.4	2.2	6.8	0.77
C	500	73.8	15.6	8.8	2.1	6.8	0.78
J	0	72.9	15.2	10.5	2.1	7.1	0.77
J	500	74.2	15.4	9.1	2.1	6.6	0.80
Pooled S.E.		0.37	0.08	0.41	0.05	0.17	0.01
P-value							
Feed		0.8	0.4	0.4	1.0	0.8	0.8
level of phytase		0.4	0.1	0.3	0.8	0.5	0.4
Feed x level of phytase		0.5	0.8	0.7	0.8	0.5	0.8

Values are mean (n=3)

1, C-0, Control; C-500, Control plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase.

Table 3.4b: Experiment 3-II (Aquaria system): Initial and final whole body chemical composition (% WB) and blood cholesterol (mg/dl) of experimental fish

Feed[1]	level of phytase	Moisture (% WB)	Crude protein (% WB)	Lipid (% WB)	Crude ash (% WB)	Gross energy (kJ g-1)	Phosphorus (% WB)	Blood Cholesterol (mg/dl)
Initial fish		79.8	14.6	2.5	2.5	3.9	0.89	-
Mean of individual treatment								
C	0	75.0	15.1	8.0	1.9	6.5	0.69	326
C	500	75.5	15.2	7.5	1.9	6.2	0.70	310
J	0	75.4	14.6	8.5	1.7	6.5	0.64	255
J	500	74.4	14.5	9.1	2.0	6.8	0.70	264
Pooled S.E.		0.25	0.11	0.24	0.03	0.11	0.01	11.23
P-value								
Feed		0.5	0.01	0.04	0.7	0.2	0.12	0.01
level of phytase		0.7	0.9	1.0	<0.001	0.8	0.03	0.9
Feed x level of phytase		0.2	0.6	0.2	0.003	0.2	0.05	0.5

Values are mean (n=4).
1, C-0, Control; C-500, Control plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase.

Table 3.5: Energy budget of experimental fish in respiration system

Feed[1]	level of phytase	Total O_2 consumed (g)	O_2 consumed (g) per BMG (g)	GE[2] (initial) (kJ)	GE (final) (kJ)	Total GE (fed kJ)	EE[3] (% of GE fed)	ER[4] (% of GE fed)	ME[5] (% of GE fed)	ER/EE	EE/CP retained	AUE[6] (% of GE fed)
Mean of individual treatment												
C	0	29.0	0.9	79.3	358.3	1004.6	42.8	25.9	70.2	0.64	57.1	29.8
C	500	27.7	1.0	80.7	338.6	960.3	42.3	25.3	68.6	0.63	55.8	31.4
J	0	21.9	2.1	80.7	228.6	836.7	38.8	11.1	56.1	0.45	73.1	43.9
J	500	21.7	1.7	75.9	213.9	818.8	38.9	13.7	55.7	0.46	67.7	44.3
Pooled S.E.		1.8	0.2	2.3	27.3	39.1	1.9	2.2	3.0	0.04	3.7	3.0
P-value												
Feed		0.1	0.001	0.8	0.02	0.1	0.4	<0.001	0.03	0.03	0.1	0.03
level of phytase		0.8	0.4	0.8	0.7	0.7	1.0	0.6	0.9	0.93	0.6	0.9
Feed x level of phytase		0.9	0.3	0.6	1.0	0.9	0.9	0.4	0.9	0.91	0.8	0.9

Values are mean (n=3).
1, C-0, Control; C-500, Control plus phytase; J-0, Jatropha meal; J-500, Jatropha meal plus phytase; 2, gross energy; 3, energy expenditure; 4, energy retention; 5, metabolisable energy; 6, apparent unmetabolisable energy.

3.4 Experiment 4

3.4.1 Water quality parameters and temperature

Water quality parameters were monitored daily during the experimental period and recorded as such: NH_3 0.1-0.2mgl^{-1}, nitrite 0.07-0.1 mgl^{-1} and nitrate 1-3 mgl^{-1}. Dissolved oxygen and pH fluctuated between 7.2 ± 0.3 mg l^{-1} and 7.2 ± 0.2 respectively. Unfortunately because of some technical problem in fish labour the temperature was not constant during the experiment period. Temperature was recorded as such: Weeks 1, 2 and 3, 26±1 ^{o}C; week 4, 5 and 6, 28±1 ^{o}C; weeks 7 and 8, 30.5±1 ^{o}C.

3.4.2 Composition of experimental diets

Table 4.3 shows the proximate composition of the experimental diets. Diets did not vary with respect to crude protein, lipid, and gross energy. Crude ash was higher in diet Control (about 10%) than diets non-toxic Jatropha (NJ), non-toxic Jatropha plus phytase and lysine (NJPL), detoxified Jatropha (DJ) and detoxified Jatropha plus phytase and lysine (DJPL) (about 6.6%, 6.8%, 6.7% and 6.9% respectively).

The HPLC results revealed that phorbolesters were not detectable after 30 minutes extraction with organic solvent. The sensitivity of the method was 5µg g^{-1}. Phytic acid levels were registered at about 3.2% in diet detoxified Jatropha (DJ) and 3.1% for diet nontoxic Jatropha (NJ).

Calculated total non-starch polysaccharides were higher in Diets detoxified Jatropha (DJ) and detoxified Jatropha plus phytase and lysine (DJPL) (about 6.8%) than diets non-toxic Jatropha (NJ) and non-toxic Jatropha plus phytase and lysine (NJPL) (about 4.2%).

Calculated mineral levels in the experimental diets showed that Ca in diets containing JM (8.6-8.7 $mgkg^{-1}$) was almost half that of diet Control (16.3 $mgkg^{-1}$). Fe was also lower in diets containing Jatropha meal (about 74.5-74.9 mg kg^{-1}) than diet Control (207.1 mg kg^{-1}). Other minerals such as Zn, Mn, Mg, Na, K and P exceeded the requirement of common carp (NRC, 1993).

3.4.3 feed utilisation and growth performance

Fish in experimental groups Control (C), non-toxic Jatropha (NJ) and non-toxic Jatropha plus phytase and lysine NJPL fed actively on the test diets suggesting high palatability of the feeds. However, after the fourth week, fish in groups detoxified Jatropha (DJ) and detoxified Jatropha plus phytase and lysine (DJPL) after feed had been provided, ingested and spat out the feed several times. It took almost 30 minutes till all of the feed had finally been ingested. At the same time, no mortality was observed during the study period.

Fig 4.1 shows the weekly SGR of experimental fish. Fish fed diets NJPL and DJPL indicated a higher SGR in the first week of experiment, however after the second week, the SGR of Fish fed DJPL was clearly reduced.

Table 4.4 presents the feed utilisation and growth performance of fish during the experimental period. At the end of the feeding period (52 days), fish fed diets NJ, DJ and DJPL indicated significantly ($\rho<0.05$) lower FI, BMG (%) and SGR than fish fed diets C and NJPL. Fish fed DJ showed the lowest SGR and highest FCR of all the experimental groups ($\rho<0.05$). Meanwhile, FCR was significantly lower for fish fed diets NJPL and C ($\rho<0.05$).

3.4.4 Chemical composition of whole body

Chemical composition of whole body of fish at the beginning and end of the experiment is given in Table 4.5. Fish fed diets Control (C) and non-toxic Jatropha plus phytase and lysine (NJPL) showed significantly ($\rho<0.05$) higher whole body moisture than those fed non-toxic Jatropha (NJ), detoxified Jatropha (DJ) and detoxified Jatropha plus phytase and lysine (DJPL) diets. Moreover, this was inversely related to whole body lipid which was significantly ($\rho<0.05$) lower in the former two groups. Fish fed diets C and NJPL had higher crude ash than fish fed the other experimental diets ($\rho<0.05$). There was no differences in crude protein of fish fed the different experimental diets ($\rho>0.05$). Gross energy was significantly higher in fish fed diets NJ, DJ and DJPL ($\rho<0.05$).

3.4.5 Retention and gain

Protein productive value expressed as percentage of CP intake was significantly higher in fish fed diets non-toxic Jatropha plus phytase and lysine (NJPL) and Control (C) (about 34.1% and 34.9% respectively) than fish fed the other experimental diets ($\rho<0.05$; Table 4.5). However, there was no significant difference in the energy retention of experimental fish. P retention was significantly higher in fish fed diets C and non-toxic Jatropha plus phytase and lysine (NJPL) (about 85.3% and 91.2% respectively) than fish fed detoxified Jatropha (DJ), detoxified Jatropha plus phytase and lysine (DJPL) and non-toxic Jatropha (NJ) (about 35.1%, 57.7% and 57.4% respectively). Protein gain and ash expressed as mg/100g ABM/day was significantly higher in fish fed diets NJPL and C ($\rho<0.05$). However, there was no significant difference in lipid gain of experimental fish. Energy gain was significantly lower in fish fed diet DJ than in other experimental fish.

3.4.6 Whole body minerals and minerals gain

Phosphorous (P) was significantly higher in fish fed diets Control (C), non-toxic Jatropha (NJ) and non-toxic Jatropha plus phytase and lysine (NJPL) than the other experimental fish ($\rho<0.05$; Table 4.6). Although replacement of fish meal with either non-toxic or detoxified Jatropha meal affected the mean values of Fe, Zn, Na and K levels in whole body of experimental fish, because of the large variation within the groups there was no significant difference among the groups ($\rho>0.05$). Mg was significantly higher in fish fed C ($\rho<0.05$). Fish fed diet DJ had significantly lower Ca levels than other experimental fish ($\rho<0.05$). Minerals gain expressed as mg/1000g ABM/day revealed that fish fed C and NJPL had significantly higher Fe, Zn and P gain per day than the other experimental groups.

Table 4.1: Proximate composition, antinutrients, non-starch polysaccharides and minerals of ingredients

	Fish meal	Wheat meal	Detoxified Jatropha meal	Non toxic Jatropha meal
Dry matter (% DM)	90.4	90.6	95.7	95.8
Crude protein (% DM)	63.5	14.5	72.1	68.2
Lipid (% DM)	8.6	1.9	2.9	1.8
Crude ash (% DM)	20.1	1.7	10.0	9.8
Gross energy (kJ g-1)	21.1	19	20.5	19.7
Antinutrients				
Phorbolesters (mg/g)	-	-	ND	ND
Phytic acids (% DM)	-	-	9.1	8.3
Non-starch polysaccharides (% DM)				
Rhamnose	-	-	0.0	0.0
Fucose	-	-	0.0	0.0
Arabinose	-	-	3.6	2.0
Xylose	-	-	6.2	1.0
Mannose	-	-	1.4	0.8
Galactose	-	-	0.9	2.9
Glucose	-	-	6.9	3.0
Glucuronic acid	-	-	0.0	0.0
Galacturonic acid	-	-	0.4	1.6
Total NSP	-	-	19.4	12.3
Minerals				
Fe (mg kg^{-1})	458	50.4	70.0	68.5
Zn (mg kg^{-1})	58.5	22.9	100.0	91.8
Mn (mg kg^{-1})	7.73	27	31.7	28.9
Mg (g kg^{-1})	3.7	4.1	11.2	10.8
Na (g kg^{-1})	11.2	0.4	2.5	1.2
K (g kg^{-1})	7.85	2.5	18.3	18.6
Ca (g kg^{-1})	33.6	6.5	8.7	8.2
P (g kg^{-1})	22.8	3.8	21.8	18.8

Values are the mean of duplicate determination.
ND, not detected.

Table 4.2: Diet formulation

	C[3]	DJ[4]	DJPL[5]	NJ[6]	NJPL[7]
Fish meal	45	6	6	6	6
Wheat meal	49	44.7	43.69	45.7	44.69
Jatropha non-toxic				37	37
Jatropha detoxified		35	35		
Starch		2	2		
Fish oil		1.3	1.3	1.3	1.3
Sunflower oil	2	7	7	6	6
L-Lysine			1		1
Phytase (Natuphos)			0.01		0.01
Mineral premix[1]	2	2	2	2	2
Vitamin premix[2]	2	2	2	2	2

1, Mineral premix (g kg^{-1}): CaCO3 336g, KH2PO4, 502; MgSO4.7H2O 162g, NaCl 49.8 g, Fe(II) gluconate 10.9g, MnSO4.H2O 3.12g, CuSO4.5H2O 0.62g, KI 0.16g, CoCl2.6H2O 0.08g, NH4molybdate 0.06g, NaSeO3 0.02g.

2, Vitamin premix (mg or IUg^{-1}): retinol palmitate 500.000 IU thiamine 5 mg; riboflavin 5mg; niacin 25 mg; folic acid 1 mg; biotin 0.25 mg; pyridoxine 5 mg; cyanocobalamine 5 mg; ascorbic acid 10 mg; choleocalciferol 50,000 IU; α-tocopherol 2.5 mg; menadione 2 mg; inisitol 25 mg; pantothenic acid 10 mg; choline chloride 100 mg.

3, Control; 4, detoxified Jatropha meal; 5, detoxifird Jatropha meal plus phytase and lysine; 6, non-toxic Jatropha meal; 7, non-toxic Jatropha meal plus phytase and lysine.

Table 4.3: Proximate composition, antinutrients, non-starch polysaccharides and minerals of experimental diets.

	C[1]	DJ[2]	DJPL[3]	NJ[4]	NJPL[5]
Dry matter	91.3	92.7	91.4	90.5	91.6
Crude protein (% DM)	38.8	38.5	38.3	37.4	37.6
Lipid (% DM)	10.2	9.7	10.7	10.5	10.9
Crude ash (% DM)	10.0	6.7	6.9	6.6	6.8
Gross energy (kJ g-1)	20.4	19.8	19.9	20.4	19.8
Antinutrients					
Phorbolesters (mg/g)	-	ND	ND	ND	ND
Phytic acids (% DM)	-	3.2	3.2	3.1	3.1
Non-starch polysaccharides (NSP, %DM)					
Rhamnose	-	0.0	0.0	0.0	0.0
Fucose	-	0.0	0.0	0.0	0.0
Arabinose	-	1.3	1.3	0.7	0.7
Xylose	-	2.2	2.2	0.4	0.4
Mannose	-	0.5	0.5	0.3	0.3
Galactose	-	0.3	0.3	1.1	1.1
Glucose	-	2.4	2.4	1.1	1.1
Glucuronic acid	-	0.0	0.0	0.0	0.0
Galacturonic acid	-	0.1	0.1	0.6	0.6
Total NSP	-	6.8	6.8	4.2	4.2
Minerals					
Fe (mg kg^{-1})	207.1	74.5	74.5	75.9	75.9
Zn (mg kg^{-1})	27.0	48.7	48.7	47.9	47.9
Mn (mg kg^{-1})	4.2	23.6	23.6	23.5	23.5
Mg (g kg^{-1})	5.9	6.7	6.7	6.9	6.9
Na (g kg^{-1})	2.3	1.4	1.4	0.9	0.9
K (g kg^{-1})	5.1	9.0	9.0	9.5	9.5
Ca (g kg^{-1})	16.3	8.6	8.6	8.7	8.7
P (g kg^{-1})	11.8	11.7	11.7	11.1	11.1

Values are mean of the duplicate determination.
1, Control; 2, detoxified Jatropha meal; 3, detoxifird Jatropha meal plus phytase and lysine;4, non-toxic Jatropha meal; 5, non-toxic Jatropha meal plus phytase and lysine.

Table 4.4

Growth performance of experimental fish after 52 days.

	C[1]	DJ[2]	DJPL[3]	NJ[4]	NJPL[5]
Initial weight (g)	3.9 ± 0.2	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1
Final weight (g)	16.0^{a} ± 2.3	10.3^{b} ± 1.2	12.9^{b} ± 2.3	12.4^{b} ± 0.9	15.7^{a} ± 1.1
Body mass gain (%)	306.5^{a} ± 40.2	159.5^{b} ± 25.8	217.0^{b} ± 60.4	211.5^{b} ± 25.6	294.3^{a} ± 16.9
Feed intake (g)	13.9^{a} ± 1.1	12.0^{b} ± 0.6	13.1^{ab} ± 0.8	12.5^{b} ± 1.1	14.1^{a} ± 1.1
Feed conversion ratio (g feed/g BMG)	1.2^{c} ± 0.2	1.9^{a} ± 0.1	1.5^{b} ± 0.2	1.5^{b} ± 0.1	1.2^{c} ± 0.1
Specific growth rate (%)	2.7^{a} ± 0.3	1.8^{c} ± 0.1	2.2^{b} ± 0.2	2.1^{b} ± 0.2	2.6^{a} ± 0.2

Values are mean (n = 4). Values for each experimental group in the same row followed by different superscripts are significantly different ($P < 0.05$).

1, Control; 2, detoxified Jatropha meal; 3, detoxifird Jatropha meal plus phytase and lysine;4, non-toxic Jatropha meal; 5, non-toxic Jatropha meal plus phytase and lysine.

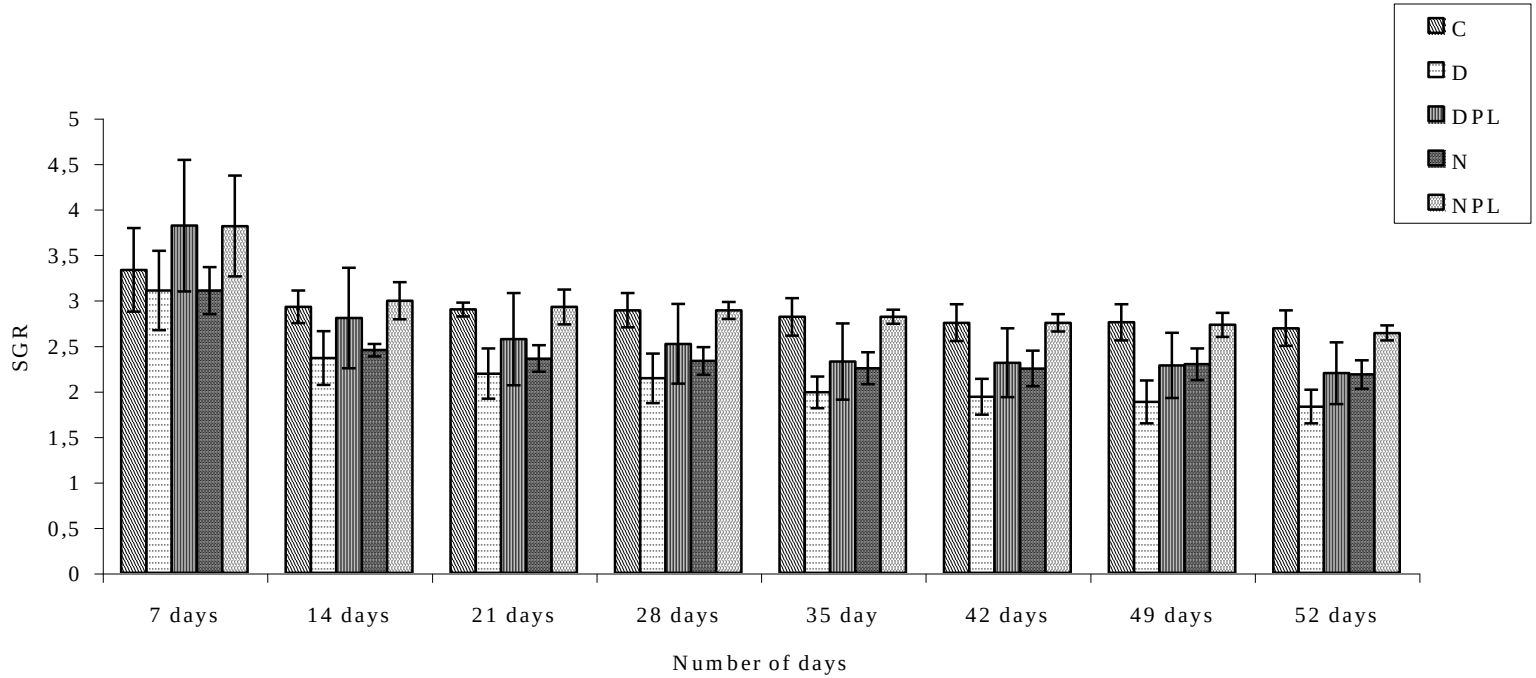


Figure 4.1, weekly SGR of experimental fish

Table 4.5

Initial and final whole body chemical composition (%WB) and component retention and gain of experimental fish

	Initial fish	C[1]	DJ[2]	DJPL[3]	NJ[4]	NJPL[5]
Chemical composition of whole body (% of wet basis)						
Moisture	81.4	$77.3^{a} \pm 0.6$	$74.3^{c} \pm 0.6$	$74.9^{bc} \pm 0.8$	$75.1^{b} \pm 0.8$	$76.4^{a} \pm 0.5$
Crude protein	13.4	14.8 ± 0.2	14.8 ± 0.2	14.5 ± 0.4	14.4 ± 0.1	14.9 ± 0.4
Lipid	2.8	$6.1^{c} \pm 1.5$	$9.5^{a} \pm 0.4$	$9.0^{a} \pm 0.8$	$8.7^{a} \pm 0.8$	$7.1^{b} \pm 1.1$
Crude ash	2.6	$2.3^{ab} \pm 0.1$	$2.1^{c} \pm 0.1$	$2.1^{c} \pm 0.1$	$2.2^{bc} \pm 0.2$	$2.4^{a} \pm 0.1$
Gross energy (kJ g^{-1})	4.0	$5.5^{b} \pm 0.3$	$6.8^{a} \pm 0.2$	$6.5^{a} \pm 0.4$	$6.4^{a} \pm 0.3$	$5.8^{b} \pm 0.2$
Retention						
Protein productive value (% of CP intake)		$34.1^{a} \pm 3.8$	$21.5^{c} \pm 2.5$	$26.0^{bc} \pm 4.2$	$26.8^{b} \pm 1.9$	$34.9^{a} \pm 2.2$
Energy retention (% of GE intake)		25.5 ± 3.9	22.7 ± 2.6	25.8 ± 4.1	25.0 ± 3.1	27.1 ± 1.7
Phosphorus retention (% of P intake)		$37.3^{a} \pm 6.3$	$15.2^{c} \pm 1.8$	$25.0^{b} \pm 3.2$	$24.4^{b} \pm 2.9$	$38.8^{a} \pm 2.5$
Gain						
Protein gain (mg/100g ABM/day)		$355.4^{a} \pm 63.9$	$192.4^{b} \pm 34.8$	$253.9^{b} \pm 61.5$	$241.0^{b} \pm 23.6$	$347.9^{a} \pm 35.8$
Lipid gain (mg/100g ABM/day)		166.8 ± 43.0	166.0 ± 30.2	201.2 ± 50.5	188.0 ± 34.7	194.5 ± 27.4
Energy gain (kJ/100g ABM/day)		$139.7^{ab} \pm 30.1$	$104.7^{b} \pm 18.5$	$130.5^{ab} \pm 30.8$	$122.5^{ab} \pm 17.7$	$146.0^{a} \pm 14.5$
Ash gain (mg/100g ABM/day)		$50.9^{a} \pm 8.0$	$21.0^{c} \pm 3.4$	$30.3^{b} \pm 7.2$	$32.0^{b} \pm 3.2$	$51.0^{a} \pm 2.1$

Values are mean (n = 4). Values for each experimental group in the same row followed by different superscripts are significantly different ($P < 0.05$).
1, Control; 2, detoxified Jatropha meal; 3, detoxifird Jatropha meal plus phytase and lysine;4, non-toxic Jatropha meal; 5, non-toxic Jatropha meal plus phytase and lysine.

Table 4.6: Whole body mineral composition of initial and final fish (mg/kg wet bases) and mineral gain (mg/1000g ABM*/day) of experimental fish

	Initial	C[1]	DJ[2]	DJPL[3]	NJ[4]	NJPL[5]
Fe	19.1	13.1 ± 2.2	14.0 ± 1.8	14.3 ± 2.1	14.2 ± 0.9	14.3 ± 4.0
Zn	52.3	42.4 ± 2.2	43.6 ± 4.7	45.4 ± 3.7	43.5 ± 2.9	43.9 ± 2.1
Mn	1.0	0.3^{b} ± 0.05	0.6^{a} ± 0.1	0.6^{a} ± 0.1	0.6^{a} ± 0.03	0.6^{a} ± 0.1
Mg	613.7	537.4^{a} ± 9.77	446.5^{b} ± 15.4	425.5^{b} ± 35.6	442.1^{b} ± 17.6	454.9^{b} ± 28.7
Na	521.7	558.7 ± 10.90	413.0 ± 151.2	529.7 ± 226.9	533.4 ± 165.7	469.7 ± 107.4
K	1446.1	1810.1 ± 18.47	1709.2 ± 111.4	1650.1 ± 217.3	1825.7 ± 139.9	1719.1 ± 249.5
Ca	3726.3	3523.7^{a} ± 16.56	2730.0^{b} ± 113.5	3412.9^{ab} ± 399.5	3772.8^{a} ± 1061.9	3765.8^{a} ± 802.0
P	955.7	1060.2^{a} ± 37.3	932.9^{b} ± 42.9	943.5^{b} ± 57.2	1014.0^{a} ± 27.5	1043.7^{a} ± 38.6
Minerals gain (mg/1000g ABM/day)						
Fe		31.1^{a} ± 5.9	15.7^{b} ± 5.1	23.4^{ab} ± 2.2	24.0^{ab} ± 11.8	29.0^{a} ± 4.8
Zn		105.1^{a} ± 9.1	60.4^{c} ± 6.3	77.5^{b} ± 6.6	80.1^{b} ± 5.8	96.3^{a} ± 14.5
Mn		1.3^{a} ± 0.2	0.03^{b} ± 0.1	1.1^{a} ± 0.3	1.0^{a} ± 0.3	1.3^{a} ± 0.3
Mg		886.2 ± 96.8	812.3 ± 221.2	716 ± 188.9	761.6 ± 93.9	921.8 ± 69.3
Na		1310.0 ± 804.1	971.5 ± 300.8	1058.7 ± 420.4	897.7 ± 501.3	893.8 ± 501.3
K		4190.3 ± 748.1	3412.9 ± 717.2	3970.3 ± 588.9	3718.3 ± 389.9	4257 ± 389.9
Ca		8070.6 ± 1287.6	5663.3 ± 527.4	7528.6 ± 3182.7	7592.1 ± 239.5	5654.6 ± 239.5
P		2672.4^{a} ± 189.7	1530.5^{d} ± 57.3	1849.4^{c} ± 135.2	2092.1^{b} ± 150.7	2539.0^{a} ± 71.1

Values are mean (n = 4). Values for each experimental group in the same row followed by different superscripts are significantly different ($P < 0.05$).
*Average body mass
1, Control; 2, detoxified Jatropha meal; 3, detoxifird Jatropha meal plus phytase and lysine;4, non-toxic Jatropha meal; 5, non-toxic Jatropha meal plus phytase and lysine.

4. Discussion

4.1 Experiment 1: The effect of replacement of fish meal with differentially treated JM (at 50% of total dietary protein) on common carp's growth performance

In present study, high protein content and well composition of essential amino acid profile have favoured the choice of *Jatropha curcas* meal as protein source in formulated practical diet for common carp. From many antinutrients available in Jatropha meal (JM), protease inhibitor and lectin can be inactivated by moist heat treatment. However, Jatropha meal contains a host of heat stable antinutrients including phytic acids, phenolic compounds and insoluble and soluble fibre. These chemical fractions might not be utilised by common carp and may diminish the nutritional value of other dietary ingredients. In experiment 1, replacement of fish meal with heat treated non-toxic Jatropha meal (JM) at 50% of total dietary protein depressed the growth performance of common carp and this is consistent with the previous study of Makkar and Becker (1999). However, it is worth mentioning that the overall performance of common carp fed Jatropha meal was higher than reported in earlier studies with other non-conventional plant protein sources such as Sesbania seed meal (Hossain et al. 2001a,b) and Mucuna seed meal for common carp (Siddhuraju and Becker, 2001) in which fish meal was replaced at lower levels.

Below the effect of further treatment of JM and its impact on nutrient utilisation of common carp is discussed.

4.1.1 Alcohol treatment of JM

In previous studies it has been shown that alcohol extraction of diets containing soybean meal or soy flour increased the acceptability of diets and supported better growth

than untreated soybean or soy flour in salmonids or yellowtail (Arai et al., 1983a, 1983b; Shimeno et al., 1992; Bureau et al., 1998). Soy saponins, oligosaccharides and soluble non-starch polysaccharides in the alcohol extract of soybean meal were considered to cause appetite suppression (Kaushik et al., 1995; Krogdahl et al., 1995; Bureau et al., 1998). Saponin containing feeds have been shown to have adverse effects on feed intake, growth and histology of fish (Kaushik et al., 1995; Olvera Novoa et al., 1990; Bureau et al., 1998; Krogdahl et al., 1995). Furthermore, it has been reported that extraction of Lupin meal with ethanol considerably elevated the nutritional quality of such meal for *Oncorhynchus mykiss* (Glencross et al., 2003). The negative effect of ethanol soluble components in Lupin meal was attributed to the high level of Lupin's oligosaccharides. Kaushik et al. (1995) also observed that alcohol-water extracted soy protein concentrate with less than 2% soluble carbohydrates was utilised well by rainbow trout, whereas a soybean meal with 18% water-soluble carbohydrates had a lower energy digestibility than the soy concentrate. However, Murai et al. (1986) reported that methanol treatment of soy flour did not improve the growth performance of common carp fed diets based on this material. In experiment 1, extraction of Jatropha meal with 80% ethanol did not improve the growth performance of common carp either (Table 1.3b). This might suggest that common carp is more tolerant to the alcohol soluble components in soybean diets or Jatropha meal than salmonids. Moreover, it might be concluded that alcohol soluble compounds in Jatropha meal (total phenolics, 0.1%; tannins, 0.04%, saponins, o.5% and soluble non-starch polysaccharides, 2.85%) were not present at such a level to describe the cause of depression of growth performance of fish fed Jatropha diet (Diet Jat).

4.1.2 Lysine supplementation

Makkar and Becker (1999) suggested that a retardation in growth performance of common carp fed Jatropha meal at levels such as those investigated here might be due to the reduced bio-availability of some essential amino acids in Jatropha meal. Table 1.3a shows that the proportion and composition of essential amino acids in all the experimental diets adequately meet the requirement of common carp as determined by the NRC (1993); even lysine marginally exceeds this requirement. Set against this, heat treatment of Jatropha meal might have implied amino acid loss or reduced bio-availability due to the Millard reaction (Makkar and Becker, 1999). Viola et al. (1983) reported that although heat treated soybean meal could be considered a good source of lysine, the lysine availability for carp feed was insufficient. Autoclaving of linseed and sesame seed meal was shown to reduce the availability of lysine up to 82% and 80% respectively for common carp (Hossain and Jauncey, 1990). In the present study, the addition of 1% L-lysine in diet J-Lys (Table 1.3a) increased the growth performance of fish to a level comparable to those of the control. Viola and Lahav (1991) demonstrated that the addition of 0.5% lysine-HCl to a soybean diet containing 25% crude protein improved body weight gain of common carp to levels similar to those of their control with 30% crude protein. Essential amino acids supplementation of diets containing soy flour similarly improved the growth performance of fingerling common carp when compared to fish fed a soy flour diet (Murai et al., 1986). On the other hand, it has also been stated that common carp show little or no growth on diets composed only of synthetic amino acids (Aoe et al., 1970; Plakas and Katayama, 1981; Becker, 1984; Murai et al, 1986); nevertheless, it seems that the supplementation of amino acid deficient plant protein sources with one or two synthetic amino acids has been effective in improving growth performance parameters in common carp.

4.1.3 The effect of phytase supplementation on growth parameters

Hossain and Jauncey (1993) demonstrated that common carp fed purified diets containing 0.5 or 1% phytic acid showed a significant reduction in growth performance and feed utilisation, calcium and zinc bioavailability as well as hypertrophy and vacuolisation in the intestine. Reduction in mineral bioavailability was also observed in rainbow trout (Riche and Brown, 1996), channel catfish (Satoh et al. 1998) and tilapia (McClain and Gatlin, 1988) fed diets containing phytic acids. Table 1.3a shows that the level of phytic acid in diets containing Jatropha meal is very high (about 2.1%). As earlier explained, the antinutrient effect of phytic acids is because of their capacity to bind essential dietary minerals such as calcium, iron and zinc and thus decreasing their bioavailability. It also reacts directly with charged groups of protein or indirectly with negatively charged groups of protein mediated by a mineral cation, and thus adversely influences protein digestion and bioavailability (Francis et al., 2001). Previous studies has shown that addition of phytase efficiently and significantly alleviated negative effects of phytic acids by improving growth, feed utilisation and phosphorous and other nutrients availability for fish (Schäfer et al., 1995; Rodehutscord and Pfeffer, 1995; Sajjadi and Carter, 2004). In Experiment 1, supplemental 500 FTU phytase increased % BWG, FCE and SGR to levels comparable to those of the control group (Table 1.4). Many studies reported that the addition of phytase to the diets has been shown to enhance growth performance. An increasing of weight gain has been reported in channel catfish fed phytase supplemented diets containing only plant protein or a combination of plant and animal protein sources (Jackson et al., 1996). Li and Robinson (1997) reported fish fed diets containing 250 FTU or above consumed more feed, gained more weight, and had a lower feed conversion ratio in comparison to fish fed the basal diet containing no microbial phytase. Based on microbial sources, supplementation of 500 to 1500 U/kg phytase improved calcium and phosphorus availability, growth performance, bone mineralisation and protein

digestibility in Nile tilapia, *Oreochromis niloticus*, (Furuya et al. 2001; Liebert and Portz, 2005 and 2007). Positive results of phytase addition on growth performance were also reported for some fish such as seabass, *Dicentrarchus labrax*, (Oliva-Teles et al., 1998), Korean rockfish, *Sebastes schlegeli*, (Yoo et al., 2005), common carp, *Cyprinus carpio*, (Schäfer et al., 1995), rainbow trout, *Oncorhynchus mykiss*, (Rodehutscord et al., 1995; Vielma et al., 1998, Forster et al., 1999), catfish, *Ictalurus punctatus*, (Jackson et al., 1996).

In Experiment 1, phytase improved the body crude protein, protein productive value and protein gain *(mg/100g average body mass/day)* of fish fed JM to a level comparable to fish fed fish meal diet. In crucian carp, application of 500 FTU phytase indicated elevated crude protein digestibility by 6.6% (Lie et al., 1999). Also studies with pangus showed an increased net protein utilisation by addition of 500 FTU/kg in contrast to diet without phytase. Reduced protein phytate complexes in the gut and increased nutrient bioavailability could be an explanation for this observation (Debnath et al., 2005).

Pallauf and Rimbach (1997) stated that since common carp do not have an acidic stomach, the *in vivo* intestinal conditions (pH > 6.5) do not offer an ideal environment for the activity of microbial phytase (optimum pH about 5.5). Therefore, they hypothesized that the reduction of phytate-P must have happened during the moistening and pelleting of rations. In our study, the pH of diets containing Jatropha meal was shown to be about 6.4, very close to the intestinal pH of common carp (Hepher, 1988). Since this pH deviates from the optimum for this enzyme, yet the growth parameters achieved with this diet indicate successful hydrolysis of phytates, it cannot be ruled out that degradation of phytic acids takes place in the intestine of common carp.

4.2 Experiment 2: The effect of replacement of fish meal with non-toxic JM and soybean meal (at 75% of total dietary protein) in diets containing sufficient inorganic phosphorous

Experiment 2 indicated that the replacement of fish meal with Jatropha meal (J-0) and soybean meal (S-0) significantly reduced the growth performance of common carp (Table 2.3). On the other hand, growth parameters such as average body mass gain, FCR and SGR were better in fish fed diets containing Jatropha meal (JM) than soybean meal. This is the first time that it has been demonstrated that common carp thrive on non-toxic JM better than soybean meal. So far, research has shown that common carp are not capable of utilising high levels of plant protein sources (e.g. more than 50% replacement). Hasan et al. (1997) reported inferior growth performance of carp fed diets based on linseed and groundnut meals when the replacement of fish meal with these ingredients exceeded more than 25% of total dietary protein. Hossain et al. (2001a) observed that replacement of fish meal with untreated Sesbania at 30% and 40% total crude protein level significantly reduced growth rate, protein efficiency ratio and protein and energy retention of common carp when compared to fish fed a control diet (fish meal).

In contrast to experiment 1, where addition of phytase to JM significantly elevated growth parameters to a level comparable to that of fish fed fish meal diet, phytase *per se* improved growth performance of fish fed diet containing JM in Experiment 2, however, this was still significantly lower than fish fed fish meal diet. In experiment 2, PPV and protein gain were only marginally improved by phytase supplementation and they were significantly lower than fish fed diets containing fish meal. The lysine level of diets containing soybean meal were expected to be sufficient when compared to the requirement for this species (Table 2.2), whereas diets containing Jatropha meal indicated lower lysine level (1.4% of diet) than the requirement level (1.7% of diet; NRC, 1993). As in section 4.1.2 explained the inferior growth performance of fish fed diet S-0 might partly be attributed to the limitation of lysine

availability. However, in fish fed J-0 and J-500 it can be stated with a high degree of certainty that deficiency in lysine affected the growth parameters. Meanwhile, since the level of substrate (Jatropha meal, JM) in this experiment was higher than experiment 1, the possible benefit of higher levels of phytase application on growth parameters of common carp cannot be with certainty answered and remains to be cleared.

Moreover, in experiment 2 the effect of phytase addition was even less evident for the soybean meal diet. In contrast, Schäfer et al. (1995) reported a significant effect of phytase supplementation on the growth rate and feed utilisation of common carp fed a soybean meal based diet. It can be concluded that in experiment 2, phytase addition was not effective at reducing the antinutritional effect of phytates available in soybean meal. Indirect evidence for this is the only marginal increase in whole body phosphorus of fish fed S-500. Moreover, reduced growth in common carp fed soybean meal might be associated with other factors than phytic acids including saponins, oligosacchardies and non-starch polysaccharides which have been reported to have adverse effects on feed intake and nutrient utilisation in different fish species (Kaushik et al., 1995; Krogdahl et al., 1995; Bureau et al., 1998).

In experiment 2, the whole body chemical composition of fish was significantly affected by the dietary treatments (Table 2.4). Fish fed diets Jatropha meal (J-0) and JAtropha meal plus phytase (J-500) had higher deposition of fat and consequently higher body energy content than fish fed a fish meal based diet (C-0) or soybean meal (S-0). This was inversely related to the whole body moisture content. Such an inverse relationship between moisture and fat content has previously been reported by Focken and Becker (1993). The general effect of JM on whole body fat level is separately discussed in section 4.6.

In experiment 2, whole body crude protein and ash were also significantly affected by dietary treatment (Table 2.4). Fish fed Jatropha diet recorded the lowest crude protein and ash levels. In our first experiment, whole body protein and ash of fish fed JM and phytase were similar to fish fed control diet. Decreasing crude protein level in fish fed JM can be stated

with high certainty as a result of deficiency in lysine. Generally, in experiment 2 the effect of addition of phytase was not apparent for nutrient utilisation of experimental groups.

4.2.1 The effect of experimental diets on liver and fore-gut histology

Despite the pathological liver and intestinal findings in fish fed Jatropha meal in experiment 2, the appearance and behaviour of the fish during the experimental period were normal. This may imply that the animal welfare was not compromised during the eight week experiment; however, whether Jatropha meal (JM) causes severe health problem in a longer feeding trial remains to be investigated. In the present study, the hepatocytes of fish fed Jatropha meal (J-0) indicated increased lipid deposition. Intracellular lipid deposition in the liver of common carp (Hossain and Jauncey, 1989a,b) and carp fry (Hasan et al., 1991, 1997) fed mustard oil cake has been reported. Higher intracellular lipid deposition in the liver is presumably due to the presence of antinutritional factors (such as phytates) present in feed which cause disturbances in the fat metabolism (Hasan et al., 1991).

In the present study, foregut of fish fed Jatropha meal indicated severe anomalies. Enteritis and poor gut morphology can lead to inefficient feed conversion. Repair of damaged enterocytes is also an energy consuming activity which in turn directs valuable resources from growth to the more immediate urgency of tissue repair and maintenance. Enteritis induction in the foregut of common carp in the present study might be caused by the high level of phytates present in Jatropha meal. This is in line with what Hossain and Jauncey (1993) previously reported, who demonstrated that common carp fed diets containing high level of phytates showed hypertrophy and vacuolisation of the cytoplasm of the intestinal epithelium (Hossain and Jauncey, 1993). Furthermore, in the present work, the addition of 500 FTU phytase was effective at restoring foregut morphology of fish fed a Jatropha diet. To our best of knowledge

this is the first demonstration of the effect of phytase on the histology of liver and intestine of fish fed plant protein sources.

Uran et al. (2008) reported that common carp fed soybean meal indicated enteritis, attributing this alteration to soy saponine; however, in the present study common carp fed soybean meal diets showed normal foregut histology similar to fish fed control diet. Later Uran et al., (2009) stated that severity of enteritis can be influenced by variation in quality between different commercial sources of soybean meal.

4.2.2 The effect of experimental diets on body morphological traits

In experiment 2, there was significant difference in HSI of fish fed the various experimental diets. Fish fed Diets Jatropha meal (J-0) and Jatropha meal plus phytase (J-500) had higher HSIs than the other experimental groups. Increased HSI in these groups is concomitant with elevated fat deposition in the hepatocytes which was discussed previously. Such an observation is in line with other studies on grass carp and common carp fed plant protein sources (Dongmeza et al., 2010; Kumar et al., 2010). Moreover, the viscerosomatic index (VSI) was significantly affected by feeds. Fish fed Diets J-0 and J-500 showed higher VSIs than other experimental fish. Refstie et al. (2006) also reported increases in gut weight of cod fed plant protein sources. In the present study, the higher VSIs were paralleled by increasing relative intestinal length (RIL) in J-0 and J-500 group. The higher RIL could be related to the poorly digestible components (such as complex carbohydrates) in the diets. These components have been reported to cause elongation of the intestine in fish (Kihara and Sakata, 1997).

4.3 Experiment 3: The effect of replacement of fish meal with non-toxic JM (at 75% of total dietary protein) in diets without supplementation of inorganic phosphorous

In Experiment 3, the overall performance of fish fed experimental diets was inferior to those of experiment 2 in which all the diets contained sufficient inorganic phosphorus. These results could imply that the amount of available P in diets was not sufficient to support both optimum growth and bone mineralisation when compared to the previous experiment. In experiment 2, addition of phytase to the jatropha meal (JM) *per se* improved percentage of body mass gain of fish; however, this parameter was not significantly different in those fish fed JM without phytase. In Experiment 3, the diets containing JM were not supplemented with inorganic P and the dietary levels of total P (Table 3.2, 12 g kg^{-1} of diet) were above that recommended for common carp but this may well have not been readily available to the fish (recommendation for available P: 0.6 g kg^{-1} of diet; NRC, 1993). The incorporation of microbial phytase into the diets containing JM improved the P availability but the effect was not apparent on weight gain, protein and energy utilisation of the experimental fish (Table 3.3a and 3.3b). These results may suggest that more basic studies with JM are required to establish the optimum level of phytase to support high growth in common carp (dose response experiment).

Although fish meal contains high levels of P, most of the P is present as hydroxyapatite and/or tricalcium phosphate (Sarker et al., 2005). Due to this chemical structure, P in fish meal is less soluble, therefore it is less available to fish lacking an acidic stomach such as common carp (Ogino et al., 1979). Ogino et al. (1979) reported that the availability of phosphorus from fish meal for common carp and rainbow trout is about 24% and 74% respectively. In Experiment 3, fish fed diets containing fish meal (C-0 and C-500) with sub-optimum inorganic phosphorus achieved about 27% phosphorus retention whereas fish fed Jatropha meal plus phytase (J-500) indicated 30% phosphorus retention. Noteworthy

to mention that during the experimental period we did not observe any morphological sign of phosphorus deficiency such as changes in skin colour, small scales or bone and head deformities in experimental fish fed either the control or Jatropha meal diets. Cheng et al. (2005) reported a deformed head of malabar grouper receiving a 0.26% P diet after a 14 week trial. Therefore, a feeding study of a longer duration might be essential to assess the development of phosphorus deficiency.

The results for whole body chemical composition obtained from fish in the aquaria and respiration systems (Tables 3.4a and 3.4b) are not consistent. In the aquaria system, the experimental diets significantly affected whole body protein, lipid and phosphorus level (Table 3.4b), whereas in the respiration system none of the whole body components were significantly affected by the experimental diets (Table 3.4a). The difference between growth parameters of fish in experimental set ups might have been induced by increased stress for fish in the respiration system. Under such conditions, the utilisation, digestion and assimilation of nutrients might be severely affected. In the present experiment the whole body lipid for all fish regardless of diets were somewhat higher than for fish in experiment 2 in which all the diets were sufficiently supplemented with phosphorus. Increases in lipid level of fish fed deficient P is consistent with previous studies on common carp, rainbow trout and red sea bream (Ogino et al., 1979; Sakamoto and Yone, 1980; Takeuchi and Nakazoe, 1981; Sugiura et al., 2004). Onishi et al. (1981) reported an increase in gluconeogenic activity and possible acceleration of fatty acid synthesis in carp fed a phosphorus deficient diet. The most likely explanation for an increase in fatty acid synthesis is impaired oxidative phosphorelation due to phosphorus deficiency, leading to inhibition of the TCA cycle and accumulation of acetyl-Co A (Sugiura et al., 2004).

4.3.1 Energy budget of experimental fish

Huisman (1976b) and Huisman & Valentijn (1981) mentioned that the growth and oxygen consumption rates are closely related. This was supported in this experiment where fish fed control diets showed higher mean O2 consumption than those fed Jatropha. However, in present study (Table 3.5), despite the clear trend in favour of the control groups, the high variability within groups may suggest that the experimental design lacked the statistical power (because of low number of fish per treatment) to prove a difference. Earlier studies also reported such high variation in the individual fish for tilapia and common carp (Becker and Fishelson, 1990; Becker et al., 1992, Francis et al., 2002).

The oxygen consumed per unit of body mass gain was significantly higher for fish fed Jatropha meal. This might implicate the higher metabolical cost needed for the digestion of Jatropha meal. It has been well documented that in teleost species heat loss is the largest component of energy consumed (approximately about 50-56%) whereas energy retention (ER) accounts for 21-35% (Cui and Liu, 1990). The present results indicated that ER of fish fed diet C-0 was about 25% which is in line with the range of previous studies. However, fish fed diet J-0 and J-500 showed severely reduced ER about 11-13.7%. The tendency for higher AUE observed for fish fed Jatropha diet**s** might imply lower absorption or higher excretion of nutrients. Moreover, fish fed diets containing Jatropha meal indicated higher EE per g protein retained in fish body. The present results demonstrate that the utilisation of Jatropha meal by common carp requires more energy than fish meal.

4.4 Experiment 4: The interactive benefit of phytase and lysine at high level of non-toxic JM and detoxified JM inclusion

4.4.1 non-toxic Jatropha meal

The result of the experiment 4 confirmed that at high level of replacement of fish meal with non-toxic JM, the deficiency of lysine is a limiting factor. Supplementation of diet JM with 1% lysine and 500FTU significantly improved all the growth parameters to levels comparable to fish fed diet C (fish meal diet). It is well known that plant protein sources are generally deficient in lysine, therefore, there is a need of inclusion of this amino acid to improve the nutritional quality of plant based diets (Watanabe, 2002; Kumar et al., 2011). Biswas (2007) reported a highly positive effect of inclusion of phytase and lysine in soybean-based diet for *Penaeus monodon* juveniles. The supplementation of lysine and phytase not only enhanced the nitrogen and P utilisation but also significantly altered the fatty acid profile of the tissue.

It has been reported that supplementation of lysine in lysine deficient diets increased the protein and lipid content in the tissue of channel catfish (Zarate and Lovell, 1997). The increased protein content in the tissue may be due to enhanced protein synthesis through lysine addition to the diet. In the present study, however, no such effect was observed. We noticed that there was no significant difference in the crude protein level of the experimental fish at the end of experiment. This is in agreement with Biswas et al. (2007) who reported no differences in whole body crude protein for *Penaeus monodon* (Fabricius) fed soybean meal diets supplemented either with lysine, phytase or both.

4.4.2 The nutritional quality of detoxified Jatropha meal

In experiment 4, the performance of fish fed detoxified JM (DJPL) even with supplementation of 1% lysine and 500FTU was inferior to fish fed diet C (fish meal) but significantly higher than fish fed DJ (detoxified JM). Although phorbol esters (PEs) in diet DJ was not detected by HPLC, the reduced growth performance of fish fed diet DJPL may imply

that detoxification was not complete. A similar result was reported for common carp fed diets containing detoxified Jatropha meal (involving a different detoxification method) at 75% replacement of fish meal by Kumar et al. (2010). These authors reported that after extraction, the PEs might be present at such a low concentration as to not be detectable by HPLC. These small amounts of PEs might be present in a strongly bound form which evades extraction by the HPLC determination (Kumar et al., 2010). PEs cause severe depression of feed intake and growth performance and consequently diarrhoea in common carp and rat (Becker and Makkar, 1998). Meanwhile, Kumar et al., (2010) indicated that common carp fed a detoxified JM diet demonstrated severe necrosis, denudation of enterocytes and infiltration of immune-cells in the intestine possibly as a result of residual PEs in feed. Therefore, the comparative results obtained here may suggest that the method used in the present study was not efficient enough to completely eliminate the detrimental effect of PEs.

4.5 The effect of phytase on minerals utilisation

4.5.1 Enhancement of P bioavailability

P is an important constituent of nucleic acids, cell membrane, skeletal tissues as well as directly involved in all energy-producing cellular reactions (Cao et al., 2007). Since the level of P in water body is negligible, diets for fish must be supplemented with inorganic P in order to support optimum growth performance. However, excessive P is a critical pollutant in aquaculture environment. Plant protein ingredients possess significantly lower level of P than fish meal. Moreover, phytate-P in plant ingredients is not available to fish. Thus the inclusion of microbial phytases in fish nutrition was prompted by increasing the availability of indigenous plant-P, thereby to reduce P excretion and less need for inorganic P addition. In the present study, (Experiments 1, 2,3-II and 4) phytase supplementation tended to

significantly increase whole body ash and P of fish fed Jatropha meal diet (JM), indicating hydrolysis of phytate either intestinally or during the pelleting of the diet. Enhanced Plant-P bioavailability by supplementation of microbial phytase has been reported for some fish such as seabass, *Dicentrarchus labrax*, (Oliva-Teles et al., 1998), Korean rockfish, *Sebastes schlegeli*, (Yoo et al., 2005), common carp, *Cyprinus carpio*, (Schäfer et al., 1995), rainbow trout, *Oncorhynchus mykiss*, (Rodehutscord et al., 1995; Vielma et al., 1998, Forster et al., 1999), catfish, *Ictalurus punctatus*, (Jackson et al., 1996) and Nile tilapia, *Oreochromis niloticus*, (Liebert and Portz, 2005). Schäfer et al., (1995) reported that soybean meal based diet supplemented with 500 and 1000 FTU/kg phytase could release 20% and 40% of phytate-P in common carp and 60% and 80% in crucian carp respectively. Cheng and Hardy (2003) demonstrated that supplementation of 400 FTU/kg in extruded full-fat soybean meal diet resulted in optimum release of P for rainbow trout. Moreover, addition of 250 FTU/kg phytase into the diet of catfish was realised to be efficient to replace dicalcium phosphate without compromising growth performance and bone P deposition (Robinson et al., 2002). It should be mentioned that it has been reported that the level of P must be reduced to a level which is below the requirement of fish (Cao et al., 2007). This is one of prerequisites for optimum activity of phytase. Excessive P in diet can repress the activity of phytase. In experiments 1, 2 and 4 the level of P in diets was not reduced, however, addition of 500 FTU/kg into the diets was efficient to improve P and mineral utilisation in experimental fish. On the other side, for economical reasons and higher benefit from phytase, there is a need to correctly formulate diet to allow for release of additional nutrients and less discharge of P into the environment. Therefore, the minimum level of inorganic P and phytase needed in dietary JM to support high growth and bone mineralisation of common carp is of high interest and remains to be clarified.

In experiments 1, 4 (with sufficient inorganic P) and 3-II (without P supplementation) whole body ash of common carp fed dietary phytase was significantly higher than those diets

without phytase supplementation (Tables 1.5, 3.4b and 4.5). Increment of whole body ash in fish fed diets containing phytase is an indication of higher mineral bioavailability in diets. Nwanna et al., (2007) reported that pre-treatment of plant feedstuffs with two type of phytase (PtN, Natuphos and PtR, Ronozyme) at 4000 FTU/Kg diet level significantly increased the apparent digestibility of mineral utilisation in common carp which resulted in elevated growth performance of fish.

In experiment 3-I (in respiration system) in which JM was not supplied with inorganic P, whole body P of fish in respiration system was affected neither by diets nor by phytase addition. Moreover, P retention in the respiration system for all experimental groups was rather lower than in the aquaria system. Although in experiment 3, all due caution was taken that the water quality should remain above optimum level, the water quality parameters indicated higher fluctuations during the day than the aquaria system. This might be explained by higher swimming activity as a result of nervousness and consequently, metabolic CO2 might have accumulated in the water with a subsequent drop in water pH. Some studies have revealed that high *p*CO2 and low pH in the water may cause chronic respiratory acidosis which may result in a compensatory calcium and phosphorus release from mineralised tissues to maintain blood pH, as shown in mice (Meghji et al., 2001). It has been shown that in Atlantic salmon parr, normal deposition of minerals in the bone matrix may be inhibited if minerals are required to contribute to buffering (Storset et al., 1997). Low pH causes mineral mobilisation from the skeleton of fish and a depletion of ascorbic acid reserves as well as reduced concentrations of proline and hydroxyproline in cartilage (Hamilton and Haines, 1989; Majewski et al., 1990). Previous studies also indicated that high levels of dissolved CO2 in the fresh water period affect the mineral status of Atlantic salmon smolts (Graff et al., 2002; Fivelstad et al., 2003a, b).

4.5.2 Enhancement of other minerals

In experiment 1 (at 50% inclusion of non-toxic Jatropha meal (JM)), supplementation of Jatropha meal (JM) with phytase significantly increased Mg, K and Fe of whole body of fish to levels comparable to those of control fish. In rainbow trout, increasing the apparent absorption of Ca, Mg, Cu, Fe, Sr and Zn in low-ash soybean meal by supplementation of phytase has also been reported (Sugiura et al., 2001). Addition of 1000 FTU/Kg phytase was also sufficient to significantly enhance Ca, Mg, and Mn content of bone in channel catfish (Yan and Reigh, 2002). Based on NRC, (1993) the requirement of common carp for Fe is reported to be about 150 mg kg-1 of diet. Though in experiment 4 (at 75% inclusion of JM with phytase and lysine, Table 4.3) the level of Fe in the diets containing JM was much lower than the reported requirement for common carp, the whole body Fe level in fish fed diets containing JM showed no significant difference to those fish fed the fish meal based diet; on the contrary, the mean value was even slightly higher (Table 4.6). It has been reported that common carp fed diets deficient in Fe indicate poor growth, skeletal abnormality, low feed efficiency, low ash in whole body and increased visceral fat (NRC, 1993). Throughout the present study we did not observe any skeletal abnormality in fish fed diets containing JM. However, whether JM imposes pathological abnormalities in fish in the long term remains to be answered.

4.6 The effect of JM and phytase on whole body fat

Generally, diets containing Jatropha seed meal (treated or not treated) significantly increased the fat content when compared to those fish fed control diet (experiments 1, 2, 3 (I & II) and 4). Kaushik et al. (2004) and Fournier et al. (2004) reported a significant increase in whole body fat of European seabass, *Dicentrarchus labrax*, and juvenile turbot, *Psetta maxima*, fed high levels of mixtures of plant protein sources. Common carp fed diets

containing mustard, sesame, linseed, copra and groundnut oil cakes reported significantly higher deposition of crude lipid in whole body (Hasan et al., 1997). Dias (1999) showed that replacement of fish meal with corn gluten meal or soy protein concentrates elevated hepatic fatty acid synthetase activity of rainbow trout and European seabass significantly. Since an increase in body fat content might affect the meat quality, it would be of interest to have a better insight into the effect of plant protein sources on lipid metabolism (Kaushik et al. 2004).

In experiment 2, fish fed diet containing Jatropha meal (JM, at 75% replacement of fish meal) with phytase supplementation did not show any difference in whole body lipid level compared to fish fed JM without phytase addition. However, in experiment 4, fish fed the NJPL diet (non-toxic JM supplemented with phytase and 1% lysine) indicated significantly reduced whole body lipid compared to fish fed NJ (non-toxic Jatropha), DJ (detoxified Jatropha) and DJPL (detoxified JM supplemented with phytase and 1% lysine). The lipid level lowering effect of dietary lysine is not well explained. Lysine and methionine are the precursor of carnitine which has been found to reduce the lipid content of several tissues such as liver, muscle and viscera as observed in European seabass juveniles (Santulli and d'Amelio, 1986; Biswas et al., 2007).

Moreover, ethanol extraction of Jatropha meal in experiment 1 also significantly enhanced whole body crude lipid when compared to those receiving only JM. Murai et al. (1986) also reported a higher body fat level in common carp fed methanol treated soy flour than in fish receiving untreated soy flour. Saponins have been shown to decrease serum cholesterol and triglyceride levels in humans (Kitagawa and Yoshikawa, 1983) and *Oreochromis niloticus* (Dongmeza et al., 2006). Therefore, removal of this component in diet JM treated with ethanol might have influenced higher fat deposition in fish fed the aforementioned diet. Nevertheless, determining which component(s) in the alcohol extract of JM might affect fat metabolism cannot be said with certainty and this remains to be clarified.

4.7 The effect of JM and soybean meal on blood cholesterol

In Experiment 3, replacement of fish meal with Jatropha meal significantly decreased plasma cholesterol of fish which is also consistent with the results of experiment 2 (Tables 2.4 and 3.4b). The hypocholesterolemic effect of plant proteins compared to animal proteins are well documented (Forsythe, 1995). Significant reduction of plasma cholesterol levels in rainbow trout, yellowtail, Atlantic salmon and common carp fed diets containing soybean meal has been reported (Shimeno et al., 1995; Kaushik et al., 1995; Refstie et al., 1999). The components of soybean thought to be responsible for this are isoflavones such as saponins, phytic acids, protein-isoflavone interactions or some aspects of the protein itself (Greaves et al., 2000). Jatropha meal has been shown to contain high level of phytic acid. It has also been demonstrated that the purified phytic acid has a direct effect on lowering the serum cholesterol in growing rats (Jariwalla et al., 1990) and diabetic KK mice (Lee et al., 2005). The mechanism of the cholesterol lowering activity of phytic acid is still not clear. It has been hypothesised that phytic acid increases the bile acid secretion. Faecal bile acid secretion and fat digestibility are negatively correlated (Xu et al., 2001). Faecal secretion of bile acids implies a loss of body cholesterol, leading to serum hypocholesterolemia (Beynen and West, 1989). Jariwalla et al. (1990) demonstrated that the cholesterol and triglyceride lowering effect of phytic acid was accompanied by a 27% decrease in the zinc/copper ratio in the plasma. However, neither in this study nor the previous one, phytase addition significantly affected the serum cholesterol levels of fish when compared to fish fed diets with no added phytase. Therefore, the hypochlosterolemic effect of Jatropha meal cannot solely be attributed to the phytic acid. It is possible that the underlying cause is more likely to be the reduction of fish meal as the main source of cholesterol.

5. **Conclusion**

This work has shown that, given proper treatment, Jatropha meal can act as a suitable replacement for fishmeal at high levels in diets for common carp. Although its culture is more problematic, non-toxic Jatropha strains are far superior to the toxic varieties since it is still very difficult to remove the potent phorbol esters in the latter that have detrimental effects on animals. Despite the non-detectability of these phorbol esters, the non-toxic variety still has to be suitably pretreated before inclusion in fish diets. The results of this work suggest that phytase addition to enhance phosphorous availability and lysine supplementation to correct the essential amino acid imbalance are two factors that have significant effects in improving Jatropha meal suitability at high levels of inclusion. However, to get full economic benefit from phytase, it is important to correctly formulate diet to allow for release of additional nutrients. It is suggested that further work (a dose response experiment) to determine the optimum levels of both components (inorganic P and phytase) should be carried out so that Jatropha meal can be implemented commercially.

6. **Summary**

The aquaculture industry is the fastest growing food producing industry (FAO 2006) and plays an important role in meeting the demand for fish. In recent years, increasing costs, low quality and a fluctuating supply of fish meal in the market have necessitated research on partial or total replacement of fish meal in fish feeds with alternative protein sources. Moreover, for many fish species, feed resources are in a transition phase from being largely dependent on few resources to becoming multisource-based (Øverland et al., 2009). Generally, plant protein sources are of greater interest because they are inexpensive and readily available. On the other hand, their use is limited by the presence of complex carbohydrates, unbalanced amino acids and a wide variety of antinutritients. Plant antinutrients and toxic substances are natural insecticides and may also adversely affect fish health, resulting in growth reduction or diseases (Francis et al., 2001). Since plant protein sources are normally much cheaper than fish meal, there is considerable scope to process the potential ingredients and produce economical products with increased nutritional value.

Jatropha curcas L. is a hardy plant which can thrive on marginal degraded lands. Large scale plantation of Jatropha has taken place in India, China, Madagascar, Myanmar and many other developing countries for the production of biofuel (Makkar et al., 2008). The seeds of Jatropha contain about 300-350g kg^{-1} oil, which is used as fuel or in transesterified form as a substitute for diesel. The protein quality of dehulled Jatropha seeds is considerable. The levels of essential amino acids (except lysine) are higher than the FAO reference for a growing child of 3-5 years (Makkar et al., 2008). However, Jatropha contains antinutrients such as lectins, trypsin inhibitors and phytic acids at high levels. Moreover, Jatropha seeds can be very toxic (except one variety identified as non-toxic Jatropha) and the toxicity is attributed to the presence of phorbolesters. The detrimental effect of phorbolesters on growth and health of common carp and rats is well documented (Becker and Makkar, 1998).

The nutritional quality and effect of heat-treatment on non-toxic Jatropha meal for common carp has already been assessed (Makkar and Becker; 1999). This study indicated that 15 minutes heat treatment (at 121°C and 66% moisture) of non-toxic Jatropha meal reduced the trypsin and lectin activity significantly. Common carp fed heat-treated non-toxic Jatropha meal had higher weight gain, protein efficiency ratio and protein productive value than those fish fed untreated Jatropha meal. However, these parameters were significantly lower in the aforementioned trial when compared to the fish fed a fish meal based diet (Makkar and Becker, 1999). According to these authors the reduction of growth performance in fish fed non-toxic Jatropha meal might be attributable to the deficiency of some essential amino acids such as lysine, to high levels of phytic acids or the presence of other antinutrients such as saponins, non-starch polysaccharides or other unknown components. These may indicate the need for additional processing of Jatropha meal in order to be viable for inclusion at high level in practical diets of common carp.

This work was therefore conducted to test various ways of further improving the nutritional quality of Jatropha meal to increase the levels of inclusion in diets for common carp. In the first experiment, four diets based on 50% replacement of fish meal with defatted non-toxic JM were formulated, one with no further Jatropha meal treatment, the second with 80% aqueous ethanol extraction before diet formulation, the third supplemented with 1% L-lysine and the fourth with 500 FTU phytase (5000G, Natuphos). These were compared to a standard, fish meal based diet. The results showed that diets with 500 FTU kg^{-1} phytase or 1% L-lysine could maintain common carp's growth performance at a level comparable to fish fed a fish meal diet. Fish fed diets containing JM and ethanol treated JM had significantly lower growth performance than the control. The addition of 1% L-lysine or 500 FTU phytase enhanced percent body weight gain, food conversion efficiency and specific growth rate to a level comparable to those of the control, furthermore, the addition of 500 FTU phytase significantly increased whole body Mg, P and K to the levels of those fish fed Diet Control.

The results of this study show that Jatropha meal can be a suitable alternative to fish meal at 50% replacement for common carp provided that the problems associated with the presence of phytate and deficiency of lysine are overcome.

When the level of Jatropha meal replacement was increased from 50% to 75% while at the same time supplementing the diets with inorganic phosphorus (Experiment 2), however, a significant decrease in body weight gain of common carp was observed both with and without 500 FTU phytase. Two other test diets based on soybean meal (with/without phytase) performed equally badly. At the end of the experiment, carp fed a fish meal based diet had significantly higher weight gain and SGR and lower FCR than the other experimental fish ($\rho < 0.05$). The addition of phytase improved the mean weight gain of fish fed diets supplemented with phytase numerically but statistically the effect was not significant. Moreover, replacement of fish meal with protein from either plant source significantly ($\rho < 0.05$) affected the whole body protein, lipid and ash content of the fish. Fish fed the JM diets had significantly lower crude protein and moisture and higher lipid levels than fish fed other experimental diets. Moreover, the histopathological examination of the livers of fish fed diet JM revealed higher levels of intracellular lipid deposition. The foregut histology of fish fed JM indicated severe enteritis. However, fish fed JM with phytase supplementation showed no histological alteration in either liver or foregut. The result of this experiment demonstrated that, although 50% substitution of fish meal with Jatropha meal is feasible, raising this level to 75% negatively affected growth parameters. Moreover, inclusion of Jatropha meal at these levels without the addition of phytase has adverse effects on liver and foregut of common carp.

In a third experiment, the phosphorous utilization in Jatropha meal diets with added phytase but without phosphorous supplementation was therefore investigated in more detail. This trial was conducted with separate groups kept in parallel in a recirculation and a respiration system. At the end of the eight week trial, in both experimental systems, phytase

supplementation had not affected body weight gain (BWG), food conversion ratio (FCR) and specific growth rate (SGR) of fish. However, whole body P, P gain and P retention were significantly improved in fish fed JM with phytase supplementation when this was compared to the fish fed JM without phytase. Moreover, fish fed Jatropha diets indicated higher O_2 consumption per gram body mass gain than carp fed fish meal diets. The experimental feeds also significantly affected energy retention (ER) and metabolisable energy (ME). These factors were higher in carp fed fish meal based diets than those fed JM with or without phytase addition. Energy expenditure per gramme protein retained was significantly higher for fish fed JM with or without phytase addition, indicating that the utilisation of Jatropha meal was associated with higher energy costs for the fish. The results of this study showed that the addition of phytase improved P bioavailability in diets containing non-toxic Jatropha meal without, however, having any concomitant beneficial effects on the growth parameters of fish.

In the final experiment, the nutritional quality of non-toxic Jatropha meal was compared to that of the toxic variety once appropriate measures had been taken to detoxify the latter. Fish meal was replaced either with either of the two at 75% of total dietary protein, for each Jatropha variety once without further supplementation and once supplemented with 500 FTU phytase and 1% lysine. The results of this experiment indicated that there was no significant differences in final body mass (FBM), percentage of body mass gain (% BMG), feed intake (FI), food conversion efficiency (FCR) and specific growth rate (SGR) of fish fed diets Control and non-toxic JM supplemented with 500 FTU phytase and 1% lysine. However, fish fed either of the two diets based on detoxified JM as well as that containing non-toxic JM without phytase/lysine supplementation showed significantly inferior growth parameters when compared to carp fed the control diet and non-toxic JM supplemented with phytase and lysine ($\rho<0.05$). At the same time, the whole body mineral analysis indicated that there were no statistically significant differences between the groups for most minerals

although the mean values for Fe, Zn, Na and K in the experimental fish differed. Whole body P was significantly higher in fish fed the control and non-toxic JM diets (with/without phytase and lysine) than fish fed either of the two detoxified JM diets ($\rho<0.05$). This experiment indicates that fish meal can be replaced at 75% of total dietary protein with non-toxic Jatropha meal when this diet is supplemented with 1% lysine and 500 FTU phytase. The depressed growth performance in the group fed detoxified JM could imply that the detoxification process was not complete and traces of phorbolesters may still be present in the diets.

This work has shown that, given proper treatment, Jatropha meal can act as a suitable replacement for fishmeal at high levels in diets for common carp. Although its culture is more problematic, non-toxic Jatropha strains are far superior to the toxic varieties since it is still very difficult to remove the potent phorbol esters in the latter that have detrimental effects on animals. Despite the lack of these phorbol esters or their reduction to non-detectable and harmless levels, the non-toxic variety still has to be suitably pretreated before its inclusion in fish diets. The results of this work suggest that phytase addition to enhance phosphorous availability and lysine supplementation to correct the essential amino acid imbalance are two factors that have significant effects in improving Jatropha meal suitability. However, to get full economic benefit from phytase, it is important to correctly formulate diets to allow for the release of additional nutrients. It is suggested that further work (a dose response experiment) to determine the optimum levels of both components (inorganic P and phytase) should be carried out so that Jatropha meal can be implemented commercially. In view of the success experienced with the use of the non-toxic variety, it is also recommended that its inclusion in the diets for other commercial fish species should be investigated.

7. **Zusammenfassung**

Die Aquakulturindustrie ist der am schnellsten wachsende Zweig der Lebensmittelindustrie (FAO 2006) und spielt eine bedeutende Rolle darin, den menschlichen Bedarf nach Fisch abzudecken. In den letzten Jahren haben steigende Kosten, geringe Qualität und ein schwankendes Angebot von Fischmehl dazu geführt, die Bedeutung der Forschung zum teilweisen oder vollkommenen Austausch des Fischmehls im Fischfutter durch alternative Eiweißquellen zu steigern. Zudem befinden wir uns in einer Übergangsphase, in der die Auswahl der Komponenten für die Futtermittel für viele Fischarten erweitert wird (Øverland et al., 2009). Generell sind pflanzliche Proteinquellen von größerem Interesse, da sie billig und allgemein erhältlich sind. Andererseits wird ihr Einsatz durch ihren Gehalt an komplexen Kohlehydraten, ihre unausgewogene Aminosäurenzusammensetzung und das Auftreten einer Reihe von antinutritiven Substanzen eingeschränkt. Pflanzliche Antinutritiva und Gifte sind natürliche Insektizide, die auch Fischen schaden, ihr Wachstum behindern und ihre Gesundheit negativ beeinflussen können (Francis et al., 2001). Da pflanzliche Proteinquellen normalerweise viel billiger sind als Fischmehl besteht das Potential, sie zu bearbeiten um ökonomische Produkte mit gesteigertem Nährwert zu erhalten.

Jatropha curcas L. ist eine robuste Pflanze welche an marginalen oder degradierten Standorten gedeit. Ihr Anbau wird in größerem Stil in Indien, China, Madagaskar, Myanmar und vielen anderen Entwicklungsländern praktiziert, um Biotreibstoff zu produzieren (Makkar et al., 2008). Jatrophasamen enthalten 300-350g kg^{-1} Öl, welches als Brennstoff oder in der transesterifizierten Form als Dieselersatz verwendet wird. Die Proteinqualität der geschälten Samen ist recht hoch. Der Gehalt an essentiellen Aminosäuren (außer Lysin) übersteigt den FAO Referenzwert für 3-5jährige Kinder (Makkar et al., 2008). Jatropha enthält jedoch auch große Mengen an Antinutritiva wie z.B. Lektine, Trypsininhibitoren und Phytinsäure. Zudem ist Jatropha auch in den meisten Formen (außer einer als nicht-toxisch bezeichneten Variante)

sehr giftig, was auf das Vorkommen von Phorbolestern zurückzuführen ist. Der schädliche Einfluß von Phorbolestern auf Wachstum und Gesundheit von gemeinen Karpfen und Ratten ist gut dokumentiert (Becker und Makkar,1998).

Die nutritive Qualität und der Effekt einer Hitzebehandlung auf nicht-toxische Jatropha wurde bereits beschrieben (Makkar und Becker, 1999). Diese Studie zeigte, daß eine 15minütige Hitzebehandlung (121°C, 66% Luftfeuchtigkeit) die Trypsin- und Lektinaktivität im Mehl der Samen dieser Variante deutlich verringerte. Gemeine Karpfen, die mit hitzebehandeltem, nicht-toxischem Jatrophamehl gefüttert worden waren, zeigten höhere Gewichtszunahmen, Proteineffizienz- und Proteinproduktionswerte als Fische, welche unbehandeltes Mehl erhalten hatten. Dennoch waren diese Parameter geringer als in Fischen, die eine fischmehlbasierte Diät bekamen (Makkar und Becker, 1999). Die Autoren waren der Meinung, daß das geringere Wachstum der mit nicht-toxischer Jatropha gefütterten Fische auf Mängel in der Zusammensetzung essentieller Aminosäuren wie z.B. Lysin, hoher Phytinwerte und dem Auftreten anderer antinutritiven Substanzen wie z.B. Saponine, stärkefreier Polysaccharide und anderer nicht weiter bekannter Komponenten zurückzuführen war. Dies würde eine weitergehende Behandlung von Jatrophamehl erforderlich machen, damit dies in höheren Mengen in kommerziellen Futtermitteln für gemeine Karpfen verwendet werden kann.

Diese Studie wurde somit durchgeführt, um diverse Methoden der nutritiven Qualitätssteigerung von Jatrophamehl zu untersuchen, um dieses Ziel zu erreichen. Im ersten Experiment wurden vier Futtermittel hergestellt, die alle auf einem 50%igen Austausch von Fischmehl durch nicht-toxisches, entfettetes Jatrophamehl basierten. Im ersten wurde das Jatrophamehl nicht weiter behandelt, im zweiten wurde es vor der Futtermittelmischung mit einem 80% Äthanol /20% Wassergemisch extrahiert, im dritten mit 1% L-Lysin und im vierten mit 500 FTU kg^{-1} Phytase (5000G, Natuphos) supplementiert. Diese Futtermittel wurden mit einer Standarddiät auf Fischmehlbasis verglichen. Die Ergebnisse zeigten, daß die

mit Lysin oder Phytase supplementierten Futtermittel das Wachstum der Karpfen auf ein ähnlich hohes Niveau wie das der mit der Fischmehldiät gefütterten Fische brachten. Die mit nicht weiter behandeltem oder äthanolextrahiertem Jatrophamehl gefütterten Fische wuchsen jedoch signifikant langsamer. Der Zusatz von 1% Lysin oder 500 FTU Phytase verbesserte die prozentuale Gewichtszunahme, Futterkonvertiereffizienz und spezifische Wachstumsrate auf ein ähnlich hohes Niveau wie die Kontrolldiät. Zusätzlich steigerte der Zusatz von Phytase die körperlichen Magnesium-, Kalium- und Phosphorwerte der Karpfen auf die Werte der Kontrollfische. Dieser Versuch zeigte, daß Jatrophamehl für den 50%igen Austausch von Fischmehl im Futter von gemeinen Karpfen eingesetzt werden kann, sofern die mit dem Auftreten von Phytin und dem Mangel an Lysin verbundenen Probleme eliminiert werden.

Bei einer Steigerung des Austauschniveaus von 50% auf 75% bei gleichzeitiger Supplementierung mit inorganischem Phosphor (Versuch 2) wurde jedoch mit oder ohne 500 FTU Phytase im Futtermittel ein deutlicher Rückgang des Gewichtzuwachses der Karpfen beobachtet. Zwei andere, auf Sojamehl basierende Futtermittel (mit/ohne Phytase) brachten ähnlich schlechte Ergebnisse. Am Ende des Versuchs hatten die mit einer Fischmehldiät gefütterten Fische eine höhere Gewichtszunahme, spezifische Wachstumsrate und geringere Futterkonvertierrate als die anderen experimentellen Fische ($\rho < 0.05$). Die Supplementierung mit Phytase brachte eine numerische Steigerung der Gewichtszunahme, aber dieser Effekt war nicht statistisch signifikant. Zudem beeinflußte der Einsatz von beiden pflanzlichen Proteinquellen die Körperzusammensetzung in punkto Eiweiß-, Fett und Aschegehalt der Fische. Die mit Jatrophamehl gefütterten Karpfen hatten signifikant geringere Protein- und Wasser- und höhere Fett- und Aschegehalte als die mit anderen Futtermitteln gefütterten Fische. Zudem zeigte die histopathologische Untersuchung der Lebern der Fische einen erhöhten Grad der intrazellulären Fettablagerung. Die Histologie des vorderen Darms wies auf eine schwere Enteritis. Fische, welche mit Phytase supplementiertes Jatrophamehl erhalten hatten, zeigten keine histologischen Anomalitäten in Leber oder Vorderdarm. Dieses

Ergebnis zeigte, daß, obwohl eine 50%ige Substitution von Fischmehl im Fischfutter durch Jatrophamehl möglich ist, die Steigerung dieses Niveaus auf 75% das Wachstum jedoch negativ beeinflußt. Zudem werden bei einer solchen Steigerung ohne einhergehender Phytasesupplementierung Leber und vorderer Darm von gemeinen Karpfen geschädigt.

In einem dritten Experiment wurde daher die Phosphoraufnahme von Fischen, die mit Jatrophamehl mit Phytase- aber ohne Phosphorsupplementierung gefüttert wurden, detaillierter untersucht. Dieser Versuch wurde mit separaten Gruppen durchgeführt, die in einem Respirationssystem und in einem Aquarienkreislauf gehalten wurden. Am Ende des achtwöchigen Versuchs hatte die Phytasesupplementierung in beiden Haltungssystemen keinen Einfluß auf Körpergewichtszunahme, Futterkonvertierverhältnis und spezifische Wachstumsrate der Fische gehabt. Diese Supplementierung hatte jedoch einen positiven Effekt auf Phosphorgehalt, -zunahme und -retention im Körper im Vergleich zu denen der Fische, die mit Jatrophamehl ohne Phytase gefüttert worden waren. Zudem hatten die mit Jatrophamehl gefütterten Fische einen höheren Sauerstoffverbrauch pro Gramm Körpermasse als die mit Fischmehldiäten gefütterten Fische. Die Testdiäten beeinflußten auch die Energieretention und metabolisierbare Energie des Körpers signifikant. Diese Parameter waren höher in den mit Fischmehldiäten gefütterten Fischen als in denen, welche Jatrophamehl mit oder ohne Phytase erhalten hatten. Der Energieaufwand pro Gramm retiniertem Eiweiß war ebenfalls höher in den mit Jatrophamehl (mit/ohne Phytase) gefütterten Fischen, was darauf hinweist, daß die Nutzung von Jatrophamehl für die Fische mit einem höherem Energieaufwand verbunden war. Dieses Experiment zeigte, daß der Zusatz von Phytase die biologische Nutzbarkeit von Phosphor im Futtermittel mit nicht-toxischem Jatrophamehl steigerte, jedoch ohne zu weiteren, damit einhergehenden Verbesserungen des allgemeinen Wachstums der Fische zu führen.

Im letzten Versuch wurde die nutritive Qualität von nicht-toxischem Jatrophamehl mit der der toxischen Variante verglichen, nachdem die nötigen Maßnahmen zur Detoxifizierung

der letztgenannten durchgeführt worden waren. Fischmehl wurde in den Testdiäten zu 75% des Eiweißgehalts mit einer der beiden Jatrophasorten ersetzt, jeweils einmal ohne weitere Supplementierung und einmal mit Zusatz von 500 FTU Phytase und 1% Lysin. Die Ergebnisse zeigten, daß es in Bezug auf Endgewicht, prozentualer Gewichtszunahme, Futteraufnahme, Futterkonvertiereffizienz und spezifischer Wachstumsrate keine signifikanten Unterschiede zwischen den mit nicht-toxischem Jatrophamehl mit Lysin und Phytase und den mit der Kontrolldiät gefütterten Fischen gab. Die mit detoxifiziertem Jatrophamehl sowie die mit nicht-toxischem Jatrophamehl ohne Lysin-/Phytasezusatz gefütterten Fische zeigten jedoch schlechtere Wachstumsparameter als die Fische, welche die beiden anderen Diäten erhalten hatten. Gleichzeitig zeigte die Körpermineralanalyse, daß es in Bezug auf die meisten Mineralien keine statistisch signifikanten Unterschiede zwischen den Testgruppen gab, obgleich die mittleren Eisen-, Zink-, Natrium- und Kaliumwerte voneinander abwichen. Der Gesamtkörpergehalt an Phosphor lag in den mit den Kontrolldiäten und nicht-toxischer Jatropha (mit/ohne Lysin und Phytase) gefütterten Fischen über dem der beiden mit detoxifiziertem Mehl gefütterten Gruppen. Dieser Versuch zeigte, daß Fischmehl zu 75% mit nicht-toxischem Jatrophamehl bei einhergehender Supplementierung mit 1% Lysin und 500 FTU Phytase ersetzt werden kann. Das deutlich zurückgeliebene Wachstum der mit detoxifiziertem Jatrophamehl gefütterten Fische könnte andeuten, daß der Entgiftungsprozeß nicht vollkommen abgeschlossen war und Spuren von Phorbolestern im Futtermittel zurückgeblieben waren.

Die vorliegende Arbeit hat gezeigt, daß Jatrophamehl bei korrekter Nachbehandlung als adäquater Ersatz für Fischmehl in Futtermitteln für gemeine Karpfen dienen kann. Obwohl ihr Anbau mit größeren Problemen verbunden ist, ist die nicht-toxische Form hierfür viel besser geeignet, da es immer noch sehr schwierig ist, die äußerst wirksamen Phorbolester der toxischen Variante zu eliminieren, die bei Konsum einen stark schädlichen Einfluß auf Tiere ausüben. Obwohl der nicht-toxischen Form diese Phorbolester fehlen oder sie hier unterhalb

der nachweisbaren und schädlichen Grenze zu finden sind, muß diese Variante dennoch zusätzlich behandelt werden, bevor sie in Fischfuttermitteln zum Einsatz kommen kann. Die Ergebnisse dieser Arbeit deuten darauf, daß eine Phytasesupplemetierung zur Erhöhung der Phosphoraufnahme und der Zusatz von Lysin zur Korrektur des unausgewogenen Aminosäuregehalts zwei Faktoren sind, die zu einer deutlichen Verbesserung der nutritiven Qualität des Jatrophamehls führen. Um dabei den maximalen ökonomischen Nutzen aus der zugesetzten Phytase zu erzielen muß für das Futtermittel aber auch die optimale Rezeptur ermittelt werden. Es wird daher angeregt, in zukünftigen Versuchen den optimalen Gehalt an Phytase und inorganischem Phosphor zu bestimmen, so daß der Einsatz von Jatrophamehl kommerziell durchgeführt werden kann. In Anbetracht des hiesigen Erfolgs mit der nicht-toxischen Jatrophavariante wird empfohlen, deren Nutzung auch für andere Fischarten zu untersuchen.

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