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DEVELOPMENT OF SUPPLEMENTAL DIETS FOR CARP IN VIETNAMESE UPLAND PONDS BASED ON LOCALLY AVAILABLE RESOURCES

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List of Abbreviations

&	and
AA	Amino Acid
AOAC	Association of Analytical Comunities
ADC	Apparent Digestibility Coefficient
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
a.m.	Morning
ANLU	Apparent Net Lipid Utilization
ANOVA	Analysis of Variance
et al.	Lain= others
CA	Crude Ash
CF	Condition Factor
CL	Crude Lipid
CP	Crude Protein
DM	Dry Matter
DO	Dissolved Oxygen
E	East
EAA	Essential Amino Acid
E-ADC	Energy Appearance Digestibility Coefficient
ER	Energy Retention
EUR	Euro
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FI	Feed Intake
Fig.	Figure
FM	Fresh Matter
FW	Final Weight

GE	Gross Energy
HSI	Hepato Somatic Index
HUA	Hanoi University of Agriculture
ISI	Intestine Somatic Index
IU	International Unit
IW	Initial Weight
L	Length
L-ADC	Lipid Apparent Digestibility Coefficient
MBG	Metabolic Body Gain
MBW	Metabolic Body Weight
MGR	Metabolic Growth Rate
MoFi	Ministry of Fisheries
N	North
n.d.	Not Determined
NDF	Neutral Detergent Fibre
NEAA	Non Essential Amino Acid
NRC	National Research Council
P/E	Protein/Energy
P-ADC	Protein Apparent Digestibility Coefficient
PER	Protein Efficiency Ratio
p.m.	Afternoon
PPV	Protein Productive Value
RIA1	Research Institute for Aquaculture Number 1
SPL	Sweet Potato Leaf
USFA	Un-saturated Fatty Acid
VAC	Vietnamese farming system, in which garden, fish pond, and livestock are integrated
W	Weight
WG	Weight Gain

Units

°	Degree
°C	Degree Centigrade
%	Percent
µl	Micro-liter
µm	Micro-meter
cm	Centimeter
d	Day
g	Gram
gf	Gravity Force
h	Hour
ha	Hectare
kcal	Kilocalorie
kg	Kilogram
$kg^{-0.8}$	Metabolic Body Mass
kJ	Kilo joule
km	Kilometer
l	Liter
m^2	Square Meter
m^3	Cubic Metter
mg	Milligram
ml	Milliliter
ppm	Part Per Million
ppt	Part Per Thousand
rpm	Revolutions Per Minute
std	Standard Deviation
mt	Metric Tonne (1000 kg)
VND	Vietnamese Dong

Chemical Terms

HCl	Hydrochloric Acid
M	Mole
TIA	Trypsin Inhibitor Activity

Exchange Rate:

1 € ≈ 20 000 VND (in 2006)

Introduction

Aquaculture has grown steadily in recent decades, from 1 million tonnes in the 1950s to nearly 52 million tonnes in 2006 which in financial terms was equivalent to almost 80 billion US$ (FAO, 2009d). In 2006, aquaculture contributed approximately 47% to the total fisheries production which increased the world consumption per capita to 16.7 kg annually. FAO (2009d) stated that 90% of food fish for China and 24% for the rest of the world came from aquaculture. With annual growth rates of 8.7% since 1970 and 6.1% during 2004 and 2006, aquaculture is maintaining the fastest growth among all animal food-producing sectors (FAO, 2009d, Delgado et al., 2003).

In aquaculture, omnivorous and herbivorous fish species play very important roles, especially in Asia and the Pacific regions. The contribution of these groups in 1991 exceeded 85% of the world aquaculture finfish production, of which more than 70% was derived from cyprinid fish species such as silver carp, common carp, grass carp, and Indian carp (FAO, 1993, Yakupitiyage, 1992). Data from FAO (2003) showed that the production of cyprinids was ten times higher than that of salmonids in 1991. However, despite cyprinids having a low value on the world market, they have contributed greatly to the diets of poor people as local animal protein sources (Dey et al., 2005, Yakupitiyage, 1992) and become the most important cultured fish species in aquaculture (Acosta et al., 2005), especially in extensive or semi-intensive systems in Asia and the Pacific (Tacon et al., 1997, Van et al., 2002, Dey et al., 2005), including China (Jian et al., 2005).

In contrast to their quantitative contribution to aquaculture, research on cyprinids is limited compared to other species such as salmonids. Data from Aquatic Sciences and Fisheries Abstracts (ASFA) showed that there were only 4600 studies on cyprinids during 1978 and 1993 whereas there were 9200 studies on salmonids during the same period (Tacon, 1993a). Tacon and De Silva (1997) alerted researchers to the fact that the volume of research on omnivorous and herbivorous fish species was not commensurate with their contribution to total fish production. In particular, they urged that scientific understanding of culture systems and their requirements should be improved.

Aquaculture in Vietnam has followed the world trend and increased dramatically in recent years. MARD (2008) reported that total fishery production has increased from 1,344 million tonnes in 2005 to 4,160 million tonnes in 2007, of which aquaculture contributed from 40 to 50 percent. Highly intensive systems of giant tiger prawn (*Penaeus monodon)* and catfish (*Pangasius hypophthalmus)* production in the South led Vietnam to be the third largest producer of tiger prawn and the leader in catfish culture. However, although these high value species contributed approximately 60 to 65% of the total fishery production in Vietnam in 2005 (MARD, 2008), they seem to be unaffordable for people in rural areas (Steinbronn, 2009), especially for the ethnic minorities in the mountainous North. It was reported that fish production contributed approximately 30 to 35% of the total animal protein intake of Vietnamese (Barg, 1997, Tung, 2000) and, in food-deficit regions, fish tends to constitute a higher share of total animal protein consumption (Deppert, 1992, FAO, 2009d, Kent, 1997) and can even rise to 45% in some poor regions in Asia (Prein et al., 2000). However, people in these poor regions are more dependent on herbivorous and omnivorous fish such as carp than on carnivorous fish and shrimp (Dey et al., 2005)

Son La is one of the poorest provinces, located in the mountainous region of North - Western Vietnam. It has a population of approximately one million, and is comprised of 12 different ethnic groups such as Thai, H'mong, Muong, Dao, Tay and others. With a total area of 14000 km^2, Son La is the third biggest province in Vietnam. However, due to a low proportion of agricultural land (less than 27%, (Sourced from Son La's statistic office, 2009, unpublished), a harsh climate and rugged terrain the livelihood of farmers in Son La is precarious, making Son La one of the poorest provinces in Vietnam (Minot et al., 2003), especially in the settlement areas for ethnic minorities. According to a report from the Vietnamese Government Statistic Organisation (GSO, 2009b), nearly 60% of Son La's population in 2004 was poor, and 40% were malnourished.

Yen Chau is a district of Son La with a population of 60 000, in which the Black Thai ethnic minority accounts for approximately 54% (People's committee Yen Chau, data referring to 2004). Black Thai farmers usually live in the valleys, where they are able to produce paddy rice, mainly for household consumption. Furthermore they grow

maize, cassava and occasionally cotton as cash crops on the hillsides. The production of fish in a small pond system is also a very typical activity which supplies an important nutritional source for humans and contributes thereby to food security in the region (Edwards, 2000, Steinbronn, 2009). In addition, products from aquaculture systems can be sold at local markets and thus lead to increased household income (Steinbronn et al., 2004, Steinbronn, 2009). The economic importance can be demonstrated by comparing the price of common carp (30,000 VND/kg) with the average monthly per capita income in Son La of approximately 13.4 US $ (= 210,000 VND, General Statistic Office 2004, data referring to 2002).

Unlike aquaculture in South Vietnam where highly intensive cultivation of catfish (*Pangasius hypophthalmus*) and tiger prawn (*Penaeus monodon*) is possible, Yen Chau tends to have extensive systems typical of mountainous regions. The systems are described as small scale models or are integrated with other activities: namely rice-cum-fish, rice-cum-prawn and duck-cum-fish. Products or by-products of this process can be sources for others (Luu, 1992). Self-recruiting species like tilapia, snakehead fish, climbing perch, and carp are the most suitable for small scale aquaculture systems in mountainous region because of their availability and hardiness (Hung, 2004).

Within the framework of the Special Research Program on "Sustainable land use and rural development in mountainous regions of Southeast Asia" (SFB 564, "The Uplands Program") supported by the German Science Foundation (DFG), the studies conducted by Steinbronn (2009) have described the pond system in the region as a poly-culture system of grass carp, other carp species and tilapia, their feed being crop residues such as leaf materials and grasses (Dongmeza et al., 2009). Such feed is mainly consumed by the macro-herbivorous grass carp. As the production of natural feed is restricted in this system, the other species usually show much lower growth rates than grass carp (Steinbronn, 2009). Thus, the productivity in this pond system is low. Even though most farmer households in Yen Chau own at least one pond each, the demand in the local market cannot be covered (Steinbronn, 2009). In addition, unknown diseases causing high mortalities could be one of many the reasons for the decrease of grass carp production (Steinbronn et al., 2005b). To avoid the risk of losing grass carp, farmers are tending to change the composition of the fish species stocked in favour of

e.g. common carp and mud carp without having an appropriate feed base for these species (Tuan et al., 2008, Steinbronn et al., 2005b).

Common carp is one of the most popular fish species in Vietnam in general (Anjum, 2005) and in Yen Chau in particular (Steinbronn et al., 2005b). Observations by Steinbronn (2009) showed that common carp is preferred to many other fresh water fish such as grass carp and mud carp. According to local people, common carp is delicious and tastes better than other fish (Phuong, 2005, Dey et al., 2005). This fish species is usually given to pregnant and lactating women (Phuong, 2005) or eaten on special occasions, for example the religious ceremony of Lares on 23rd December of the lunar calendar. They also fetch higher prices in the local market than grass carp, Indian carp, or tilapia (Steinbronn, 2009). Culture of common carp has greatly increased in many lowland regions in Vietnam. However, in order to achieve high production through such a traditional small aquaculture system in Yen Chau, farmers must still overcome many difficulties, especially when they lack a knowledge of aquaculture (Steinbronn, 2009).

On the other hand, Yen Chau's farmers often produce redundant surpluses of agricultural products such as cassava and maize. The survey done by Thanh and Foerster (2003) showed that maize with a yield of approximately 6 tonnes per hectare in the main season was the major source of cash income for more than 90% of farmers in Yen Chau. GSO (2009a) reported that Sonla produced approximately 326000 and 208000 tonnes of maize and cassava respectively in 2007. Most of these products were sold to traders from the lowlands at relatively low prices. During the main season in 2008, the price of maize decreased to 2200 VND/kg which was half the price of the year before (Viet, 2008). Many of these products are used as ingredients to formulate feed for animals as well as fish species in the lowlands. Therefore, if farmers in Yen Chau could process those crops by themselves for use in fish culture, they could turn crop surpluses which presently earn them very little into 'value added' products. This study entitled "Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources" was conducted not only to contribute to an improvement of income and food security in the region but also to improve scientific knowledge of cyprinids.

2. Literature Reviews

2.1 General Aquaculture

In 2006, world fish production was estimated at 143 million tonnes, of which approximately 110 million tonnes (77%) was for human consumption. This increased apparent per capita supply to 16.7 kg live weight aquatic products (Table 1) (FAO, 2009d). Overall, the contribution of fish to the total animal protein intake per capita increased from 14.9% in 1992 to 16.0% in 1996. This index was 18.5% or even higher in the food-deficit regions (FAO, 2009d).

Table 1: World fisheries and aquaculture production

	2002	2003	2004	2005	2006
INLAND (million tonnes)					
Capture	8.7	9.0	8.9	9.7	10.1
Aquaculture	24.0	25.5	27.8	29.6	31.6
MARINE (million tonnes)					
Capture	84.5	81.5	85.7	84.5	81.9
Aquaculture	16.4	17.2	18.1	18.9	20.1
Total capture	93.2	90.5	94.6	94.2	92.0
Total aquaculture	40.4	42.7	45.9	48.5	51.7
Total world fisheries (million tonnes)	133.6	133.2	140.5	142.7	143.6
Per capita food fish supply (*kg per capita per year*)	16.0	16.3	16.2	16.4	16.7

Source: FAO (2009d), *excluding aquatic plants.*

However, despite an increase in total fisheries production, the contribution of capture fisheries seems not to have grown during the last three decades when most of the world's fish stocks have been over exploited or exploited to their maximum potential. During the 1950s and 1960s, capture fish production increased by an average of 6 per cent per annum, but this rate declined to 2% during the 1970s and 1980s, falling almost to zero during the 1990s (FAO, 2000). Recently, FAO (2009d) reported that about 52% of stock were fully exploited while 28% were either overexploited, depleted or recovering from depletion. As a result, it might be difficult to increase yields from capture fisheries in the future (Williams, 1996).

In contrast, aquaculture grew steadily in the last few decades. World aquaculture production, excluding seaweeds, increased from 31 million tonnes in 1998 to 51 million tonnes in 2006 (FAO, 2006a). It is generally accepted that the scope for growth in capture fisheries is minimal; hence growth in the aquatic food supply will have to be realized through aquaculture (FAO, 2005, Reantaso et al., 2008). During the past three decades, aquaculture has expanded, diversified and intensified. It accounted for 51.7 million tonnes, the equivalent of US$ 78.8 billion in 2006 (Subasinghe, 2006, FAO, 2006b) and continues to be the fastest growing animal food producing sector. Growth in global aquaculture averaged 7.3% annually (Lowther, 2006) and now accounts for nearly 45% of the world's consumption of seafood (FAO, 2006b).

The development of aquaculture industries has been considered as a means of supplying the future demand for aquatic products; in particular, to provide a major world-wide protein source (Shpigel et al., 1993). Another report pointed out that millions of people were dependent on aquatic production as their main protein source (Becker and Focken, 1998). It was predicted that aquaculture would have to provide more than 50% of the total demand for seafood products (Tidwell et al., 2001) and reach at least 100 million tonnes per annum by 2030 to maintain the current per capita consumption (FAO, 2006b).

Within the Asian countries, fish protein provides approximately 45 percent of the total protein consumed (Prein and Ahmed, 2000). Although Japan, the European Union and USA have higher per capita consumption of fish and fish products, the share of fish

protein in terms of total animal protein consumption is far less than that in many developing countries (Gupta et al., 2005).

Among all fish species, Cyprinid fish have an irreplaceable role in human nutrition in Asian countries. It is reported that cyprinids represent 70% of world culture fish production, of which Asia accounts for more than 85% (Tacon and De Silva, 1997). FAO (2009d) has recorded that silver carp, grass carp, common carp, bighead carp and crucian carp belong to the top 10 species in world cultured aquatic production. World aquaculture production is dominated by the production from the Asia-Pacific region, which accounts for 89 percent in term of quantity and 77 percent in terms of value. This region accounts for 88 percent of shrimp and 98 percent of carp, approximately 9 million tonnes of which come from China and India (FAO, 2009d).

2.2 Aquaculture of Vietnam

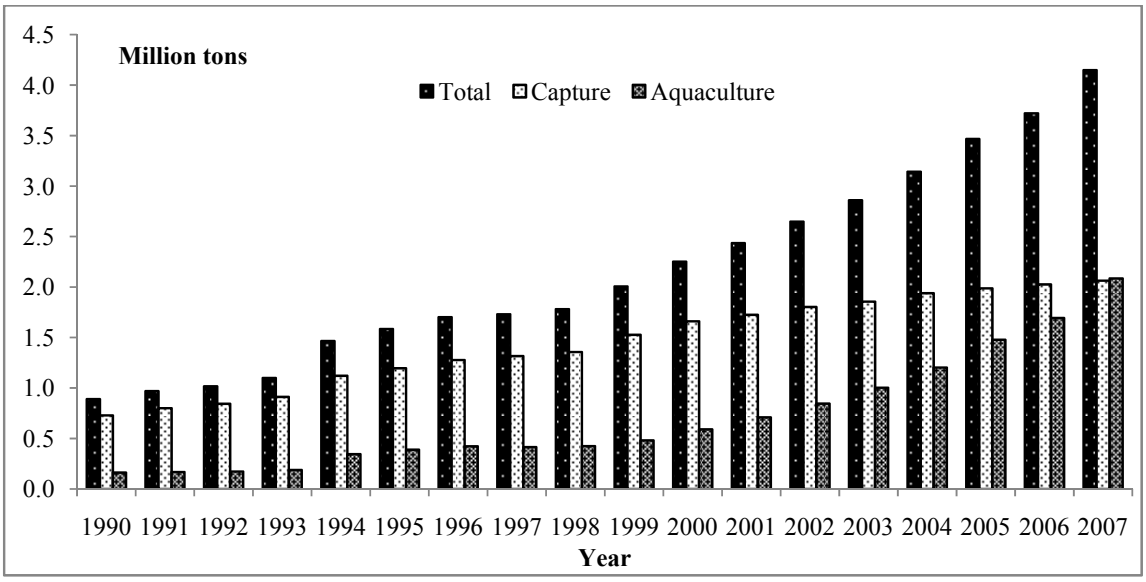

Figure 1: Fishery production of Vietnam (GSO, 2009b)

Aquaculture in Viet Nam began with small scale extensive culture in the early 1960s (Nguyen et al., 2005b, Son, 1996), but rapid growth has been achieved during recent decades (Duong, 2004, Phuong et al., 2007). The main culture areas are located in the Mekong river delta with two major commercial cultured species, giant tiger prawn (*Penaeus monodon*), and catfish (*Pangasius hypophthalmus*) (Phuong et al., 2007).

These species contributed approximately 60 to 65% of total fishery production in Vietnam in 2005 (MARD, 2008). MARD (2008) reported that total fishery production has increased from 1.344 million tonnes in 2005 to 4.160 million tonnes in 2007, of which aquaculture contributed from 40 to 50% (Figure 1).

Aquaculture in Vietnam has great potential and continues to grow at a high annual rate reaching 8% per year in 2007 (Chinh, 2008). Vietnam was recorded as the third largest aquaculture producer in the world with a total of 1.6 million tonnes in 2006 (FAO, 2009d). Total fishery production in 2007 was over 4.1 million tonnes, equivalent to an increase of 6 times compared to with 1980. Aquaculture accounted for 2.1 million tonnes (>50%), 10 times higher than that of 1980 (MARD, 2008). In 2007, total export value reached 3.7 billion USD and aquaculture has increased in the last decade, from 41% of total exports in 2000 to 60% in 2007. Vietnam is the leader in catfish production, the third country in giant tiger prawn production and the fifth aquatic product exporter in the world. Depending on the available resources, the Vietnamese government is planning to export about 7.5 to 8 billion USD in the year 2020 with a total production of 4.5 to 5 million tonnes, of which aquaculture will contribute 5.5 to 6 billion USD.

Aquaculture farming systems in Vietnam are diverse. The southern part of the country has the greatest variety of farming activities. For example, giant tiger prawn and catfish are cultured in intensive systems in the South with pond, fence and cage culture systems. Whereas the central region concentrates on the intensive culture of giant tiger prawn and the marine cage culture of fin fish or lobster species, the northern region is dominated by freshwater fish ponds, rice-cum-fish and marine cage culture (Phuong et al., 2007). It is the differences in topography, climate, as well as social conditions that have caused an unequal development of aquaculture in Vietnam between regions and between the species cultivated. Although, fish production has grown very fast in many regions, small-scale aquaculture in Vietnam has not grown in proportion to its potential (Tung, 2000).

2.3 Rural aquaculture and current aquaculture system in Yen Chau – Son La - Vietnam

2.3.1 Role of aquaculture in rural development

Aquaculture plays an important role in rural development. It has strongly influenced the livelihood of farmers in many ways by providing food, employment, income and security (Muir, 1999, Halwart et al., 2003, Halwart, 2005, Charles et al., 1997). In rural areas, aquaculture contributes directly or indirectly to alleviate poverty (Edwards, 1999). It has been reported that about 30% of total animal protein intake by Vietnamese people comes from fish (Tung, 2000). Requirements for fish products in the rural or food-deficit areas seem to be larger than in other regions (FAO, 2009a, Kent, 1997). However, due to many constraints, the potential of small scale farming systems in South Asia have not been fully exploited (Edwards, 1999, Deppert, 1992, Tung, 2000).

One of many constraints is the unwillingness of farmers to adopt new aquaculture technology. Generally farmers prefer to use limited available resources and low cost by-products and work on a small scale rather than to aim for high productivity of fish at a high level of intensification (Tacon and De Silva, 1997). In fact, a high technology or a high level of intensification could be introduced to improve the system but semi-intensive aquaculture seems to be more suited to farmers in mountainous areas (Tacon and De Silva, 1997). The total profit per unit of an intensive system may be higher than those of extensive and semi-intensive systems but profit the margin of extensive and semi-intensive systems is probably higher than that of intensive ones (Edwards, 1999). According to observations by the same author, farmers will not apply new technology as long as its benefits have not been clearly proved (Edwards, 1999). Letting farmers watch their neighbours, who have similar potential, gaining success is a good method to convince them adopting new techniques (Edwards, 1999).

The study of (Huisman, 1990) claimed that poor farming households are more likely to seek new income generating activities than simply contributing to household subsistence. This is in agreement with the observation in Yen Chau by Steinbronn

(2009) who stated that farmers often kept big grass carp for selling in the market rather than eating them. At home they consumed other, smaller or scarcer fish instead.

2.3.2 Aquaculture in Yen Chau

An overview of rural aquaculture was given by (Edwards, 1997) and details of aquaculture systems in Yen Chau were described in studies by Steinbronn (2009). Herbivorous and omnivorous species such as grass carp, tilapia, Indian carp, and common carp were predominantly cultured in a poly-culture system, calling VAC (V: vuon-gardening; A: ao-pond; and C:chuong-animal husbandry) (Luu, 1992). In this system, fish production integrated with other activities such as crop cultivation and animal production. Water in the system was not only used for fish culture but also for other purposes such as irrigation for vegetable and/or using for livestock. Steinbronn (2009) stated that more than 65% of Yen Chau's farmers owned at least one pond which has the average size of 800 m^2.

Rural aquaculture is often practised as semi-intensive culture in which manure and feed are used as supplements (Sinha, 1985, Edwards, 1999). According to criteria applied by Martinez Espinosa (1995) to cost and output, aquaculture in Yen Chau can be ranked at the extremely low level "poorest of the poor". Observations by Silke (2009) showed that the current system was facing a multitude of constrains such as a high mortality of grass carp due to disease problems, low water quality and limited feed in terms of both quality and quantity. Manure was occasionally applied to the fish pond but it can be washed away by floods, especially in the rainy season. To date, Yen Chau's farmers have hardly applied any high value supplemental feed to their aquaculture ponds. Even some cheap feed ingredients such as rice bran, maize or cassava were rarely used for fish ponds. Green fodders were used for ponds, but only grass carp can digest such high fibre-containing leaves (Steinbronn, 2009, Tuan et al., 2008). Some green fodders such as bamboo leaves, cassava leaves etc. even contained high amount of various anti-nutrients which resulted in a low digestibility of the plant material and poor growth of the fish (Dongmeza et al., 2005, Dongmeza et al., 2009). Moreover, due to the limitations of these crop leaves, grass carp could be in

competition with ruminants, especially in the dry season. The current aquaculture system in Yen Chau is described in figure 2:

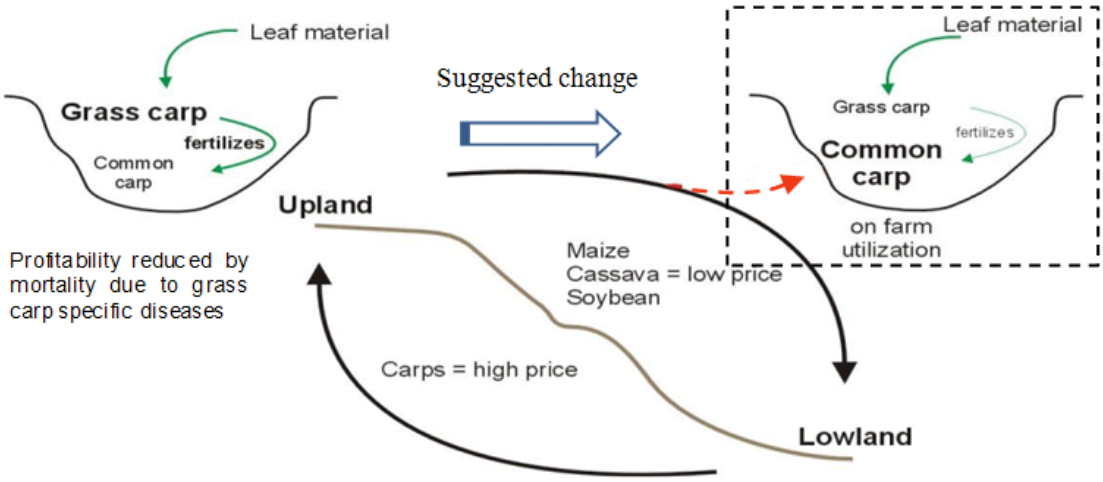

Figure 2: The current farming system in Yen Chau – Son La (Tuan et al., 2008)

According to the data from GSO (2009b), total fish production in Son La is increasing slightly through the expansion of cultured areas, but not as a result of intensification. The current production can supply less than 4 kg of fish per head (Table 2) (GSO, 2009b, MoFi, 1999), which is much lower than the average level of consumption in Vietnam as a whole and far below the potential of a semi-intensive aquaculture system described by Luu (Luu et al., 2002, Luu, 1992). Development of supplemental feed is necessary to improve the system, as well as improvement of farmer knowledge and better treatment of disease.

Son La in general and Yen Chau in particular were recorded as a granary of cassava and maize in North of Vietnam. GSO (2009a) estimated that Son La produced approximately 441 and 270000 tonnes of maize and cassava respectively in 2008 (Table 2). Most of these products were sold at relatively low prices; less than 1000 VND/kg of cassava in 2005 (Steinbronn, 2009) and approximately 2200 VND/kg of maize in 2008 (Viet, 2008). If a small proportion of these products were used for local animals including fish, the province can develop the production of animals as well as aquaculture far beyond the capacity of current systems.

Table 2: Some data on status of fish production in Son La

Year	Aquaculture area	Fishery production	Aquaculture production	Aquaculture productivity	Total fish production per capita
	ha	tonnes/year	tonnes/year	tonnes/ha	kg/ capita/ year
1995	900	1649	1050	1.2	2.0
1996	900	3159	1595	1.8	3.8
1997	1000	3293	1592	1.6	3.9
1998	900	1826	1476	1.6	2.1
1999	900	2089	1578	1.8	2.4
2000	1000	2181	1638	1.6	2.4
2001	1000	2335	1713	1.7	2.5
2002	1500	2942	2248	1.5	3.1
2003	1500	3065	2381	1.6	3.2
2004	1500	3205	2433	1.6	3.3
2005	1600	3325	2582	1.6	3.4
2006	2000	4021	3273	1.6	4.0
2007	2100	4296	3549	1.7	4.2

Source: (GSO, 2009b)

Table 3: Production of some common crop in Son La (x 1000 tonnes**)**

Year	Rice	Maize	Cassava	Soybean
2000	99.4	135.8	128.5	9.5
2001	101	151.6	142.8	9.4
2002	110	196.1	159.1	11.5
2003	100.6	200.9	183.0	12.1
2004	99.4	217.8	199.1	14.8
2005	108.1	228.0	192.3	13.6
2006	112.1	269.0	201.0	11.1
2007	119.2	444.0	210.6	11.5
2008 (estimated)	128.6	441.3	270.4	10.1

Source: GSO (2009a)

2.4 Feed technology and roles of fish meal in fish feed worldwide

Aquaculture has developed quite fast in recent years. The increase in global aquaculture production has resulted in an increase in the demand for compound feeds (Williams, 2007). The demand for fish meal in the aquatic animal feed industry has been steadily increasing while the global production of capture fisheries, which is used to produce fish meal, has not increased since 1985. It was reported that the global capture fisheries production fluctuated between 88 and 96 million tonnes year^{-1}(FAO, 2008). Meanwhile, researchers estimated that approximately 20 million tonnes of compounded aquatic animal feed were used in the aquaculture industry in 2004 and this would need to increase to 25 million tonnes by 2010 (FAO, 2006b). As a consequence, large amounts of fish meal would be needed to formulate that amount of compound feed. It was reported that in 2003 the aquatic animal feed industry

consumed 42% of the total world production of fish meal (FAO, 2006b, Tacon et al., 2008). Another report, Pike (2005) showed that about 46% of fish meal and 81% of fish oil worldwide were used in aquaculture (Figure 3). The aquaculture sector consumed approximately 68% of total global fish meal production and about 90% of fish oil production in 2006 (Tacon and Metian, 2008). The demand in the aquatic animal feed industry for both fish meal and fish oil has been steadily rising while the global production of these marine products has not increased. If this trend is continued, more than 60% of the total global production of fish meal will be used up by the aquatic animal feed industry by 2020 and the total global supply of fish oil will be exhausted. Thus, research to reduce the dependence on fish meal and fish oil in formulating compound aquatic animal feed is of high importance (Williams, 2007). In fact, many studies have been conducted to evaluate the potential of different plant protein sources and have showed much variability.

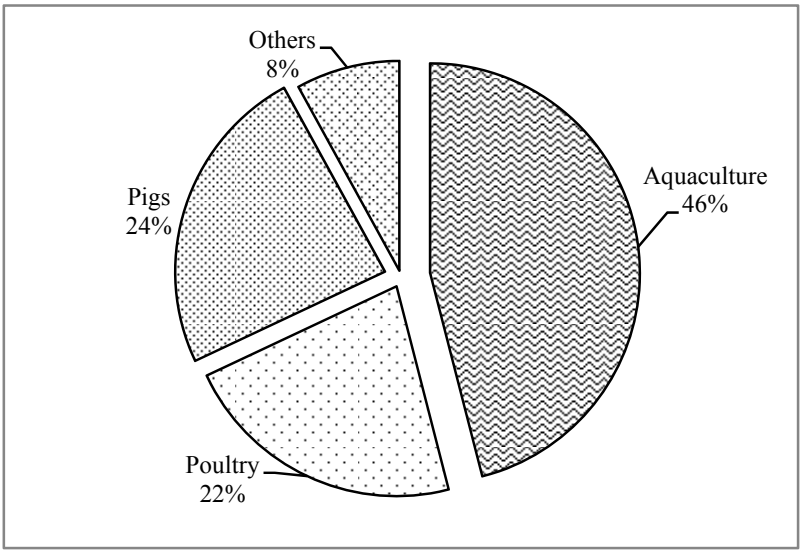

Figure 3: Summary of global use of fish meal in 2002 (Pike, 2005)

Undoubtedly, fish meal is eminently important in aquatic feed. It has a high level of protein with a balanced profile of amino acids and essential amino acids which make it the first choice as a protein for animal feed (Hall, 1992). In addition, fish meal and fish oil have high palatability, which make them suitable as efficient attractants in fish feed production (Nunes et al., 2006, Smith et al., 2005). Although plant protein can partially or fully replace the protein in fish meal, the flavors of plant protein can not

attract fish as well as fish meal (Boonyaratpalin et al., 1998). A small supplement of fish meal, such as squid oil can significantly improve the palatability of feed, feed acceptance and consequently increase feed intake (Espe et al., 2006). The same author inferred that the absence of fish meal in the diet could decrease lipid utilization in salmon, resulting in low growth and lean fish. Moreover, many studies claimed that the digestibility of fish meal was always higher than that of other sources, even in comparison with soybean, one of the best plant protein sources (Boonyaratpalin et al., 1998, Degani et al., 1997). All these characters led fish meal to become indispensable in aquatic feed production.

Unfortunately, fish meal production is insufficient to cover worldwide demand. The uneven geographical distribution of fish meal as well as the frequently large distances between the producing and consuming locations are reasons for the unavailability of fish meal in many countries (Tacon, 1985, Hossain et al., 2001). Peru and Chile are the two major fish meal producers, that accounted for 25% of total fish meal in the world (Hardy, 2006) while aquaculture in Asian accounted for 90% of global aquatic production (FAO, 2009d). Hardy (2006) pointed out that any change in Peru's fish meal production can strongly impact worldwide fish meal supplies. However, Schipp (2008) inferred that fish meal based aquaculture seems to be impossible since a primary source is unavailable.

An increase in the demand for fish meal causes prices for this product to rise (Asraf Mohamed et al., 2007). Hardy (2006) stated that the price of fish meal in 2006 was nearly double that over the previous several decades. Therefore, it is better for aquaculture to focus on production of omnivorous and herbivorous species, such as tilapia, milk fish or carp, which require less protein in general and fish meal in particular (Schipp, 2008, Borgeson, 2005, Hardy, 2006). Furthermore, fish meal is becoming limited due to increasing demand and decreasing marine fishery resources (Tacon, 1985). This makes efforts to reduce the dependence on fish meal in formulating compounded aquatic animal feed extremely important (Asraf Mohamed et al., 2007, Williams, 2007).

2.5 Alternative protein sources and constraints

Looking for alternative protein sources is the priority target of many nutritionists throughout the world (Erfanullah et al., 1998, Goda et al., 2007, Craig et al., 2007). Previously, alternative proteins, including plant and animal proteins, have been studied by many aquaculture nutritionists and the feed industry (Glencross et al., 2007, Tacon, 1985). Many plant protein sources have been investigated to assess their capacity to replace fish meal such as cotton seed, linseed, casein (Soltan et al., 2008, Lim et al., 2009, Koumi et al., 2009, Deyab et al., 2003), duck weed (Hassan et al., 1992, Yılmaz et al., 2004, Azim et al., 2003, Tavares et al., 2008, Leng et al., 1995, Bairagi et al., 2002), blue-green alga *Spirulina platensis* to *Ictiobus cyprinellus* fish (Belay et al., 1996, Stanley et al., 1976), cassava leaves (Keong et al., 1989, Fasuyi et al., 2005) and peanut leaves (Mario et al., 2008, Almazan et al., 1996). Among these, soybean and lupin appeared to be the best plant protein sources which can replace fish meal in fish and shrimp formulated diets (Robaina et al., 1995, Lim and Lee, 2009, Jose et al., 2006, Glencross et al., 2004, Glencross et al., 2005, Chien et al., 2003, Nyirenda et al., 2000, Boonyaratpalin et al., 1998). However, although rapid expansion of livestock production in many regions has increased demand by consuming almost all the feedstuff produced (Siddhuraju et al., 2002), the use of plants as a protein source, including soybean meal, has brought about variable results. Most plant products show imbalances in nutritional values, lack of some amino acids and, especially lack of essential amino acids such as lysine and methionine. Espe (2006) claimed that the balance of amino acids in the basal diet was so important that supplemented crystalline amino acids in plant protein diets could not improve dietary protein utilization in salmon. Gomes et al. (1995) reported that plant protein substitution has often had to be limited due to anti-nutrients and poor palatability. According to many researchers, it is the anti-nutrients such as protease inhibitors, lectins, tannins, phytates antigenic or estrogenic factors and oligosaccharide that prevent the use of plants as a replacement for fish meal (Francis et al., 2001b, Escaffre et al., 1997, Becker et al., 1999, Almazan, 1995, Makkar, 2007, Liener, 1989). The effect of trypsin inhibitor, one of these factors, has been extensively described in fish (Krogdahl et al., 1983). Similarly, Escaffre (1997) conducted an experiment to determine the influence of trypsin

inhibitor from soybean (SBTI) on growth performance as well as on the activities of digestive enzymes in common carp larvae. While heat treatment, soaking, fermentation or germination can deactivate most anti-nutrients (Makkar, 2007), some anti-nutrients like oligosaccharides and phorbol esters seem to be stable and could harm cultured species (Makkar, 2007).

Looking for animal protein sources other than fish meal is also an important alternative option. Replacing fish meal by other animal proteins has often been tried. Yang et al. (2004) were relatively successful in replacing fish meal by meat and bone meal. Nwanna et al. (2004) and Rachmansyah et al. (2004) used shrimp head meal instead of fish meal. Many other authors tested other by-products such as blood meal, poultry by-products etc. (Wang et al., 2006, Shapawi et al., 2007, Rawles et al., 2006, Hu et al., 2008b). Another report showed that frogs, termites and earthworms were suitable for fish culture as protein sources since they contained high proportions of protein (Phonekhampheng et al., 2008, Phonekhampheng, 2008).

2.6 Earthworm and potential of vermi-culture

Earthworms are one of the most ancient invertebrate animals on earth. The use of earth worms started around 230 years ago in Asia to treat human disease caused by bacteria and viruses (Titov et al., 2006). In nature, earthworms live in the surface soil and feed on organic material and micro-organisms. Earthworm activities can not only conserve soil but can also enhance soil structure and aeration as well as water holding capacity (Suthar, 2009b, Janagan et al., 2003). Decomposing waste by earthworms is considered as an eco-friendly, innovative and sustainable technology (Suthar, 2009b, Suthar, 2009a). Vermi-culture naturally reduces the degradation time of organic matter. Moreover, during the degradation process low nitrogen organic waste is converted into nutrient-rich biomass (Janagan et al., 2003, Gupta et al., 2009). Furthermore, this biomass has high potential as a protein source for animal feed (Guerrero, 1983, Hartenstein, 1981b). People in many countries have used earthworms to clean their environment by using them to decompose waste, (Frederickson et al., 1997).

Table 4: Amino acids composition of earthworm

A.A. content	(%DM)	A.A. content	(%DM)
Arginin	6.51	Methionine	2.20
Histidine	2.57	Tyrosine	2.97
Isoleucine	4.69	Phenylalanin	4.01
Leucine	7.59	Glutamic acid	14.20
Lysine	7.56	Glycine	5.00
Tryptophan	1.23	Alanin	5.53
Threonine	4.79	Proline	5.30
Valine	5.00	Serine	5.03
Cysteine	1.83		

Source: Anphu (2009)

Earthworms were initially raised as fish bait in sport fisheries (Mason et al., 2006). It could be inferred that the flavour of earthworms attract fish and that they are a source of nutrients in animal feeding (Kirk, 1971). Paoletti (2003) stated that the nutrient quality of earthworms is comparable with that of dairy milk and eggs. Earthworms have even been used as food for human due to their high nutritional value. Native people in Alto Orinoco in Venezuela consume earthworms (*Andiorrhinus kuru, Andiorrhinus motto*) as one of their important protein sources. Worm meal is rich in protein, lipid and low in ash content. Paoletti (2003) analysed earthworms and found that they contained from 64 to 73 percent protein. Similarly, Stafford and Tacon (1988) also reported that various species of earthworms have about 60-80% crude protein and a variety of lipids, ranging from 2.9% (*L. terrestis*) to 20% (*D. veneta*). In addition, the compositions of amino acids and fatty acids in earthworms were similar to those in fish meal and fish oil. Dynes (2003) reported that the lipids in worm meal contained high ratios of poly-unsaturated fatty acids in the form of omega 3 (ω3). A study from Stafford and Tacon (1988) stated that earthworms contained very little ash

which makes them suitable as fish feed or as an ingredient of fish feed. On the other hand, by-products from vermi-culture, containing high level of ammonia and digested organic materials, are among the best composts for horticulture as well being plant growth stimulators (Edwards et al., 2004). It is of no doubt that these by-products contain high levels of ammonia (Dynes, 2003) and can also be used as fish pond fertilizers.

Earthworms naturally fragment organic matter into fine particles to make it more available for micro-organisms. Researchers have pointed out that micro-organisms play an important role as food for earthworms. Fungi are the most important micro-organisms for earthworms, followed by protozoa, and bacteria. However, earthworms grow best in mixtures of these micro-organisms (Edwards et al., 1988). With a little pre-treatment, a wide range of organic materials such as kitchen wastes, agriculture by-products, animal manures and green leaves etc. can be used as feed to grow earthworm (Edwards and Arancon, 2004, Curry, 2004).

Variability in the availability of organic waste leads to different levels of intensification of vermi-culture. Earthworms can be easily raised under simple conditions because of their high growth rate and prodigious reproduction. Thus, earthworms are considered not only as bio-degraders of organic wastes (Fosgate et al., 1972, Hu et al., 2008a) but also as a potentially economical source of protein (Hartenstein, 1981b). Earthworms are semi-continuous or continuous breeders, producing ova during most of their lifetime (Dynes, 2003). Observations on the reproduction of *E. foetida* fed on horse manure (Hartenstein (1981b) showed that one-half of an earthworm population, on emerging from their cocoons, became mature after 4.5 to 5.5 weeks. Partial and regular harvesting can accelerate growth and reproduction of earthworms. A transfer frequency of 6 to 8 weeks is optimum for maximizing production of biomass (Hartenstein, 1981b). Under suitable conditions, the earthworm's propagation capacity is directly proportional to the food supplied (Curry, 2004). According to one report, if one waits two months after the first stocking, the biomass of the earthworms can then be doubled every 40 to 45 days of culture and the products can be used fresh or processed for storing by drying or freezing (Quy, 2008).

Even though earthworm production has developed rapidly in recent years, it is still facing some constraints. The presence of anti-nutrients in worm meal could limit the use of this protein source. The first experiment on using earthworms for trout by Tacon et al. (1983) showed that high proportions of earthworms in supplemental diets could make the feed unpalatable, resulting in a reduction in feed intake and growth (Stafford et al., 1984). Researchers suggested that the yellow pigment excreted by worms as a lubricant to aid body movement (Edwards and Arancon, 2004, Laird et al., 1981) or as an antibiotic (Pan et al., 2003, Kauschke et al., 2007) could be cytotoxic (Kauschke et al., 1987) or could act as an anti-nutrient (Nandeesha et al., 1988, Tacon et al., 1983, Andrews et al., 1975). Researchers have also voiced suspicions about the possibility of contamination of cultivated worms by parasites or heavy metals. A study by Stafford et al. (1984) showed that earth worms supplemented maximally with domestic sewage could cause high levels of some heavy metals in trout carcasses when fed to these fish. However, the same authors did not find any difference in concentrations of zinc, lead, copper, or cadmium in the whole fish carcass when they were fed diets containing a low level of earthworm supplementation. Post-harvest techniques, for example: washing, blanching and purging can remove or minimize the accumulation of heavy metals, and heat treatment can deactivate anti-nutrient factors in the yellow fluid (Nandeesha et al., 1988, Tacon et al., 1983). The biggest constraint to earth worm production nowadays is that it is proving difficult to produce earthworms economically on a large commercial scale where the most costly process is the actual harvesting of the worms (Yaqub, 1997, Dynes, 2003). Nevertheless, earthworms can be produced at an economic price in low-input small scale operations (Pfiffner et al., 2007) and where routine labour is available.

2.7 Common carp

2.7.1 Common carp biology

Common carp was first described by Linnaeus in 1758. The Common carp or European carp (*Cyprinus carpio*) is a widely distributed freshwater fish closely related to the common goldfish (*Carassius auratus*), with which it is capable of interbreeding (Taylor et al., 1977). They are native to Asia through Europe and North America (Balon, 1995, Panek, 1987).

Kingdom: *Animalia*

Phylum: *Chordata*

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Genus: *Cyprinus*

Species: *C. carpio*

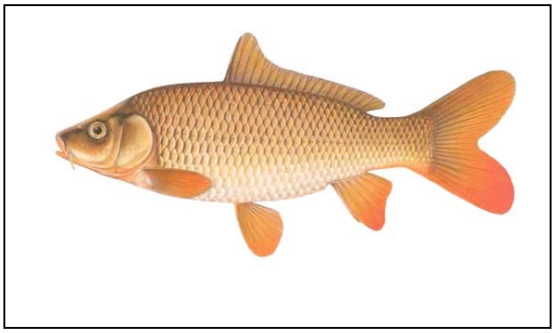

Figure 4: Common carp

Carp often grow to between 30 and 60 cm in length and weight from 0.5 to 4 kg (Tomelleri et al., 1990); it is not uncommon for common carp to reach 15 to 20 kg (McCrimmon, 1968). Males are usually distinguished from females by their larger ventral fin. Carp are characterized by their deep body and serrated dorsal spine (Nelson, 2006). The mouth is terminal in the adult and subterminal in the young (Nelson, 1992, Page et al., 1991). Colours and proportions are extremely variable, but scales are always large and thick. Three sub-species with slightly different scale patterns have been described. *C. carpio communis* (scale carp) has regular concentric scales, *C. carpio specularis* (mirror carp) has large scales running along the side of the body in several rows while the rest of the body is naked, and *C. carpio coiaceus* (leather carp) has few or no scales on the back and a thick skin (McCrimmon, 1968). They typically inhabit shallow areas of lakes and streams and generally avoid swift waters. Wild common carp live in the middle and lower streams of rivers, in inundated

areas, and in shallow confined waters, such as lakes, oxbow lakes, and water reservoirs (FAO, 2009a). Common carp are mainly bottom dwellers but also search for food in the middle and upper layers of the water body (FAO, 2009a).

Although they are very tolerant to most culture conditions, common carp prefer large bodies of slow flowing or standing water with soft sediments. As schooling fish, they prefer to stay in groups of 5 or more. They are best adapted a temperate climate and fresh or brackish water. Carp are rapidly growing fish species and are able to tolerate less than ideal environmental conditions. Best growth is obtained when water temperature ranges between 23°C and 30°C (FAO, 2009a). The fish can survive cold winter periods. Salinity up to about 5‰ is also tolerated and the optimal pH range is 6.5-9.0. The species can survive low oxygen concentration (0.3-0.5 mg/l) as well as super saturation. Common carp will readily survive winter in a frozen over pond, as long as there remains some free water. Carp can survive summer water temperatures of 32°C for short periods. Their ideal environmental temperature lies between 20 and 24°C (FAO, 2009a).

Common carp are omnivorous and have a preference for animal food, such as water insects, insect larvae, worms, molluscs and zooplankton (FAO, 2009a). Zooplankton consumption is dominant in fish ponds where stocking density is high. Additionally, carp consume stalks, leaves and seeds of aquatic and terrestrial plants, decayed aquatic plants, etc (FAO, 2009a). The common carp can eat a vegetarian diet of water plants, but prefers to scavenge the bottom for insects, crustaceans (including zooplankton) and benthic worms. They also eat fungi.

2.7.2 Common carp culture

Common carp have been cultured for more than 2000 years in China, where they were kept in un-drainable ponds (FAO, 2009a). The ponds were stocked regularly with fry from rivers and a natural food-based poly-cultural rearing technology was applied (FAO 2009). Common carp production rapidly expanded, especially in Asian regions and China (Takeuchi et al., 2002) and carp were always the most important cultured fish species (Michielsens et al., 2002) as well as the most valuable (FAO, 2001). In

the early stages of the development of aquaculture, juvenile common carp were collected from the wild for grow-out. Later, common carp were produced naturally in ponds (Müller, 1981). The first hatchery production of seed was achieved in the 1960s, resulting in the elaboration of mass production hatchery methods (Tamás et al., 1977). These technologies are now well established for the culture of common carp, and most seed for grow-out farms is supplied by the hatcheries in many parts of the world.

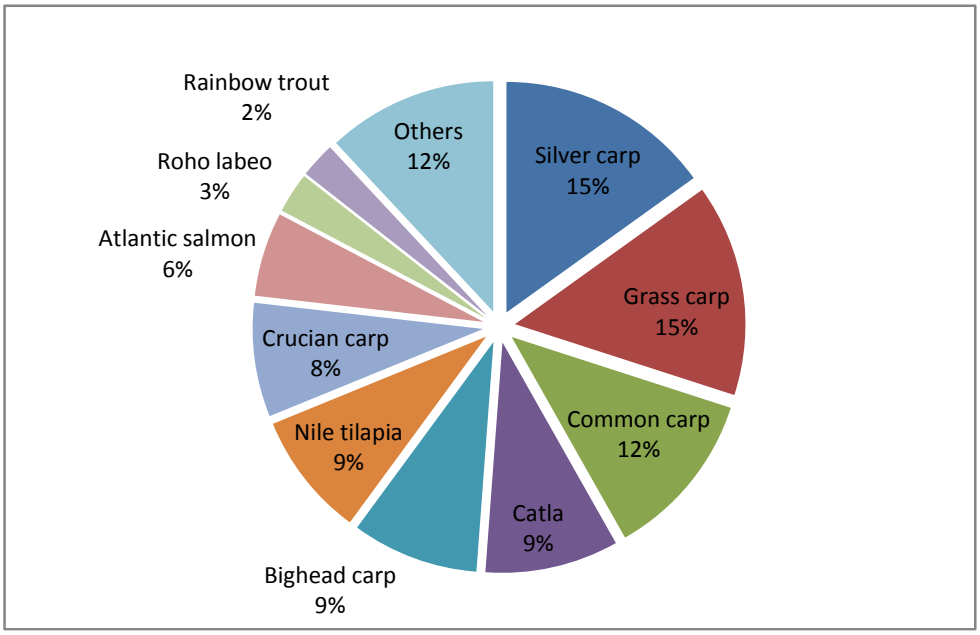

Figure 5: Top ten cultured fish species in 2007 (FAO, 2009d)

Carp was one of the first types of fish to be grown in aquaculture systems. Presently, common carp are widely farmed throughout the world and are cultured primarily in earthen ponds. The level of culture intensity ranges from highly extensive (low stocking densities with no supplemental feeding or fertilization) to relatively highly intensive (high density stocking, maintenance of water quality, provision of complete diets, mechanical aeration, management of phytoplankton blooms).

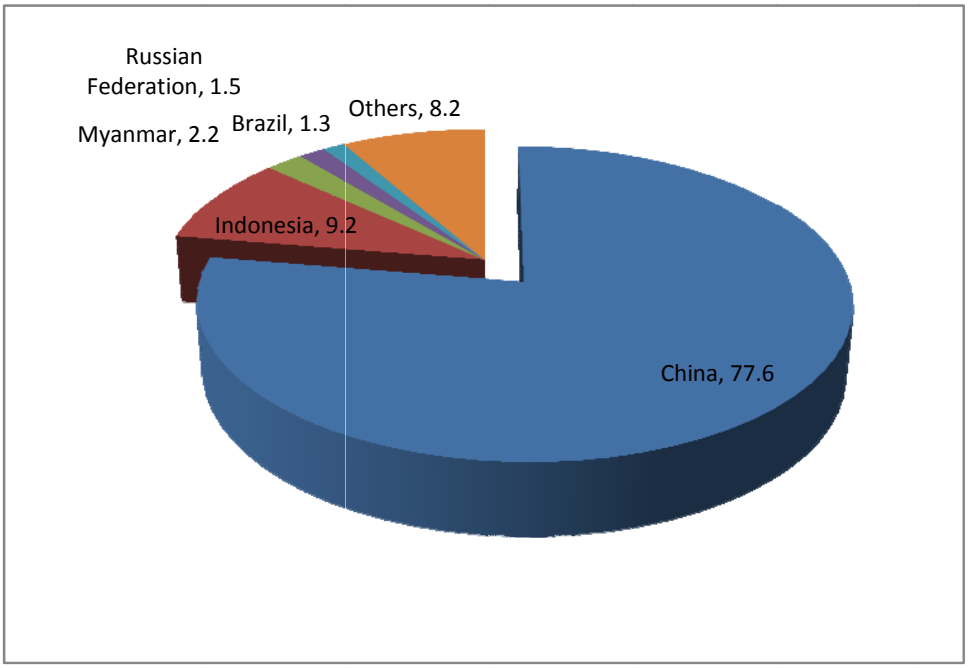

Figure 6: Main producer countries of *Cyprinus carpio* (FAO, 2009c)

Extensive monoculture of common carp is the traditional method in Europe (mainly Western Europe) and is performed in large ponds of from one to several hundred hectares. Under temperate conditions, this method requires three growing seasons to produce a marketable fish of l.0-1.5 kg in Western and Central Europe, and two growing seasons to obtain a 0.5-0.8 kg marketable fish in Eastern Europe. In this extensive system, common carp is the main species, sometimes associated with other cyprinids (tench, roach, rudd, gudgeon) and piscivorous fish (pike) as high value products (Kestemont, 1995).

The main countries involved in common carp production are China, Indonesia, Malaysia, Russian, Brazil, Bangladesh and Iran (FAO, 2009c). In addition, common carp has been introduced to aquaculture in Iran, French Polynesia and Israel (FAO, 2009a). The global aquaculture production of common carp in 2007 was nearly 3 million tonnes, with a total value of about US $3 billion (FAO, 2009c). The production of common carp mainly came from Southeast Asia, and in general from small farms (FAO, 2004-2009). Common carp aquaculture practice often involves poly-culture of freshwater fishes (Milstein, 1992, Nguyen et al., 2005a, De Silva et al., 2006) such as with silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*)

(Dimitrov 1984), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), catla (*Catla catla*), bighead (*Aristichthys nobilis*), omnivorous species such as black buffalo (*Ictiobus niger*) (Dimitrov 1987), and Nile tilapia (*Oreochromis niloticus*) (Shrestha et al., 1999). Common carp can be cultured in inland-based earthen and/or cement ponds, farm or irrigation-pond culture, running-water-pond culture (Suzuki, 1986, Shrestha and Bhujel, 1999), floating-net-cage culture in lakes (Suzuki, 1986), reservoirs (cage culture) (Petersen et al., 2005) and seasonal water bodies (Felsing et al., 2003).

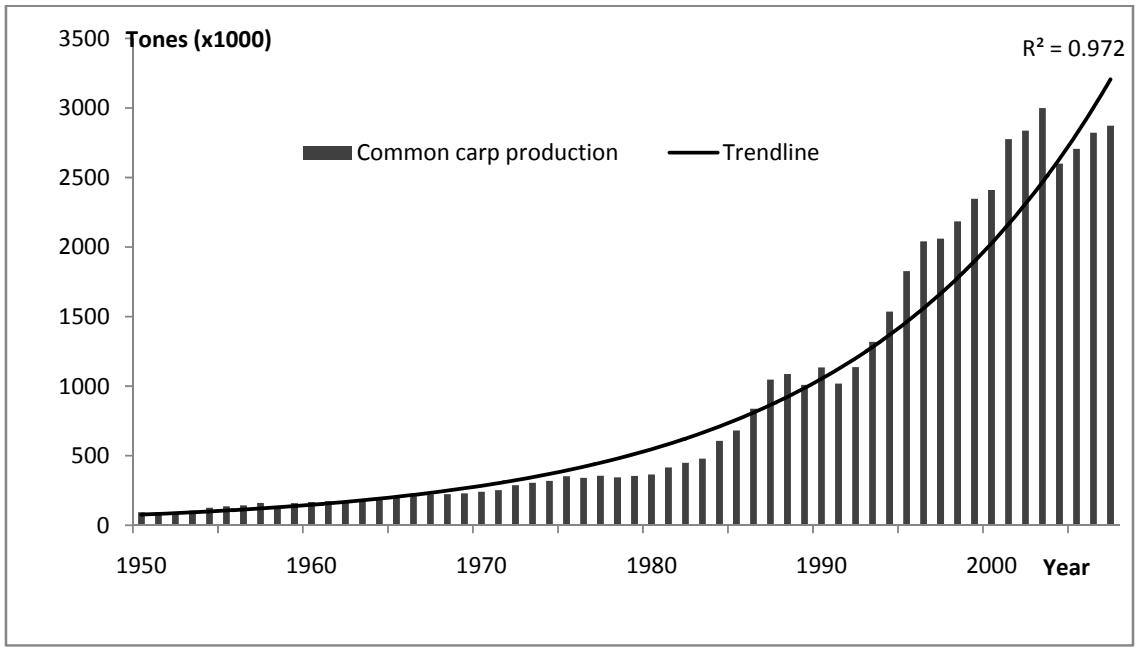

Figure 7: Global aquaculture production of *Cyprinus carpio* (FAO, 2009c)

In addition, common carp are mono-cultured (Kestemont, 1995), polycultured or cultured in integrated aquaculture-agriculture systems (Pant et al., 2004). In Asian countries, common carp can be produced with rice in the same areas or in rotational culture (after a single annual crop of rice) (Kestemont, 1995). Some studies reported that common carp was one of the major components in rice cum fish systems in mountainous areas (Penman et al., 2002, FAO, 2009b), pig cum fish or duck cum fish. Systems integrating carp culture with livestock have been designed using different species such as the pig-fish system (Sinha, 1985, Little et al., 1987), poultry-fish systems in Far East and Eastern European (Woynarovich, 1979, Little and Muir, 1987)

and the duck-fish system (Sinha, 1985). These integrated systems can increase productivity to 5 to 6 tonnes/ha/year (Luu, 1992, Delmendo, 1983).

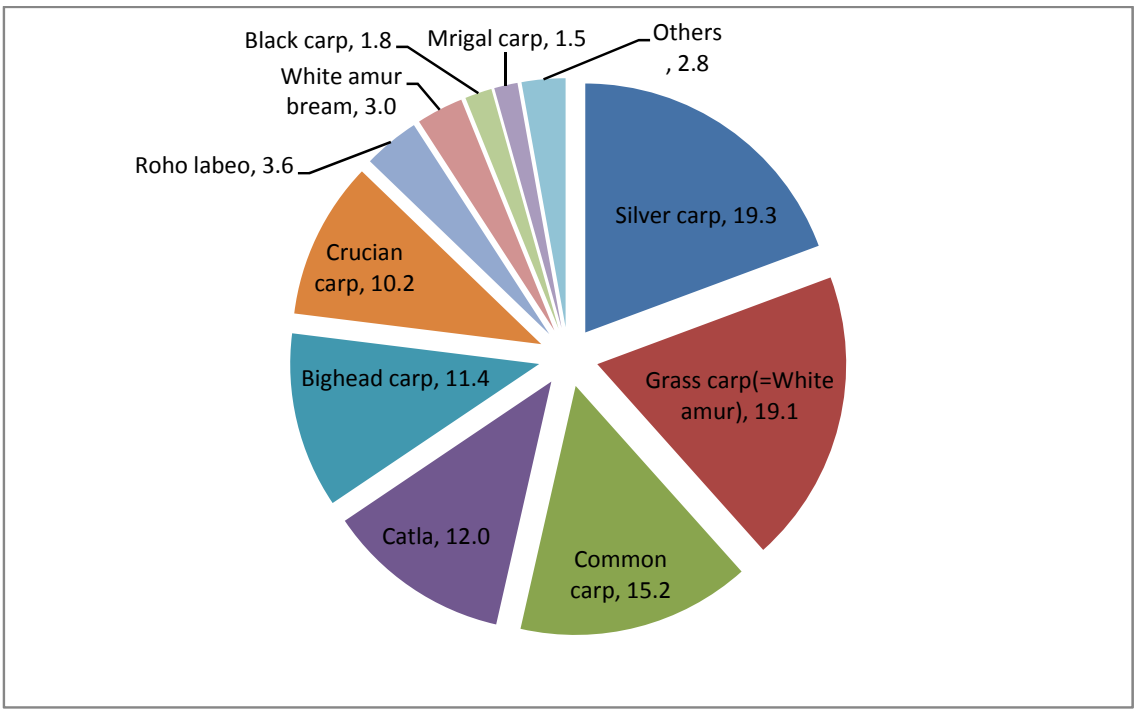

Figure 8: Contribution of some major carp species in Asian aquaculture 2007 (FAO, 2009c)

Adult carp tend to be omnivorous, feeding on snails, molluscs, worms, algae, aquatic plants, seeds and detritus (FAO, 2009a).They have been incorporated into rice paddies to feed on the insects and other organisms associated with rice culture. Carp are omnivorous and zooplankton consumption is dominant in fish ponds where the stocking density is high. The pond farming of carp is based on the ability of the species to accept and utilize cereals supplied by the farmers. The daily growth of carp can be 2 to 4% of body weight. In subtropical/tropical areas, carp can reach 0.6 to 1.0 kg body weight within one season in the poly-cultural fish ponds (FAO, 2009a).

In Europe, female carp need about 11 000 to 12 000 degree-days to reach maturity in the temperate and subtropical climatic zones. Male carp are matured within a period that is 25-35% shorter. The maturity period of Asian carp strains is slightly shorter. The spawning of European carp starts when the water temperature is 17-18°C (FAO,

2009a). Asian strains start to spawn when the ion concentration of the water decreases abruptly at the beginning of the rainy season. Wild carp are partial spawners. Domesticated carp release all their matured eggs within a few hours. After hormonal treatment carp release their ripe eggs within a much shorter period, which makes stripping possible. The quantity of released eggs is 100 to 230 g/kg body weight.

Among the different carp species, common carp is the best species to rear in intensive monoculture, similar to intensive channel catfish and salmonid production (Kestemont, 1995). In temperate regions, the stocking density of common carp varies largely with respect to the level of intensification: from 300 to 600 fish ha^{-1} in unfed, unfertilized ponds to 900 fish ha^{-1} in fertilized ponds and 4000 fish ha^{-1} in ponds which receive pelleted feeds (Kestemont, 1995).

In Vietnam, common carp appear naturally in the North, but are now distributed throughout the whole country as a result of translocations for aquaculture (Nguyen et al., 2001). Culture of this species started in the 1960s. However, commercial common carp aquaculture has only developed since late 1980s as part of an international project on selective breeding namely "Genetic improvement of carp species in Asia". Lately, a serial study was conducted to investigate carp genetics and to improve seed selection of the species (Thai et al., 2007, Anjum, 2005, Thai et al., 2006, Mair et al., 2002). The hybrids, arising from crosses between three common carp strains: Vietnamese white scale, Hungarian and Indonesian common carp showed faster growth and better adaptability to local conditions (Tuan et al., 2007, Thien et al., 1992, Gupta et al., 2005). A simple method of seed production is an initial condition for the spread of any species (Halwart, 2005) and common carp is one that satisfies this precondition. Common carp can mature and reproduce under pond conditions. Because they are so adaptable, common carp are cultured in combination with other activities such as rice cultivation or animal production. Together with grass carp and Indian carp, aquaculture of common carp plays a significant and important role in providing a valuable source of protein, especially in rural communities (Edwards, 2000, Petersen et al., 2005). Culture of common carp requires only low levels of inputs, making these fish affordable as a source of protein for poor people (Penman et al., 2002).

2.7.3 Nutritional requirements of common carp

2.7.3.1 Protein and amino acid requirements

Table 5: Essential amino acid requirement of juvenile common carp

Amino acid	Protein in diet	Requirement	
		% of protein	% of dry matter
Arginine	38.5	4.3	1.6
Histidine	38.5	2.1	0.8
Isoleusine	38.5	2.5	0.9
Leusine	38.5	3.3	1.3
Lysine	38.5	5.7	2.2
Methionine	38.5	3.1	1.2
Phenylalanine	38.5	6.5	2.5
Threonine	38.5	3.9	1.5
Tryptophan	38.5	0.8	0.3
Valine	38.5	3.6	1.4

Source: NRC (1993)

Proteins are large molecules, composed of up to 20 different amino acids connected by peptide bonds. The dietary requirement for protein is defined as the minimum amount needed to meet the requirement for amino acids as well as to achieve maximum growth. The protein requirement of fish can be predicted from their whole body amino acids composition (Gatlin et al., 1986, Cara et al., 2007). NRC (1993) listed the protein requirements of numerous species. According to this publication, common carp require about 31 to 38% of crude protein (dry matter) in their diet. However the same crude protein value can result from different combinations of amino acids. Thus, any specification for dietary protein should also specify the protein's amino acid

composition. In general, the 10 amino acids which have been found to be essential for other fish are also assumed to be required for common carp. NRC (1993) summarised the amino acid requirement of carp in the table below.

The protein requirement of fish can be affected by numerous factors, such as the size of the fish, water temperature, feeding rate, stocking density and metabolisable energy level of the diet. When a diet containing high protein and low energy in comparison with requirements is fed to the fish, the excess protein will be used as an energy source rather than for growth, and the excretion of ammonia will subsequently increase (Webster et al., 2002, Catacutan et al., 1995). Therefore, protein energy ratio (P/E) in the diet is an economically as well as nutritionally important factor; because protein is the most costly component in fish diets (Cheng et al., 2003b, Leng et al., 1995).

Although protein plays an essential role in fish growth, levels of amino acids that are too high can cause problems such as amino acid toxicity. A disproportionate intake of some amino acids may also adversely affect the absorption and utilisation of the others (Harper et al., 1970). In fish, amino acid composition of the diet is one of the major factors influencing the optimal dietary protein level (Wilson, 1989).

2.7.3.2 Lipid and fatty acid requirements

Lipid is the term used to refer to fats, oil, and waxes, in which fatty acids are the key components. Based on the nature of the link between carbon atoms, fatty acids are separated into two types: namely saturated and unsaturated. Some specific unsaturated fatty acids are essential for most of fish species. Takeuchi et al. (1978) suggested that 6% of fish oil can be added to common carp diets as a source of energy. In another study, less than 12% of lipid level was recommended for most cyprinid carp (Kaushik et al., 1995). Carp, like many other fish species, require both n-3 and n-6 highly unsaturated fatty acids. Watanabe et al. (1975) and Takeuchi et al. (1977) reported that common carp need 0.5 to 1.0 percent of linoleic and linolenic acids, in the diet. In short, dietary lipids play an important role in fish nutrition to generate energy and to maintain biological structure and function of all membranes (Sargent et al., 1999).

It is well-known that fish can convert protein to lipid and/or carbohydrate to gain energy. One gram of lipid can liberate 39.3 kJ, which is much more than the same amount of carbohydrate (17.2 kJ/g) or protein (22.6 kJ/g) (Webster and Lim, 2002). Within certain limits, increasing dietary lipid levels improves diet utilization (Watanabe et al., 1979, Johnsen et al., 1993, Peres et al., 1999) because the extra lipid is used to provide biologically useful energy thus sparing protein. (Watanabe, 1982, Beamish et al., 1986), and reducing losses of organic matter and nitrogen (Lee et al., 1973).

It is possible to use a high fat diet in salmon aquaculture. However, high lipid levels in diets for many other fish species must be considered carefully because they may lead to the production of large fat deposits. In studies on some types of fish it was found that lipid had little or no protein sparing effect e.g. turbot (*Scophthalmus maximus*) (Andersen et al., 1993) or other turbot like species (*Psetta maxima*) (Regost et al., 2001). According to Du et al. (2005), the growth performance and feed utilization of grass carp increased with increasing dietary lipid levels up to 40 g kg^{-1} dietary lipid. However over 40 g kg^{-1}, decreased growth performance and feed utilization occurred because carp is a fish with a low energy requirement (Du et al., 2005). In conclusion, excess dietary lipid levels should be avoided.

2.7.3.3 Carbohydrate requirements

Carbohydrates are cheaper and more abundant than protein. Absence of an appreciable quantity of digestible carbohydrate from the diet means that fish have to metabolise more fat for energy (Ufodike et al., 1983). It would therefore be economical and beneficial if cheap carbohydrate foods could be incorporated into fish feed without compromising growth and conversion efficiencies. Together with lipid, availability of digestible carbohydrate could minimize the use of protein as an energy source, which is the most expensive component (Alvarez-González et al., 2001).

Despite a number of studies on carbohydrates in fish, no exact level of requirement has been given for any fish species. However, some studies assumed that warm water fish can utilize more carbohydrate than cold water fish. Fish can digest low molecular carbohydrates more effectively than high molecular ones (Storebakken et al., 1998,

Singh et al., 1967). Erfanullah and Jafri (1998) reported that Indian fingerling carp can utilize effectively many different carbohydrate rich feedstuffs, such as yellow corn (cook), rice bran or cooked potato starch. Fu (2007) proved that catfish (*Silurus meridionalis*) fed on a diet containing 15% corn starch grew better than fish fed non-cooked potato starch or glucose. When Burel et al. (2000) fed trout on a diet containing 23% starch they found that 97% of the starch was digested.

Common carp is very much like other teleosts in the way they digest carbohydrates. In the study of Ufodike (1983), common carp grew well when fed on diets containing up to 45% of rice. Digestibilities of up to 97% were recorded. Very low protein as well as carbohydrate digestibility in the control (neither cassava nor rice added) indicated that carp need carbohydrates. However, a high proportion of carbohydrate in diets could lead to high lipid deposition (Keshavanath et al., 2002). Common carp like most other fish species cannot digest high molecular carbohydrates. Kaushik (1995) found that common carp cannot digest cellulose. Lesel et al. (1986) did not find any cellulase activity in trout, goldfish or grass carp.

2.7.3.4 Vitamin and mineral requirements

Even though vitamins and minerals are only needed in small amounts they are essential for normal growth in fish. Requirement for vitamins and minerals tend to vary between fish species. Many minerals such as phosphorus (Kaushik et al., 1995), and manganese (Satoh et al., 1989, Schwarz, 1995) are considered as essential nutrients for carp because of their importance in eutrophication. Takeuchi et al. (1993) demonstrated the importance of phosphorus in common carp. An increase of available phosphorus in the diet from 0.5 to 1.0% in fingerlings resulted in a doubling of growth rate. However, excessive amounts of tricalcium triphosphate (7%) can lead to reduced absorption of Zn and Mn. Satoh (1991) listed the requirements of common carp for minerals and vitamins (Table 6):

Table 6: Available data on the mineral and vitamin requirements of common carp

Minerals		*Water-soluble,vitamins*	
Phosphorus	0.6-0.7%	Thiamine	required
Magnesium	0.04-0.05%	Riboflavin	7-14 mg/kg
Zinc	15-30 mg/kg	Pyridoxine	5-6 mg/kg
Manganese	13 mg/kg	Pantothenic acid	30-50 mg/kg
Copper	3 mg/kg	Niacin	28 mg/kg
Cobalt	0.1 mg/kg	Biotin	1 mg/kg
Iron	150 mg/kg	Choline	4000 mg/kg
Fat-soluble vitamins		Inositol	440 mg/kg
Vitamin A	10 000 IU	Vitamin C	required
Vitamin E	100 IU		

Source: NRC (1993)

2.7.4 Feed for common carp in practice

Carp are primarily selective benthic omnivores that mainly eat invertebrates living in sediments (Lammens et al., 1991). Newly hatched carp initially feed on algae and zooplankton; specifically rotifers and copepods (McCrimmon, 1968). Year old carp feed on a variety of macro-invertebrates including chironomids, caddis flies, molluscs, ostracods, and crustaceans (McCrimmon, 1968). Adult carp are known to eat a wide variety of organisms, including insects, crustaceans, annelids, mollusks, fish eggs, fish remains, plant tubers and seeds (McCrimmon, 1968, Lammens and Hoogenboezem, 1991). Carp feed by sucking up mud from the bottom ejecting it and then selectively consuming edible items suspended in the water (McCrimmon, 1968). The 'feeding galleries' of carp are easily recognized in shallow waters as depressions in the sediment (Cahn, 1929). Foods of animal origin eaten by common carp include fish, eggs, carrion, insects, molluscs, terrestrial worms, aquatic crustaceans and zooplankton, while plant foods are leaves, roots and tubers, seeds, grains, nuts, algae and macroalgae (Chumchal, 2002).

The nutritional and economic importance of natural food organisms within the overall nutritional budget of pond raised fish has been well documented by researchers in Israel (Hepher, 1988, Viola, 1989, Schroeder et al., 1991, Hepher, 1989). From a nutritional standpoint, it is important to remember that common carp belong to the group of filter-feeder fish and therefore have the ability to filter fine particulate matter (bacterial laden detritus, phytoplankton, zooplankton, etc.) directly from the water column (Colman et al., 1987). During the 1990s, common carp and the other carp species such as silver carp, grass carp, bighead carp and milkfish have all been produced within semi-intensive or extensive pond farming systems using fertilisation and/or supplementary diet feeding (Tacon, 1995).

Table 7: Protein and energy digestibility of selected ingredients

Ingredients	Protein (%)*	Energy (%)*
Meat meal (34% ash)	53.9 ± 3.9	58.2 ± 6.5
Meat meal (24% ash)	63.5 ± 3.4	66.5 ± 3.4
Poultry offal meal	78.8 ± 3.5	76.7 ± 5.6
Canola meal (solvent-extracted)	81.0 ± 2.3	56.1 ± 3.0
Soybean meal (full-fat)	84.8 ± 3.8	75.9 ± 7.8
Soybean meal (solvent-extracted)	86.0 ± 0.8	69.4 ± 1.7
Peanut meal	91.9 ± 8.0	68.7 ± 5.0
Lupin (*L. angustifolius*) kernel meal	98.1 ± 1.3	61.5 ± 1.8
Wheat gluten	101.9 ± 1.6	98.8 ± 3.1

* Mean ± standard error; Source: McMeniman (1998)

Ponds are often fertilised to stimulate development of plankton species. Organic fertilisation is used to increase the amount of natural food available in carp

monoculture in Central and Eastern Europe (Czechoslovalia, Hungary and Poland) (Stickney, 1986). Generally, fertilisation has been used as the main nutritional input in fish ponds (Tang, 1970, Edwards, 1980). By applying fertilisation/manure, dense populations of zooplankton species such as *Paramecium Moina, Daphnia* and rotifers can be established which are sources of natural food for fish culture (Edwards et al., 1994, Zoccarato et al., 1995, Barash et al., 1984, Shevgoor et al., 1994). Frequent application of manure is necessary to maintain the plankton population in the semi-intensive aquaculture systems in Asia (Knud-Hansen et al., 1991, Shan et al., 1985). However, to achieve high growth rates of fish, supplemental feed is needed.

Tacon (1993b) reported that soybean meal can partially or totally replace animal protein sources in the diets of most species both marine and freshwater. Soybean meal is an ideal and palatable plant source used in aquaculture feeds and has a desirable amino acid profile (Li, 1998). The response of carp to diets containing soybean meal has been investigated by various authors (Jafri et al., 1995, Chamundeswari et al., 1999). According to Jose et al. (2006), soybean meal is an easily available, acceptable and cost effective protein source in formulated feeds for Indian major carp. Soybean meal can be included at 40% of dry matter (35% of protein) for optimal growth of *Cirrhinus mrigal* (Jose et al., 2006). Total substitution of fish meal by a soy protein concentrate in the diet of rainbow trout has been tested by Kaushik et al. (1995). However, in other cases, high levels of replacement of fish meal by soy bean meal have led to reduced growth (Jose et al., 2006). Escaffre et al. (1997) conducted trials to determine acceptable inclusion ratios of soy as a major protein source in larval common carp diets. The results showed that there was no effect on the survival or growth of carp larvae even if up to 40% of soybean protein concentration was incorporated in the diet (Escaffre et al., 1997). For rainbow trout (*Oncorhynchus mykiss*), solvent extracted meal was reported to effectively replace about 60% of the fish meal in the diet (Refstie et al., 1997). Varsha (2001) observed that diets containing 20% of soybean meal induced better growth in rohu as compared to a pure fish meal-based control. Nandeesha et al. (1989) investigated the influence of soybean meal at an inclusion level of 30% along with squilla meal on the growth of common carp and

observed better performance with the soybean based diet as compared with that on a fish meal based diet.

2.7.5 The need to develop of low-cost diets, with reference to conditions in Yen Chau

In a study by Phuong et al. (2007), feed cost was the main factor affecting the gross margin for aquaculture for all farming categories (except in extensive systems), varying according to different levels of intensification and amount of protein in the feed. Manufactured feed has a high unit price and a high total cost per hectare. The use of this type of feed requires a high level of investment. Phuong et al. (2007) reported that feed costs in the catfish culture system in Vietnam accounted for 74 to 93 percent of the total cost with an average of 84 percent. In this study, 75% of farmers using traditional methods for culturing catfish and 65% of those using semi-intensive methods stated that the high price of feed was a problem. The same author claimed that technologies that provide reasonable financial incentives to farmers are more likely to be adopted.

Fish meal features prominently in diet recipes on account of the good growth response it provokes in cultivated species. However, in economic terms, high quality of feed and maximal growth rate of fish do not always bring the highest benefit for farmers (Jose et al., 2006). An experiment done by Asgah et al. (1984) showed that feed containing 53% of crude protein did not give a higher growth than feed that contained 43%. Protein content at these levels was in inverse proportion to growth and a high expenditure of protein was uneconomical and also wasted dietary energy due to the interaction of digestible energy with protein. High levels of intensive farming cannot be followed by small scale and less capital endowed farmers (Phuong et al., 2007). Using low cost feed based on local resource is an option recommended by many researchers and policy makers (Aziz et al., 2002, Mazid et al., 1997). These authors state that despite some disadvantages of farm-made feed, it gives better net returns than manufactured pellet feed. However, it can lead to more environment pollution. Fortunately, his concern is unlikely to be a big problem for aquaculture in Yen Chau where farmers use water outflow and pond sediment for plant and vegetable

cultivation (Steinbronn, 2009). In this system, nutrients can be best utilized by integration of aquaculture with other activities.

Phuong et al. (2007) proved that the gross margin and the net return to labour were highest for traditional farms followed by semi-intensive and intensive systems. It implies that the development of low-cost, nutritionally balanced diets that can support increased production levels is necessary for many regions. To find out the maximum production potential of pond based aquaculture using low-cost diets in mono and mixed culture of freshwater fish such as catfish (*Pangasius sutchi*), silver barb (*Barbodes gonionotus*) and Genetically Improved Farmed Tilapia (GIFT), researchers have tested many low-cost pelleted feeds made from different locally available ingredients such as mustard oil cake, wheat bran and rice bran (Hossain et al., 1989, Kedar Nath et al., 2008). Many researchers state that farmers are usually more willing to use available and low cost materials to gain acceptable yields rather than high quality manufactured feeds (Tacon, 1993a, Jantrarotai et al., 1993, Nuov et al., 1993). Therefore, low cost feeds seem more suitable and feasible than high cost industrial feeds (Steinbronn, 2009, Tuan et al., 2008).

3. Materials and Methods

3.1. Experimental procedure

In total 4 trials were carried out. Three trials took place in laboratory aquarium systems at Hohenheim University (Germany) and the fourth one was carried out in a pond at Hanoi University of Agriculture in Vietnam. Figure 9 shows the experimental plans in the present study.

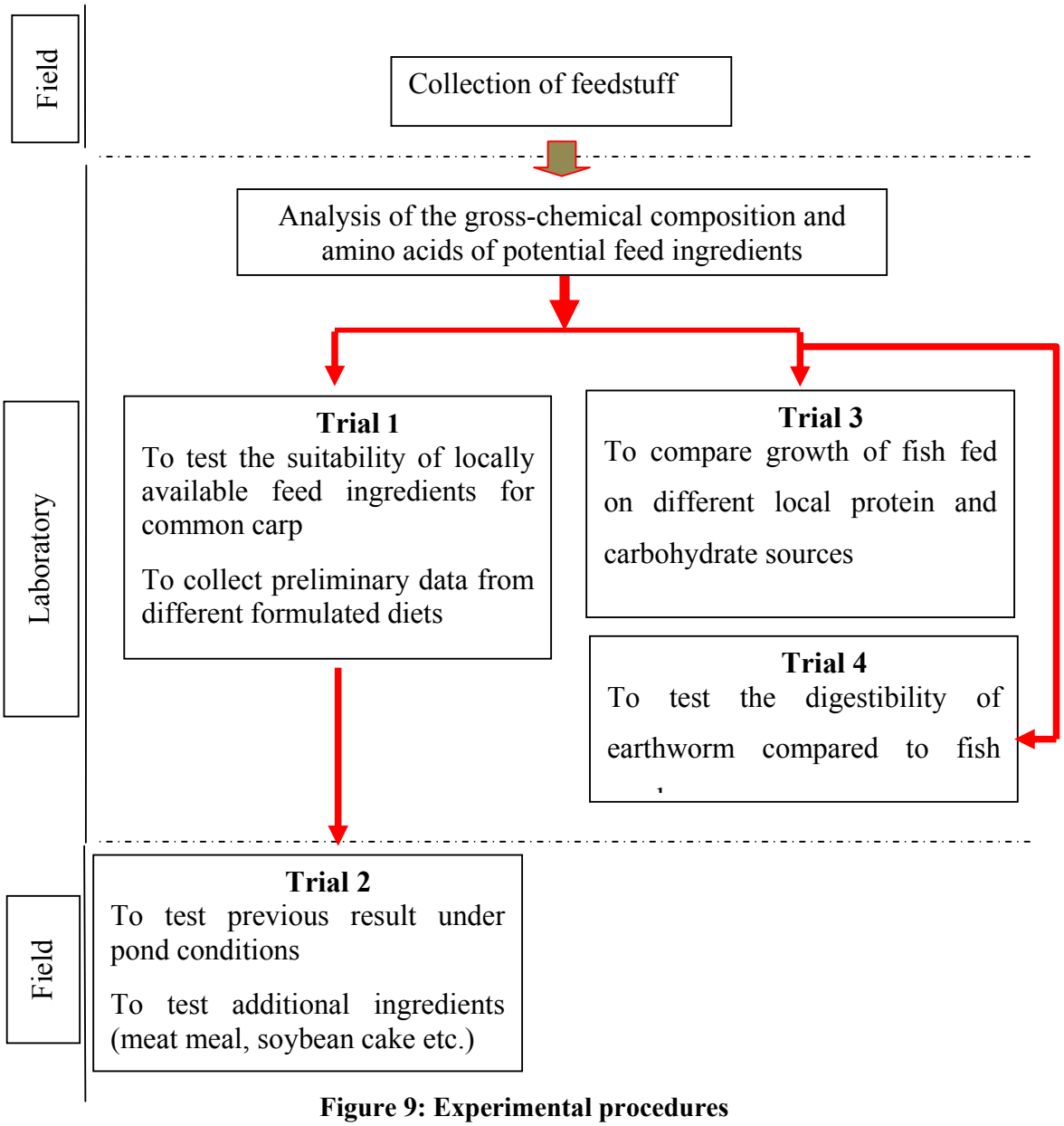

Figure 9: Experimental procedures

3.2. Trial animals

Common carp (*Cyprinus carpio*) were used for all trials. For laboratory trials carp fingerlings were sourced from Ahrensburg in Germany. Fish were kept in a 1.0 m^3 plastic tank for acclimation within the laboratory until the trials started. The tank was connected to a recirculation system. Fish were fed on Hohenheim standard feed at maintenance level before the beginning of the trials. At least one week before the start of the trials, fish of a similar size were transferred to the aquarium units so they could adapt to conditions in the small aquaria.

In the field trial, the common carp used came from Research Institute for Aquaculture No.1. The so-called V1 strain was used. These are hybrid common carp obtained by crossing Vietnamese, Hungarian and Indonesian carp.

Figure 10: Acclimation nets used for pond trial

Fish were kept in a net in the pond for one month before the beginning of the trial. A picture of those nets is shown in Figure 10. They were fed at 3% of body-weight on a commercial feed for common carp (40% of crude protein).

3.3. Trial diets

3.3.1 Ingredients

3.3.1.1 Collection of ingredients

During the second phase of the Upland Programs (from 2003 to 2006), a number of feeds which are commonly used in Northern Vietnam such as leaf materials (cassava leaves, maize leaves, banana leaves and grass), grain (rice, maize), cassava root and manure from various sources were investigated for their nutrient profiles and for their for inclusion in compound feeds. In the present study, locally available feed

ingredients such as fish meal, meat meal, rice bran, soy bean, and plant leaves (sweet potato leaves) were collected for the analysis of gross chemical composition and for the preparation of experimental diets. Fresh sweet potato leaves were collected (old stems were removed), dried in the shade and transported to Hohenheim for determination of the proximate composition. Fish meals were sourced from markets in Yen Chau and Hanoi. Due to the poor quality (low crude protein and high crude ash content) of the fish meal from Yen Chau, the fish meal from Hanoi was used in all trials.

3.3.1.2 Proximate composition of feed

All feed ingredients were analyzed for the proximate composition such as dry matter (DM), crude protein (CP), crude lipid (CL), crude ash (CA), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). In addition, the amino acid profiles and gross energy (GE) were determined.

3.3.2 Diet formulation

Based on the results of the proximate compositions of the feed materials, all experimental diets were formulated to provide 30% of CP and 10% of CL. Ingredients were well mixed and moistened to get about 40% of water. Pellets were made with diameters 1.5 mm for the laboratory trials and 2.5 mm for the pond trial. The pellets were dried in an oven at 40°C, packed and stored for later use. Hohenheim standard feed which is based mainly on fish meal and wheat flour was used as a control diet for the laboratory trials.

3.3.2.1 Trial 1

For Trial 1, experimental diets were prepared, in which either 25% (F1.1), 50% (F1.2) or 75% (F1.3) of crude protein was replaced by protein from plant sources. The major plant protein source was soybean meal (moisture heated) in the diets. The proportions of ingredients were adjusted to give the desired crude lipid and crude protein content. The compositions of the experimental diets (Trial 1) are shown in Table 8.

Table 8: Proportion of feed ingredients (%) in the experimental diets of Trial 1

Ingredients	Control	F1.1	F1.2	F1.3
Rice bran	-	19.7	2.9	2.0
Soybean meal	-	7.9	26.1	42.7
Maize powder	-	17.2	25.8	32.0
Cassava powder	-	14.7	16.0	6.0
Fish meal*	30.7	30.4	20.3	10.2
Wheat meal	60.3	-	-	-
Sunflower oil	5.0	3.1	1.9	0.2
Mineral**	2.0	2.0	2.0	2.0
Vitamin***	2.0	2.0	2.0	2.0
Cellulose	-	3.0	3.0	3.0

* Fish meal from Hohenheim University was used for control feed. Fish meal 1 (from Vietnam) was used for experimental diets.

** Vitamin premix (g or IU kg−1 premix); retinol palmitate, 500,000 IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50,000 IU; a-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25.

*** Mineral premix (g kg^{-1}): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67;$CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02.

3.3.2.2 Trial 2

Table 9 shows the proportion of feed ingredients as well as the chemical composition of the diets used in Trial 2. The pond control feed (P-control) was formulated by using the same ingredients as were used for the control feed in Trial 1. However, wheat meal in this diet was replaced by rice bran and maize powder. At that time wheat meal was temporarily unavailable due to the world food crises. The diet F2.1 corresponds to the diet F1.3 used in Trial 1. However, due to the high price of soybean meal (dry heated) compared to other protein sources, soybean meal in the diets F2.2 and F2.3 was replaced by other cheaper sources such as meat meal, soybean cake and wheat gluten (Table 9).

Table 9: Proportion of feed ingredients (%) in the experimental diets of Trial 2

Ingredient	Control	F2.1	F2.2	F2.3
Rice Bran	21.7	7.2	26.2	25.2
Soya meal	-	44.0	-	-
Cassava	5.0	5.0	5.0	5.0
Maize	35.0	31.0	15.0	15.0
Fish oil	3.2		5.0	5.0
Wheat gluten	-	-	-	8.0
Meat meal	-	-	5.0	5.0
Soybean cake	-	-	31.0	29.0
Fish meal *	33.0	10.0	10.0	5.0
Mineral - vitamins**	2.0	2.0	2.0	2.0
Antioxidants***	0.07	0.07	0.07	0.07
Di-calcium phosphate	-	0.78	0.78	0.78

* Fish meal 2 was used for both control and for experimental feed.

** Vitamin premix (g or IU kg−1 premix); retinol palmitate, 500,000 IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50,000 IU; a-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25. Mineral premix (g kg^{-1}): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67;$CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02.

*** Butylated hydroxytoluene.

Minerals and vitamins were added in lower amounts compared to the diets used in the laboratory experiments since in the ponds it was expected that the available natural food would provide the balance of these nutrients. Fungicide was added (0.07%) to the diets to avoid fungal infestation during the humid summer season.

Due to lack of facilities and equipment at Hanoi University of Agriculture (HUA), the feeds for the pond trial were formulated in the Department of Applied Biology at the Research Institute for Aquaculture No. 1 (RIA-1). After drying, the different feeds were carefully packed and transported to the hatchery station at the Department of Aquaculture in HUA where the trial was carried out.

3.3.2.3 Trial 3

Table 10: Proportion of feed ingredients (%) in the experimental diets of Trial 3

Ingredients	Control	F3.1	F3.2	F3.3	F3.4	F3.5	F3.6	F3.7
Soybean meal	-	40.5	49.0	-	40.5	31.5	-	-
Maize powder	-	44.5	-	50.3	44.5	-	-	36.0
Cassava powder	-	-	34.9	-	-	25.3	28.9	-
Wheat meal	62.8	-	-	-	-	-	-	-
Fish meal*	30.2	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Soybean cake	-	-	-	29.2	-	-	23.3	18.2
Sweet potato leaf	-	-	-	-	-	25.0	25.0	25.0
Sunflower oil	5.0	-	1.1	5.5	-	3.2	7.8	5.8
Mineral**	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin***	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
TiO2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

* Fish meal 3, from Hohenheim University, was used for control feed. Fish meal 1 (from Vietnam) was used for experimental diets.

** Vitamin premix (g or IU kg−1 premix); retinol palmitate, 500,000 IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50,000 IU; a-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25.

*** Mineral premix (g kg−1): CaCO3, 336; KH2PO4, 502; MgSO4. 7H2O, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; MnSO4. H2O, 3.12; ZnSO4. 7H2O, 4.67;CuSO4. 5H2O, 0.62; KI, 0.16; CoCl2. 6H2O, 0.08; ammonium molybdate, 0.06; NaSeO3, 0.02.

Trial 3 was conducted to compare different protein and carbohydrate sources. In this trial, one control and 7 experimental diets were prepared. All diets contained the same proportion of fish meal (10%), except the control diet. The treatments F3.1 and F3.2 have different carbohydrate sources (maize and cassava powder). The levels of heated soybean meal and sunflower oil were adjusted to maintain the proportions of CP and CL in these diets. F3.3 and F3.4 were formulated to compare the performance of fish fed on different protein sources (soybean meal and soybean cake). The proportion of maize powder was adjusted to maintain CP and CL levels in the feed. Sweet potato leaves (SPL) were tested in the diets F3.5, F3.6, and F3.7. These diets contained 25% of sweet potato leaves. All the diets contained 1% of TiO_2 as a marker to determine the

apparent digestibility of the feeds. The ingredients of the experimental diets are shown in the Table 10.

3.3.2.4 Trial 4

Table 11: Ingredients of the diets in Trial 4 (%)

Feed	Control	F4.1	F4.2	F4.3
Fish meal*	30.3	21.1	9.0	-
Earthworm	-	8.8	20.2	28.7
Wheat meal	61.7	61.6	61.7	61.7
Sunflower oil	5.0	5.5	6.2	6.7
Mineral**	2.0	2.0	2.0	2.0
Vitamin***	2.0	2.0	2.0	2.0
TiO2	1.0	1.0	1.0	1.0

* Fish meal 3, from Hohenheim, was used for both control and for experimental feed.

** Vitamin premix (g or IU kg−1 premix); retinol palmitate, 500,000 IU; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10;cholecalciferol; 50,000 IU; a-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25.

*** Mineral premix (g kg−1): CaCO3, 336; KH2PO4, 502; MgSO4. 7H2O, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; MnSO4. H2O, 3.12; ZnSO4. 7H2O, 4.67;CuSO4. 5H2O, 0.62; KI, 0.16; CoCl2. 6H2O, 0.08; ammonium molybdate, 0.06; NaSeO3, 0.02.

This trial was carried out to test the potential for replacing fishmeal by earthworm meal. Three treatments were conducted (F4.1, F4.2 and F4.3), in which 30, 70 or 100% of fishmeal was replaced by earthworm powder. Earthworms were cleaned, deep-frozen and dried by freeze dryer before vacuum packing and transporting to the University of Hohenheim. Marker TiO_2 (1%) was also added to the diets to determine apparent digestibility. Ingredients and chemical compositions of the different diets are shown in Tables 1.

3.4. Experimental setup

3.4.1 Laboratory trials (Trial 1, Trial 3, and Trial 4)

All laboratory trials were carried out in a recirculation system (figure 11). The recirculation systems consisted of 12 aquaria (for Trial 1 and 3) and 24 aquaria (for Trial 2). Each aquarium had a volume of 40 l. Water flow through the aquariums was maintained at 6-7 liters per minute. Water temperature, DO and pH were kept at 25-27°C, above 4 mg/l and around 7.0-8.0, respectively. The photoperiod was set up at 12 hrs light : 12 hrs dark.

The out flowing water from the aquariums was filtered by mechanical and biological filtration in order to ensure that NH_3-N and NO_2-N were not too high for carp (below 0.1 mg/l and 0.5mg/l, respectively). The aquariums and the filter media of the recirculation system were regularly and thoroughly cleaned during trials to remove accumulated of fish excreta. Each tank was covered with a glass lid to minimize disturbances and prevent fish from escaping.

Figure 11: Recirculation system for laboratorial trials

The trials were run with three or four treatment groups. A random block design was used for all trials. Treatments were assigned to the experimental tanks using numbers generated by the random number generator function in Microsoft Excel.

3.4.1.1 Trial 1

Sixty common carp (8.9 ± 1.0g) were selected randomly and transferred from a 1000 liter holding tank to the 12 experimental aquaria (5 fish per aquarium). The weights of individual fish were taken by using a balance accurate to. The four treatments (Control, F1.1, F1.2 and F1.3) with 3 replicates of each treatment were transferred to the individual aquariums. The feeding trial was conducted for an 8-week period.

The fish in each feeding group were fed at a rate of 5 times maintenance requirement in 5 rations per day using automatic feeders. Growth of fish was monitored weekly and the feed amounts were adjusted afterwards to ± 0.01g. At the end of the trial, weights and lengths of fish were measured. Fish were slaughtered and the weights of livers as well as the weights and lengths of fish intestines were determined. All parts of the fish carcass of each individual fish were put together and stored at -18°C. Later, the fish were autoclaved at 121°C for 15 minutes and then homogenized. Homogenized fish samples were frozen and then freeze dried. Afterwards, the dry weights of the fish samples were determined, and the fish were homogenized again using a coffee grinder. All samples were analyzed for their proximate chemical composition at the University of Hohenheim.

3.4.1.2 Trial 3

In total, 120 common carp (3.3 ± 0.3 g) were selected and transferred from the 1000 liter adaptation tank to the experimental aquarium units. Eight treatments (Control, F3.1 to F3.7) with three replicates and 5 fish per treatment were allocated to the individual aquariums. The feeding trial 3 was carried out for 8 weeks. At the beginning of the feeding trial, all fish were weighed individually to ± 0.01 g. Fish were fed 5 times per day at a level of 5 times of maintenance requirement calculated from the initial body weight. The amount of feed offered was recalculated at the beginning of each week based on the current weight of the fish after a 24 h fast.

During the last two weeks of the trial, fish faeces from each treatment were collected twice a day (at 10 a.m. and 4 p.m.). The aquariums were cleaned after feeding to ensure that no feed residues remained. Fish faeces were collected immediately after excretion using a siphon. The faeces were then transferred to a test tube for centrifugation at 4000 g for 10 minutes. Afterwards, water was removed and the faeces were collected and stored at minus 18°C before freeze-drying.

At the end of the trial, the weights and lengths of all fish were determined, their liver and intestines were weighed and the lengths of their intestine were measured. Flesh and viscera of all fish from each aquarium were put together and stored at -18°C. All samples were then autoclaved at 121°C for 15 minutes. Samples were homogenized,

then frozen and freeze dried. Dried weights of the fish samples were determined and then the samples were ground using an electrical coffee grinder. All samples were analyzed for proximate and chemical composition at the laboratory in University of Hohenheim.

3.4.1.3 Trial 4

Trial 4 was conducted in order to assess the digestibility of earthworm powder as supplemental fish feed. The general design of this trail did not differ from Trials 1 and 3. Each treatment consisted of 3 replicates with 5 fish per replicate. The common carp (~ 8 g) in Trial 3 were fed the different diets for 8 weeks.

Fish were fed 5 times per day at a level of 5 times of maintenance requirement. The fish were weighed weekly. Fish faeces from each treatment were collected twice a day (at 10 a.m. and 4 p.m.) in the last two weeks of the trial. Aquariums were cleaned after feeding to ensure that no feed residue remained in the aquarium. Fish faeces were collected immediately after excretion by siphon. Sedimentation was transferred to a centrifuge and centrifuged at 4000 g for 10 minutes. Water was removed and faeces were accumulated and stored in a freezer before freeze drying.

At the end of the trial, fish were weighed and slaughtered. Also, the weight of the fish livers as well as the weight and length of the fish intestines were determined. All fish carcasses from each aquarium were pooled and stored at -18°C and then autoclaved at 121°C for 15 minutes for homogenization. Homogenized fish samples were frozen and freeze dried. Dry weights of the fish sample were determined before being ground again using an electrical coffee grinder. All of the samples were analyzed for proximate chemical composition at the laboratory in the University of Hohenheim.

3.4.2 Field trial (Trial 2)

3.4.2.1 Description of the experimental pond

The experimental pond was situated on the campus of the Hanoi University of Agriculture (21°01’ N; 105°55’ E). The pond was earthen, 3600 m^2 (60 m x 60 m) in size and was divided into two parts by a concrete dike in the middle of the pond

(Figure 12). The trial took place in one of these sections, which was used as a tilapia nursery pond. Water was able to flow between the two parts of the pond through small holes in the concrete dike.

The water for the pond was supplied from a small river, namely Cau Bay River, which carries water from the Red River to irrigate rice fields in Gia Lam district. As the water flows through urban areas, it is occasionally polluted with wastes. To sediment suspended particles, the water was first accumulated in another pond and the cleaned water then pumped into the experimental pond.

To fertilize the pond, a total of 2 tons of pig manure (~ 5.6 tons/ha) was applied, of which 500 kg was loaded before filling the pond. The remaining manure was kept in jute bags that were located in the corners of the pond so that nutrients could leach slowly over a longer period of time.

Figure 12: Pond in the field trial

Figure 13: Cages in the field trial

After the first fertilization, about 40 thousand tilapia fry were released into the pond. The fish were fed every day. In the first 3 weeks, they were fed on soybean meal at a level of 15% of body mass, while for the following weeks an industrial feed was given (rate: 7% of fish body mass). Occasionally tilapia fingerlings were sold from this pond.

In Trial 4 a total of twelve nets were placed in the middle of the pond (Figure 13). The nets were 3 x 3 x 1.2 m with a mesh size of 3x3 mm. The net floors were kept at a height of 10-15 cm above the mud at the bottom of the pond. Nets were cleaned weekly to ensure a continuous water exchange through the mesh.

3.4.2.2 Set up

The pond trial consisted of four feeding groups (3 test diets and 1 control), each having three replicates. The trial started three weeks after the release of tilapia fry (14th May 2008). By this time, the tilapia had reached a size, which made it impossible for them to enter the net cages and interfere with the feeding trial. In each net, 45 common carp (initial weight 85.9 ± 5.7 g) were stocked, so that in total 540 fish were used in this trial. Fish were fed three times per day by hand from a small boat (8 a.m., 12 a.m., and 16 p.m.) at a feeding rate of 5 times maintenance requirement. Feed amount was adjusted every ten days after weighing the fish. The total trial lasted 2 months.

3.4.2.3 Water quality management

Twice daily at 6 a.m. and 2 p.m. DO, temperature, pH, and Secchi-Disk transparency of water were determined by oximeter Oxi-340i (WTW, Germany). Water transparency was determined by a Secchi-Disk. Each parameter was measured at three different places in the middle of the pond. Except for the Secchi depth, the other parameters were always measured at the middle level of water column in the pond (about 40 cm deep). The average results will be presented in the 'Results' section.

Once a week (at 6 a.m.), nitrite (NO_2^-), nitrate (NO_3^-), ammonium-N (NH_4^+), and phosphorus (PO_4^{3-}) were determined using Merck Spectoquant reagent tests (NO_3: test 09713; NO_2: test 14776; NH_4: test 14752; PO_4: test 14848) and by measuring photo-metrically with the photometer PhotoLab S12 (WTW, Germany). Water samples for these parameters were collected at surface level. Water samples were collected in 500 ml plastic bottle filled absolutely to the top. Samples were analyzed immediately to prevent degeneration of the samples.

3.4.2.4 Fish sample preparation

Every ten days, 20 fish were selected randomly from each net to monitor growth. Before sampling, all fish were starved for 24 hours in order to avoid stress during the sampling and to minimize inaccuracies in weighing caused by remnants of food in the gut.

At the end of the experiment, all fish were counted and the total weight of body mass in each net was determined. In addition, the weight and length of 20 randomly selected individual fish from each net was determined. From those 20 fish, 5 fish were further randomly selected for slaughtering and determination of the weight of liver as well as the weight and length of intestines. All parts from each individual fish carcass were put together and further prepared for proximate analysis. Fish were deep frozen at -18°C at the institute. Samples were then autoclaved at 121°C for 15 minutes. Fish could be easily homogenized when they were still warm. The homogenized fish samples were spread in a thin layer on glass plates and dried at 60°C in an oven. Dried weights of the fish sample were determined, and homogenized again by electrical grinder. All of the samples were transfer to laboratory in Hohenheim University to analysis chemical compositions.

3.5. Chemical analyses

3.5.1 Analysis of proximate compositions

Biochemical analyses were carried out for the diets, fish flesh and faeces. Analyses of dry matter (moisture), crude protein, crude fat, crude fibre and ash were performed according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1995). All samples were analysed in duplicate.

3.5.1.1 Moisture

Approximately 5 g of ground sample was placed into each-pre-weighted (W_0) dried moisture dish, the weight of the moisture with sample was recorded as W1. The moisture dish with sample was dried in a convection oven at 105°C for 16 hours. The dish was removed from the oven the following day and cooled in a desiccator for 1-2 hours with the final weight recorded as W_2. The percent moisture was calculated using the following formula:

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1 - W_0} \text{x } 100$$

3.5.1.2 Ash

Approximately 5g of ground sample was transferred into each pre-weighted (W_0) crucible. The weight of the crucible with sample was recorded as W_1. The crucible with sample was ignited in a muffle furnace at 490°C for 6 hours. The crucible was removed from the furnace and cooled in a desiccator for 1-2 hours with the final weight recorded as W_2. The ash content was calculated using the following formula:

$$\text{Crude Ash (\%)} = \frac{W_2 - W_0}{W_1 - W_0} \text{x } 100$$

3.5.1.3 Crude protein

CP of samples in the trial 1 and 2 were analysed by the method of Kjeldahl. Duplicates of about 0.5 g of each of the two feed were weighed onto a nitrogen-free paper. This was folded and placed into a digestion vessel. The crude protein content was determined by the Kjeldahl procedure according to the experimental instructions. The sample was digested by concentrated sulphuric acid to convert the nitrogen in all nitrogen compounds quantitatively to ammonium sulphate. Using a Büchi 321 distillation unit, all the nitrogen in the ammonium sulphate was removed as ammonia by distillation into boric acid solution after neutralisation of the acid digestion mixture with sodium hydroxide. The ammonia was then titrated with standard sulphuric acid using an automatic digital titrator (Metrohm Titrator E526 Switzerland). The amount of nitrogen in the original sample was calculated from the result of the titration and crude protein calculated as N (g) x 6.25 or, if 0.1 N sulphuric acid was used for the final titration,

$$\text{CP (\%)} = \frac{0.8756 \text{ x ml. } H_2SO_4}{\text{Sample weight}}$$

The CP of samples in the trial 3 and 4 were analysed by C-N-analyser, Variomax CN, made by Elementar, Germany.

3.5.1.4 Crude fat

Total lipid (CF) of the feed ingredients was determined by the Soxhlet method. Crude lipid in fish and faeces samples was determined by the method of Smedes (1999) as modified by Schlechtriem et al. (2003):

About 200 mg of sample together with 2 glass beads were added to a round bottom plastic tube (13ml). Then, 2 ml of isopropanol and 2.5 ml of cyclohexan were added and well mixed for 30 seconds by vortexing and 2 minutes by hand shaking. The sample was sonicated with an ultra-sonic probe for 15 minute before adding 2.75 ml of Nanopure-water. After mixing, the sample was centrifuged for 10 minutes at 3800 rpm. The supernatant was separated using a Pasteur pipette. The procedure was repeated with 2.5 ml of a mixture of Cyclohexan and Isopropanol. The supernatant was dried to determine the weight of lipid.

3.5.1.5 NDF, ADF, ADL

Neutral detergent fiber (NDF), was measured by boiling a sample of forage in a special detergent at a neutral pH (pH = 7). After filtering to remove the soluble fraction that contains starch, sugar, protein, and other compounds, the remainder was dried and weighed as NDF. The NDF was calculated as a percentage of the original forage sample after drying.

Acid detergent fiber (ADF) was determined in the same way, except a different detergent was used in acid (pH = 2) conditions. The sample was boiled and filtered as in the NDF procedure. In acid conditions, hemi-cellulose and soluble cell components dissolve. The residue is ADF and consists mainly of cellulose and lignin.

Acid detergent lignin (ADL) was measured by further treating ADF with strong acid, which dissolves cellulose, and oxidizes (removes) the lignin. Either approach allows calculation of the amount of lignin.

3.5.1.6 Gross energy

Gross energy of the samples was determined by using an iso-peribolic calorimeter (IKA calorimeter 7000C, Janke & Kunkel IKA-Analysetechnik, Germany).

Approximately 0.5 g of sample was burnt in the combustion cell of the IKA calorimeter 7000C at 30 bar O_2. Energy was calculated automatically from heat production.

3.5.2 Analysis of amino acids of ingredients, diets and fish

The composition of Amino Acids (AA) of feed ingredients were analysed by the State Institute for Agriculture Chemistry and are shown in Table 14. Based on the AA data, the ingredients were adjusted to develop diets meeting the requirements of common carp. The amino acid contents of the feeds and feed ingredients were determined following EU standard methods 98/64/EG and 2000/45/EG. Table 14 shows the details of the AA determinations.

3.5.3 Soybean treating and trypsin inhibitor analysis

Soya bean was treated to deactivate trypsin inhibitor before being incorporated in the experimental diets. In the laboratory experiments, soybean meal was steam heated at 121°C and 1.5 atm for 15 minutes. The heated soybean meal was cooled, deep frozen and then freeze dried. In the pond trial, due to the large amount of feed needed and the limited equipment at HUA, soy bean was heated dry instead of being steam heated as in the laboratory trial.

The levels of trypsin inhibitor in steam treated and dry treated soybean cake were determined and compared with that of untreated soybean. The method used followed that of Smith et al. (1980) with some modifications by Liu and Merkakis (1989).

About 250 mg of fat free sample was suspended in 12.5 ml of 0.01M NaOH. Then the sample was ground by ultra-turax for 30 seconds and the pH adjusted to 9.4 – 9.6 using 1 M HCl. The sample was ground again by ultra-turax for 5 minutes at 0C in an ice bath. The homogenised sample was centrifuged at 4000 g for 15 minutes. The supernatant was collected carefully by pipetting between the residue and fatty layer. A 250 µl sample was pipetted into a test tube containing 250 µl of distilled water and, 1.25 ml BAPNA was added. The solution was incubated at 37°C for 10 minutes. Trypsin were added and left to react for exactly 10 minutes. The reaction was stopped

by adding 30% acetic acid. The contents of the vial were centrifuged at 4000 g for 10 minutes. The supernatant was taken and the absorbance of the contents measured by spectrophotometer at 410 nm. The trypsin inhibitor activity was calculated using the following equation:

$$\text{Trypsin inhibitor activity (TIA)} = \frac{2.63 * \text{dilution factor} * \text{AI}}{\text{sample weight (g)}}$$

where: AI is a difference in the absorbances of the ingredient and standard samples
$\text{Al} = \text{absorbance of sample} - \text{absorbance of standard}$

3.5.4 Tannin analysis

After the calibration and extraction of phenols, a so called "total-tannin-assay" was established. The supernatant from the plant sample was diluted with solvent and precipitated with BSA solution. Because its tannin content was likely to be low, the supernatant from the hay sample was not diluted before the addition of the bovine protein.

The resulting tannin-protein complexes were centrifuged and the palette washed with a mixture of solvent and acetate buffer. The precipitate was dissolved in SDS to prepare it for the "total-tannin-assay". In the final stage of the assay, the absorbance of the solution was measured in a calibrated photometer at 510 nm.

With the help of the Excel programme a calibration curve was created to receive the mathematical equation y = 0.0076x – 0.0108. The tannin amount in µg was calculated via the formula: TA = (NA – Intercept) / Slope. From the value of TA the tannin curve was built up to estimate the amount of tannin in a volume of 500 µl. After that, the amount of tannin was corrected by multiplying by the dilution factor (= 10 for plants and 1 for hay).

The final tannin concentration in mg/g plant material is related to the amount of sample and the DM of the plant. The following formula is used:

$$TC = \frac{TA\ x\ DF}{EV\ x\ EC\ x\ 1000}$$

Where: TA = tannin amount (µg) EV= extract volume (µl); DF = dilution factor and EC = extract concentration (mg/ml).

3.5.5 Determination of TiO_2

Duplicate sub-samples of experimental feed or faeces (approximately 30 to 40 mg) were weighed to determine TiO_2 marker. Samples were digested in Kjeldahl flasks with 10 ml 96% H2SO4 and 1.5 g powdered Kjeldahl catalyst (K_2SO_4 + $CuSO_4$ - $5H_2O$) at 400°C for 4 h in order to oxidize the organic matter and dissolve the marker. All the digested sample was carefully transferred and made up to 25 ml with distilled water, and then left overnight. Two 1.0 ml aliquots of the diluted distillate were pipetted into two separate test tubes and 0.1 ml of 35% H_2O_2 was added to each. The solutions were mixed thoroughly and allowed to stand for 1 h. The optical absorption of the yellowish colour complex (TiO_2 and H_2O_2) formed as a result of the oxidation with peroxide, was measured at 405 nm using a spectrophotometer (Brandt, Poedjivo & Allam 1983). The quantity of TiO_2 ($\mu g.ml^{-1}$) in the 1 ml aliquot was computed by using the following equation (Richter et al. 2003):

$$\text{Marker } TiO_2\ (\mu g.\,mL-1) = 108.1 \text{ x } Abs_{405} - 0.155$$

The average of the values given by the two sub-samples was used to determine the total concentration of TiO_2 marker in the experimental feed or faeces.

Calculation of the apparent digestibility coefficient (ADC) in the experimental feed was determined according to the following equations:

$$\text{ADC of dry matter in feed} = 100 \text{ x } \left(1 - \frac{\%\text{ of } TiO_2 \text{ in feed}}{\%\text{ of } TiO_2 \text{ in faeces}}\right)$$

$$\text{ADC of feed nutrient} = 100 \text{ x } \left(1 - \frac{\%\text{ of } TiO_2 \text{ in feed}}{\%\text{ of } TiO_2 \text{ in faeces}} \text{ x } \frac{\%\text{ of nutrient in faeces}}{\%\text{ of nutrient in feed}}\right)$$

3.6 Formulas used

Metabolic body weight

Metabolic body weight is calculated by following formula:

$$\mathrm{MBW} = \mathrm{BWkg}^{-0.8}$$

Survival

Survival rate was determined according to the following formula:

$$\text{Survival (\%)} = \frac{\text{number of surviving animals}}{\text{initial number of animals}} \text{ x } 100$$

Growth

Body weight gain: was determined using the following formula:

$$\text{Body Weight Gain (BWG, g)} = \text{Final body weight (g)} - \text{Initial body weight (g)}$$

Specific growth rate: the initial live weight and final live weight were used to calculate and specific growth rate for the period of the trials using the following equations

$$\mathrm{SGR} = \frac{\mathrm{lnW_t} - \mathrm{lnW_{ini}}}{\mathrm{t}} * 100$$

where: t is duration of feeding experiment in days; W_t is the weight at the time t; and W_0 is the weight at the start of the trial.

Feed efficiency

Feed conversion ratio (FCR) which is essentially a measure of the feed utilization for growth, calculated as:

$$\mathrm{FCR} = \frac{\text{Feed consumed}}{\text{Body weight gain}}$$

Protein efficiency ratio (PER) is calculated by

$$\mathrm{PER} = \frac{\text{Body weight gain}}{\text{CP consumed}}$$

Protein productive value (PPV) is calculated by

$$\text{PPV} = \frac{\text{Final CP} - \text{initial CP}}{\text{CP consumed}} \text{ x } 100$$

Appearance net lipid utilization (ANLU) is calculated by the formula:

$$\text{ANLU} = \frac{\text{Final CL} - \text{initial CL}}{\text{CL consumed}} \text{ x } 100$$

Energy retention is calculated as the formula:

$$\text{Energy Retention (ER, \%)} = \frac{\text{Final GE} - \text{initial GE}}{\text{GE consumed}} \text{ x } 100$$

Other parameters

Condition factor

$$\text{CF} = \frac{\text{W}}{\text{L}^3} \text{ x } 100$$

where: W is body weight in grams, and L is standard length in centimetres.

Hepato – somatic index

$$\text{HSI} = \frac{\text{W}_{\text{liv}}}{\text{W}_{\text{bod}}} \text{ x } 100$$

where: W_{liv} is weight of fish liver, and W_{bod} is body mass (weight) of fish.

Intestine somatic index

$$\text{ISI} = \frac{\text{W}_{\text{int}}}{\text{W}_{\text{bod}}} * 100$$

where: W_{int} is weight of intestine, and W_{bod} is body mass (weight) of fish.

3.7 Data analysis

All data were checked for normal distribution before analysis. Results were presented as means ± std (standard deviation). The Microsoft Excel and Statistica program (versions 6.0) were used for data analysis in all the trials. Analysis of Variance (ANOVA), and Tukey-*post hoc* tests were used to determine any significant differences among treatment means. All statistical tests were performed at a significance level of $P < 0.05$.

4. Results

4.1 Nutritive values of potential feed ingredients in Yen Chau

4.1.1 Proximate composition

Table 12: Chemical composition (% of DM) and gross energy (kJ/g) of feed ingredients in the study

Ingredients	CA	CP	CL	NDF	ADF	ADL	GE
Fish meal 1	19.8	73.9	10.6	nd	nd	nd	21.2
Fish meal 2	21.8	62.1	5.7	nd	nd	nd	20.5
Fish meal 3	19.1	67.4	12.8	nd	nd	nd	21.6
Rice bran	9.7	8.4	6.0	37.0	31.4	13.8	18.9
Polished rice	0.5	17.0	6.1	nd	nd	nd	16.6
Soybean (steamed)	4.7	43.7	15.2	21.6	16.2	2.7	24.2
Soybean cake	6.6	58.6	0.7	nd	nd	nd	16.7
Maize powder	1.4	10.9	6.5	13.6	3.7	0.3	20.9
Cassava powder	2.0	3.4	1.2	7.4	5.5	1.7	17.9
Wheat meal	1.8	15.5	1.8	nd	nd	nd	19.3
Sweet potato leaves	13.1	31.9	2.6	35.2	22.5	4.1	17.9
Wheat gluten	16.3	61.0	2.1	nd	nd	nd	20.6
Meat and bone meal	30.8	55.0	8.4	nd	nd	nd	18.3
Earthworm	7.3	71.30	7.8	nd	nd	nd	21.4

Note: Fish meal 1 was used for laboratory, Fish meal 2 was used for Trial2, and Fish meal 3 sourced from Hohenheim and used for control feed in all laboratorial trials.

CA: crude ash; CP: crude protein; CL: crude lipid; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; GE: gross energy

nd: not determined.

Protein is the most important and expensive component in fish feed. It can be sourced from both plant and animal materials. But till now, the most common protein source used for aquaculture worldwide is fish meal. The first fish meal sample collected in

Yen Chau - Vietnam was the cheapest fish meal. But because it was so cheap, its quality was so poor that could not be used. The crude protein content of this sample was very low (less than 6%) but high crude ash (above 80%), indicated that the fish meal was mixed with inorganic materials. Using this material could neither promise any good growth rate of fish nor high profit for farmers. Thus, this fish meal was not used for any further investigation and it was not mentioned in the table of feed ingredients (Table 12).

However, results from laboratory analysis showed that other fish meal in Viet Nam had very good nutritive profiles and were able to make compound feed for fish culture in the region. Both fish meal 1 and fish meal 2 had a very high proportion of protein as well as a high energy content. Fish meal 1 was the richest protein fish meal and contained approximately 74% crude protein, which was higher than that of fish meal 2 (62.1%) and fish meal 3 from Hohenheim (67.4%). As regards gross energy content, Fish meal 1 had a heat of combustion of about 21.2 kJ/g and fish meal 2, 20.5 kJ/g. These values were comparable to that of the standard fish meal from Hohenheim (fish meal 3: 21.6 kJ/g). However, local fish meal was slightly lower in lipid and ash than the fish meal 3 from Hohenheim. The crude ash contents of fish meal 1 and 2 were 21.8% and 19.8% respectively, which were higher than that of fish meal 3 (16.5%). Also the fish meal from Viet Nam (both 1 and 2) contained less lipid than the fish meal 3 from Hohenheim, especially the Fish meal 2 with only 5.7% crude lipid, which was half of that of fish meal 3 (12.4%). However, this was offset by the fact that fish meal 2 was in general cheaper than both fish meal 1 and fish meal 3.

Among common crop products, full fat soybean turned out to be the richest protein and lipid source. Analytical data showed that soybean in Yen Chau contained approximately 44% crude protein and more than 15% crude lipid which resulted in high energy content (24.2 kJ/g). Soybean cake, a by-product from oil extraction, also had a great amount of protein with nearly 60% crude protein. However, the energy content of this material was lower (16.7 kJ/g) due the low lipid content. Anyway, the biggest constraint to using soybean as animal feed was the presence of anti-nutrients. On analysis, one gram of raw soybean in the study was found to contain about 32.1 mg of trypsin inhibitor which fortunately can be deactivated by heat treatment. As can be

seen from Table 13, more than 97% of trypsin inhibitor activity was removed from soybean meal after being treated by moist-heat treatment at 121°C for 15 minutes (Table 13). In contrast, soybean cake had a quite low trypsin inhibitor activity, indicating that most of trypsin inhibitor was remove during the fat extracting process. In conclusion, soybean full fat and soybean cake in the current study were almost free from trypsin inhibitor and could be incorporated into compound feeds without further treatment.

Table 13: Trypsin inhibitor activity of full fat soybean and soybean cake before and after heat treatment (mg/g)

	Before treatment	After treatment
Soybean (full fat)	32.1	0.9
Soybean cake	1.7	0.6

Another plant ingredient, sweet potato leaves also showed promising potential as a good alternative protein source. The crude protein content of sweet potato leaves is relatively high (almost 32%) compared to the other conventional plant products such as maize, rice bran, etc. Moreover, the crude ash content of sweet potato leaves is low (13.1%) compared with other plant ingredients like wheat gluten (16.3%). Also, this leaf had high NDF (35.2%) and low ADL (4.1%) which indicates that its digestibility in animals would be high. As with other plant materials, the disadvantages of sweet potato leaves are a low lipid content and the presence of anti-nutrient such as tannins and phytates. Analysis showed that sweet potato leaves contained about 0.3 mg of tannin per 1 g of sample.

Maize and cassava are not rich in protein but they are considered as good and cheap sources of carbohydrate and energy. Analysis showed that cassava powder contained low proportions of crude protein, neutral detergent fibre, acid detergent fibre and acid detergent lignin (3.4, 7.4, 5.5, and 1.7% respectively). These results were taken as indicative of a high proportion of starch. On the other hand, the energy content of cassava is high (17.9 kJ/g), higher than that of polished rice bran and sweet potato

leaves. Maize showed even better results with a lower amount of ADL (only 0.3%) but a higher content of NDF (13.6%) in comparison with cassava. In addition, other parameters of maize such as crude protein, crude lipid, and gross energy were much higher than those of cassava. Results in Table 12 show that maize from Yen Chau contains 11% of CP, 6.5% of CL and almost 21 kJ of energy in each gram of this material.

Many other protein sources such as meat and bone meal, and wheat gluten were analyzed for chemical composition. These ingredients were rich in protein, ranging from 55 to 61%, which is comparable to fish meal and could be good alternative protein sources in compound feed production. However, meat and bone meal contained about 30.8% crude ash which was the highest crude ash of all the feed ingredients.

The analytical results in Table 12 also show the high potential of earthworms in formulating fish feed because of their excellent chemical composition. The protein content of earthworm meal was 71.3% which was exceeded only by that of fish meal 1 but was higher than that of all the other ingredients. Moreover, earthworms have a very low proportion of crude ash which at 7.3% is only one third of that of fish meal 2 and is also much lower than that of the other ingredients, including plant materials. Although the lipid content of earthworm meal was not higher than that of fish meal 1 and 3, it was still higher than that of Fish meal 2 and of many other ingredients such as maize, cassava, and rice bran. Another advantage of earthworm production was that farmers can still use the by-product soil from vermi-culture (so called vermi-cast) as manure for fish ponds or plantations.

4.1.2 Amino acid composition of feed ingredients

The amino acid composition of feed ingredients was determined and is presented in Table 14. In this table, only one fish meal from Vietnam (Fish meal 1) was included. As with the protein analysis results, most amino acids in Fish meal 1 were present in higher amounts than those in fish meal 3 (Hohenheim) such as valine, cystine, methionine and leucine. Lysine is of most interest to nutritionists. This amino acid in

Fish meal 1 was approximately 25% higher than in Fish meal 3. Similarly, the cystine and methionine contents of Fish meal 1 were about 25% higher than that of fish meal 3. Only the amounts of serine, glycine, and aspartic acid in Fish meal 1 were lower than those in Fish meal 3 (from Hohenheim).

Again, the data in Table 13 shows that earthworm meal was the best ingredient because of its outstanding amino acid composition. The content of many EAA in earthworm meal was noticably higher than that of fish meal, such as valine, cystine, leucine, and tryptophan. There were only a few amino acids which were rarer than in fish meal such as glycine and alanine. However, these are non-essential amino acids.

Among plant materials, cassava was the poorest ingredient in terms of amino acid composition. The content of all amino acids of the cassava sample was low, especially the essential amino acids such as methionine, isoleusine, and tryptophan (< 0.5%). A better amino acid composition was found in rice bran and maize. Most of the amino acids levels in maize were lower than those in fish meal and full fat soybean but they were still higher than those in cassava.

Among plant feed stuffs, soybean emerged as the best ingredient with an excellent amino acid profile. This material contained approximately 8% Glutamic acid, which was the highest amino acid concentration in soybean. Although contents of other amino acids in soybean were lower than those of fish meal, they were much higher than those of other plant products such as sweet potato leaves and maize. Almost all the essential amino acids in soybean such as leucine, iso-leucine, cystine + methionine were more abundant than in cassava and maize. The concentration of lysine in soybean was especially high. This amino acid, usually the most deficient in plant products, was 5 times higher in soybean than in maize and more than 20 times higher than in cassava.

Table 1: Amino acid profiles of selected feed ingredients (% of DM)

Ingredients	Crude Protein	**Arginine**	**Cystine**	**Histidine**	**Isoleucine**	**Leucine**	**Lysine**	**Methionine**	**Phenylalanine**	**Threonine**	**Tyrosine**	**Valine**	Alanine	Glutamine acid	Glycine	Proline	Serine	Tryptophan
Fish meal 1	73.9	3.52	0.67	2.04	2.46	4.60	4.99	1.87	2.47	2.47	1.91	3.12	3.96	9.47	4.20	2.64	2.46	0.67
Fish meal 3	67.4	3.53	0.43	1.77	2.28	4.16	4.09	1.60	2.18	2.30	1.48	2.93	4.33	7.94	5.98	3.69	2.55	0.49
Earth worm	71.3	4.29	0.94	1.69	3.02	5.28	4.69	1.26	2.95	3.18	2.08	4.97	3.35	7.79	3.14	2.82	3.38	0.88
Wheat meal	15.5	0.54	0.51	0.34	0.42	0.91	0.33	0.20	0.65	0.37	0.33	0.29	0.46	4.49	0.56	1.45	0.63	0.14
Soybean meal	43.7	3.08	0.65	1.51	1.54	3.06	2.34	0.57	1.92	1.67	1.38	1.73	1.64	8.03	1.67	2.14	2.43	0.53
Soybean cake	58.6	3.38	2.13	1.47	1.95	3.62	2.96	0.67	2.37	1.79	1.57	0.64	2.22	8.79	2.05	2.70	2.43	0.63
Maize	10.9	0.54	0.23	0.38	0.35	0.96	0.42	0.20	0.44	0.39	0.31	0.48	0.60	1.85	0.48	0.70	0.56	0.09
Cassava	3.4	0.28	0.05	0.10	0.07	0.16	0.11	0.04	0.09	0.09	0.06	0.09	0.16	0.54	0.09	0.13	0.11	0.03
Rice bran	8.4	0.54	0.18	0.32	0.24	0.55	0.34	0.18	0.34	2.28	0.21	0.43	0.46	1.20	0.41	0.42	0.43	0.09
Sweet potato leaf	31.9	1.16	0.93	0.63	0.65	1.22	0.89	0.28	0.98	0.66	0.54	0.18	1.02	2.40	0.87	0.82	0.76	0.39
Mulberry leaves	12.7	0.45	0.12	0.27	0.41	0.83	0.47	0.17	0.51	0.42	0.29	0.54	0.59	1.15	0.55	0.51	0.45	0.18

Fish meal 3: normal fish meal used in Hohenheim for standard feed

4.1.3 Chemical composition, amino acid composition and estimated cost of feed in the study

As mentioned in the previous section (Materials and methods), based on the analytical results of ingredients, all feeds were formulated to contain 30% CP and 10% of CL. Whole compound feeds were analyzed either to confirm calculations or to assess feed utilization more accurately. The compositions of the experimental feed are shown in Table 14, 16, 17, and 19 respectively for the 4 trials. Costs of the feed were also estimated for evaluation of cost efficiencies later on.

4.1.3.1 Proximate chemical and amino acid composition of feed in Trial 1

Table 15: Chemical composition, gross energy content and cost of the diets in Trial 1

Variables	Control	F1.1	F1.2	F1.3
CA (% of DM)	7.9	10.1	7.4	6.1
CP (% of DM)	29.9	30.2	30.5	30.4
CL (% of DM)	9.7	10.2	10.6	10.8
GE (kJ/g)	19.7	20.1	20.2	20.9
Price (x 1 000VND/kg)	18.3	11.5	9.9	8.4

* 1Euro = 20.000VND (2006). CA: Crude Ash, CP: Crude Protein, CL: Crude Lipid, GE: Gross Energy

Table 15 shows that the chemical composition of the feed in trial 1 was closest to the composition calculated from its ingredients. Both the protein and the lipid content of the feed fluctuated within a narrow range, from 29.9 to 30.5% for crude protein and between 9.7 and 10.8% for crude lipid. Results for the protein and lipid contents of all the feeds in the Trial 1 were comparable. The only difference within these feeds was the estimated cost. The control feed had the highest cost of ingredients with approximately 18300 VND/kg due to the high proportions of fish meal and wheat meal. Both were imported ingredients and therefore very expensive in Viet Nam. The cost of the other feeds was decreased by reducing the proportion of fish meal in the diets. The most expensive feed

made from local ingredients cost about 11500 VND/kg (F1.1) whereas the cheapest (F1.3) cost only 8400 VND/kg (Table 17).

Table 16: The amino acid requirements of common carp and the amino acid composition of the crude protein content of feed in Trial 1 (% of CP)

Amino acid	Requirement*	Control	F1.1	F1.2	F1.3
Essential amino acids					
Threonine	1.50	0.98	0.99	1.01	0.99
Valine	1.40	1.23	1.17	1.17	1.22
Cystine/Methionine	1.20	1.00	1.01	0.96	0.92
Phenylalanine/Tyrosine	2.50	1.91	1.82	2.02	2.19
Lysine	2.20	1.65	1.70	1.72	1.65
Arginine	1.60	1.42	1.53	1.64	1.85
Isoleucine	0.90	0.98	0.99	1.06	1.00
Leucine	1.30	1.90	2.05	2.09	1.97
Histidine	0.80	0.93	1.03	1.00	0.98
Tryptophan	0.30	0.32	0.28	0.32	0.33
Non-Essential amino acids					
Asparagine	-	2.14	2.32	2.67	2.76
Serine	-	1.15	1.05	1.26	1.31
Glutamine	-	5.05	4.22	4.74	4.74
Glycine	-	1.44	1.58	1.56	1.33
Alanine	-	1.42	1.56	1.56	1.45
Proline	-	1.71	1.19	1.46	1.43

*Source: NRC (1993)

The amino acid composition of the crude protein content of feed in Trial 1 is presented in Table 16. In this table, most of amino acids in the feeds (both control feed and experimental) are present in small amounts. Concentrations of almost all essential amino

acids such as threonine, valine, cystine + methionine, arginine, and lysine are lower than the common carp's requirements. Among these amino acids, threonine seemed to be the most limiting one in all diets since only two thirds of the threonine requirement was met. Cystine and methionine were also limited although, among plant ingredients, soybean was reported to contain the highest amount of methionine.

4.1.3.2 Proximate chemical and amino acid composition of feeds in Trial 2 (the field trial)

In the field trial, the protein content of feed in trial 2 was slightly higher than calculated, ranging from 33.4 to 34.0%. The difference in protein between the calculated value and the actual composition of the feed could be attributed to the application of inaccurate data based on the chemical analysis of the ingredients by the laboratory at RIA 1. Accordingly, all the feed seemed to be comparable for both crude protein and crude lipid (Table 17).

Table 17: Chemical compositions, gross energy content and estimated cost of the diets in Trial 2

Variables	Control	F2.1	F2.2	F2.3
CA (% of DM)	8.9	8.2	8.9	9.7
CP (% of DM)	33.5	34.0	33.7	33.4
CL (% of DM)	10.1	10.1	9.5	9.7
GE (kJ/g)	19.3	19.3	19.3	19.3
Estimated cost (1 000 VND)	10.5	7.4	7.2	6.6

* 1Euro = 20.000 VND. CA: Crude Ash, CP: Crude Protein, CL: Crude Lipid, GE: Gross Energy

In trial 2, feed F2.1 was made from the ingredients of feed F1.3 in trial 1 with some minor changes to adapt to the local conditions. This feed was estimated to cost about 7300 VND/kg feed. The control feed cost approximately 10500 VND/kg and was cheaper than the control feed in the trial 1 due to the replacement of wheat meal by polished wheat bran. The other feeds were cheaper than F2.1 and the cheapest was F2.3 which was estimated to cost only 6600 VND/kg.

4.1.3.3 Proximate chemical and amino acid composition of feed in Trial 3

Despite the fact that all feeds were calculated to contain 30% crude protein, the analytical results showed that the control feed contained nearly 33% of crude protein (Table 17). The protein contents the feeds containing non-sweet potato leaf were very close to the calculated values excepted for feed F3.3 which contained only 28.3% crude protein. However, the feeds with the lowest protein contents according to their proximate analysis were F3.6 and F3.7 with only 27.8 and 28.2% of crude protein respectively. Crude lipid contents of feed in this trial varied between11.1 and 12.1%, slightly higher than that of the control (9.9%).

Table 18: Chemical compositions (% of DM) and energy (kJ/g) of feed in Trial 3

Feed	Control	F3.1	F3.2	F3.3	F3.4	F3.5	F3.6	F3.7
CA	10.4	7.6	8.2	7.5	7.4	10	10.3	9.3
CP	32.8	30.1	30.8	28.3	30.3	29.7	27.8	28.2
CL	9.9	12.1	11.7	11.1	11.4	12.1	11.1	11.3
GE	20.5	21	20.7	20.1	20.6	20.2	19.8	19.9

CA: Crude Ash, CP: Crude Protein, CL: Crude Lipid, GE: Gross Energy

As with the control feed, the amino acid composition of the other feeds was lower than the requirements of common carp. Feed F3.3 seemed to have the lowest concentrations of almost all amino acids, including the non-essential ones. Only the leucine, isoleucine and arginine contents of this feed exceeded the carp's requirements. However, the amino acid composition of the feeds in Table 19 did show one result of interest. The levels of almost all the amino acids in the feed containing sweet potato leaf were higher than those of the control feed or the requirements of the common carp.

Table 19: Amino acids composition of feeds in Trial 3 (% CP)

Amino acid	Req.*	Control	F3.1	F3.2	F3.3	F3.4	F3.5	F3.6	F3.7
Essential amino acids									
Arginine	4.3	4.33	6.11	6.36	5.69	6.07	8.59	8.56	8.23
Cys + Met	3.1	3.05	3.12	2.86	2.97	3.10	3.77	3.74	3.90
Histidin	2.1	2.99	3.26	3.18	2.61	3.23	4.48	4.10	4.22
Isoleucine	2.5	3.05	3.42	3.34	3.57	3.40	4.71	5.00	4.93
Leucine	3.3	6.01	7.08	6.56	6.93	7.03	9.02	9.14	9.43
Lysine	5.7	5.03	5.42	5.45	5.44	5.38	7.24	7.48	7.30
Phe + tyr	6.5	5.82	7.01	6.85	6.78	6.96	10.24	10.36	10.35
Threonine	3.9	2.99	3.65	3.57	3.39	3.63	4.92	4.86	4.86
Valine	0.8	3.75	4.09	3.86	4.03	4.06	6.09	6.26	6.31
Non-Essential amino acids									
Alanine		4.33	4.42	4.09	2.47	4.39	6.63	5.25	5.78
Asparagine		6.52	8.97	9.16	3.32	8.91	21.01	17.41	17.94
Glutamine		15.40	16.68	16.46	6.64	16.57	20.24	12.59	14.22
Glycine		4.39	4.35	4.12	2.33	4.32	6.20	4.75	5.18
Proline		5.21	4.78	4.42	2.19	4.75	6.03	4.03	4.75
Serine		3.51	4.92	4.81	1.87	4.88	6.06	3.74	4.29
Tryptophan		0.98	1.06	1.10	1.06	1.06	2.12	2.19	2.16

* Source: (NRC, 1993); Req.: requirement; cys + met: cystine + methionine; phe + tyr: phenylalanine + tyrosine.

For example, leucine levels in feeds F3.5, F3.6, and F3.7 were 9.02, 9.14, and 9.43% respectively which were much higher than that of the control feed (6.01% of CP) or were

even twice of that of requirements. Only some amino acids such as threonine, cystine, and lysine were slightly lower than requirements, nevertheless they were higher than those of the control feed (Table 19).

4.1.3.4 Proximate chemical and amino acid composition of feed in Trial 4

Table 20 shows the chemical composition and gross energy values of feed in trial 4. Chemical compositions of feed in this trial were very similar. All feeds including the control feed had the same amount of crude lipid (9.7%) and the crude protein content varied only slightly (between 25.5 and 27.8%).

Table 20: Chemical composition and gross energy content of feed in Trial 4

Feed	Control	F4.1	F4.2	F4.3
CA (% of DM)	8.3	8.4	8.1	8.2
CP (% of DM)	27.8	27.7	27.5	27.6
CL (% of DM)	9.7	9.7	9.7	9.7
GE (kJ/g)	19.4	19.4	19.4	19.4

CA: Crude Ash, CP: Crude Protein, CL: Crude Lipid, GE: Gross Energy

As with previous results for amino acid composition, Table 21 shows that the amounts of amino acids in all the feeds in trial 3 including the control feed were lower than the requirements for carp and the most limiting were cystine and methionine. The control feed could meet only 30% of this requirement. Feed F3.3 contained the most cystine and methionine but still could supply only 50% of the carp's requirement. Phenylalanine, tyrosine, and lysine were also very limited. Consequently, to meet the requirements, supplementation of these amino acids was needed.

Table 21: Requirements and amino acid composition of feed in Trial 4 (% CP)

Amino acids	Req.	Control	F4.1	F4.2	F4.2
Essential amino acids					
Arginine	1.6	5.04	5.42	5.45	5.80
Cys + Met	1.2	1.44	1.81	1.82	2.17
Histidine	0.8	2.52	2.53	2.55	2.54
Isoleucine	0.9	3.24	3.61	4.00	3.99
Leucine	1.3	6.47	6.86	7.27	7.61
Lysine	2.2	5.04	5.42	5.45	5.43
Phe + Tyr	2.5	3.96	3.97	4.36	4.35
Threonine	1.5	3.24	3.61	4.00	3.99
Valine	1.5	3.96	4.33	5.09	5.80
Non-Essential amino acids					
Alanine		5.40	4.69	4.36	5.80
Asparagine		8.27	8.30	8.36	8.33
Glutamine		18.35	18.41	18.18	18.84
Glycine		6.83	5.42	4.36	7.97
Proline		6.83	6.50	6.18	7.25
Serine		4.32	4.69	5.09	4.35
Tryptophan		1.08	1.08	1.09	0.72

Req.: requirement; source: (NRC, 1993); cys + met: cystine + methionine; phe + tyr: phenylalanine + tyrosine.

4.2 Effects of the locally formulated diets on fish growth performance under different conditions (laboratory conditions (Trial 1) and field conditions (Trial 2)

Local ingredients were evaluated throughout two trials, Trial 1 was conducted in the laboratory and the other one (Trial 2) was carried out under pond conditions at Hanoi University of Agriculture in Vietnam.

4.2.1 Environmental parameters in the feeding trials

Trial 1 was conducted under laboratory conditions at Hohenheim University (Germany). Thus, all the water parameters were maintained within an optimal range for fish culture (see in chapter/section of materials and methods). Therefore, only water parameters in Trial 2 were monitored and presented in this part (Table 22).

Table 22: Water parameters in Trial 2

Week	1	2	3	4	5	6	7	8	9
T °C	27.8	28.6	30.0	30.0	30.3	30.3	29.7	30.3	30.0
pH	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Trans cm	19.6	18.7	17.7	16.6	15.4	14.7	14.1	13.9	13.2
NO_2-N mg/l	0.09	0.11	0.13	0.23	0.28	0.32	0.26	0.23	0.27
NO_3-N mg/l	0.11	0.24	0.82	0.93	0.76	0.65	0.60	0.82	0.74
NH_4-N mg/l	0.24	0.425	0.51	1.04	0.74	0.29	0.27	0.71	0.42
NH_3 mg/l	0.01	0.03	0.05	0.11	0.09	0.08	0.07	0.2	0.04
PO_4-P mg/l	0.73	0.78	0.81	1.12	1.03	0.77	0.93	1.03	0.79

T: temperature; Trans: water transparency/Secchi depth.

4.2.1.1 Temperature

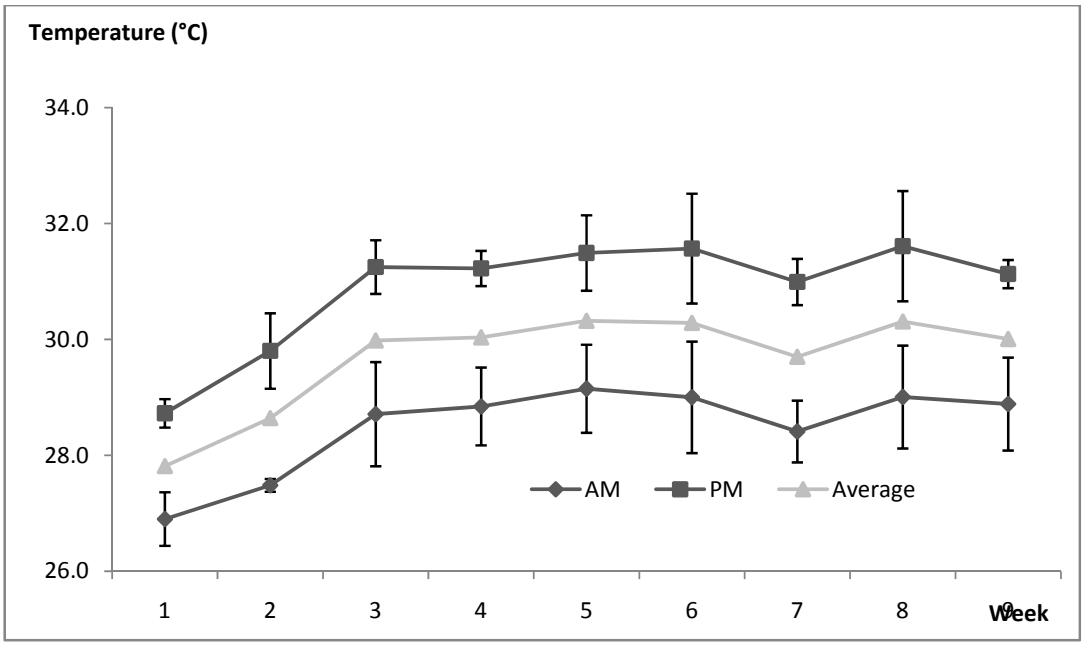

Figure 14: Temperature in Trial 2

Among all environmental factors, temperature is always considered as the most important factor influencing animal growth, especially for poikilothermic animals. Trial 2 (field trial) was carried out in summer, when the temperature was rather high. The average weekly water temperature was 29.7 ± 0.9°C, varying from 26.8°C to 31.6°C during the experimental period. The lowest average temperature at the beginning of the trial was 26.8°C which then increased to 27.5°C in the second week before reaching a peak in the third week (28.7°C). In later weeks water temperature fluctuated in a higher range, keeping above 30°C, accept for the 7th week when the temperature fell below 30°C because of long heavy rain. Overall, the average weekly temperature remained in an acceptable range for common carp (Figure 13).

4.2.1.2 pH

The water pH in trial 4 varied according to time. At the beginning of the trial, the pH was at its lowest (7.6). It increased rapidly to 7.9 after two weeks of stocking and reached a peak by the fifth week. From this time on, the pH fluctuated slightly around 8.2 and 8.3 before going down steeply to 7.9 in the 8th week (Figure 14). But in the last week of the

trial it increased to return to the highest pH value (8.3). Overall however, the pH values stayed within an acceptable range for carp (7.5 to 8.5) (Boyd, 1982).

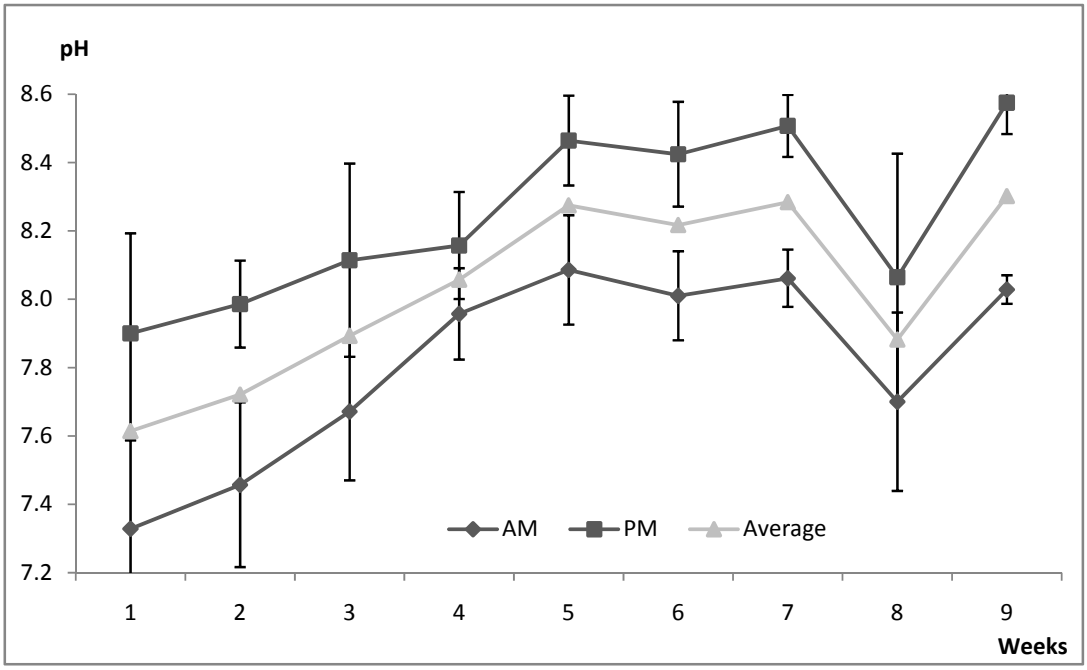

Figure 15: pH in Trial 2

4.2.1.3 pH, oxygen and temperature in 24 hours

The daily fluctuations of pH, oxygen, and temperature are shown in figure 16. The data presented in figure 16 are the averages of two continuous 24h measurements taken at the beginning of the fourth week. The lowest temperature was at midnight (1 to 3 a.m.) and the highest temperature occurred between 12a.m. and 2 p.m. During this period both pH and dissolved oxygen reached their highest levels. The lowest pH was at 5 a.m. and the highest at 2 p.m. While pH did not change much, ranging from 6.5 ± 0.1 to 9.4 ± 0.1, dissolved oxygen behaved very differently between day and night time. The data show that oxygen in the pond decreased to the lowest level of 2.4 ± 0.1 mg/l at 4 to 6 a.m. and as soon as the sun rose, dissolved increased rapidly. The highest level of dissolved oxygen reached was 7.5 ± 0.1 mg/l.

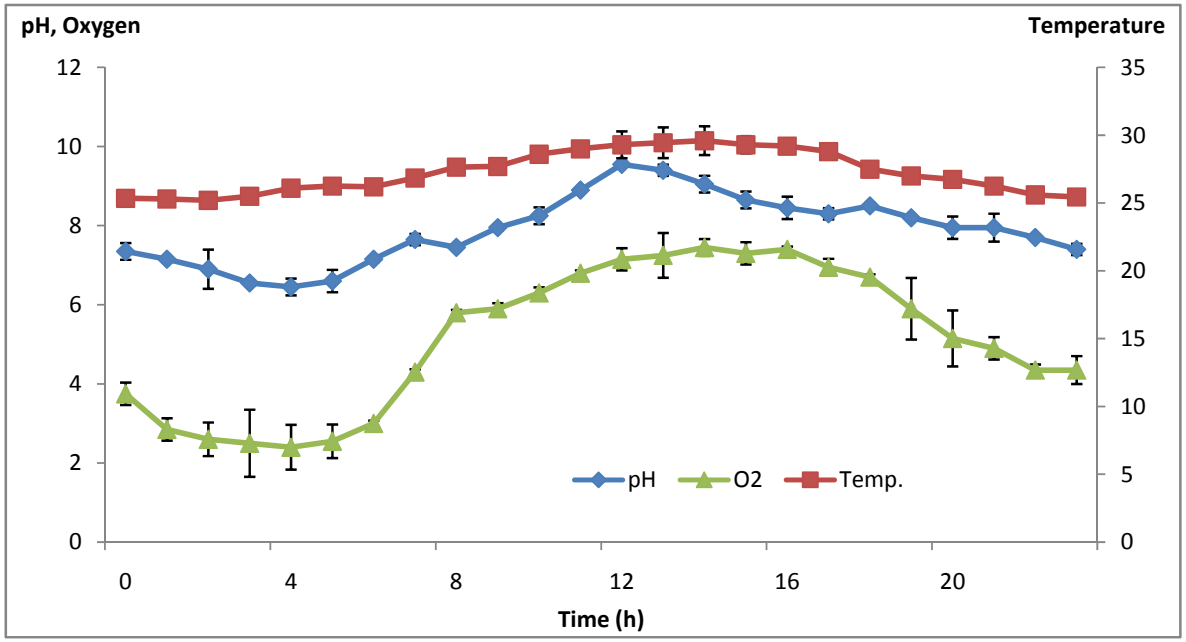

Figure 16: pH, oxygen and temperature in 24 hours in the Trial 2

4.2.1.4 Water transparency (Secchi depth)

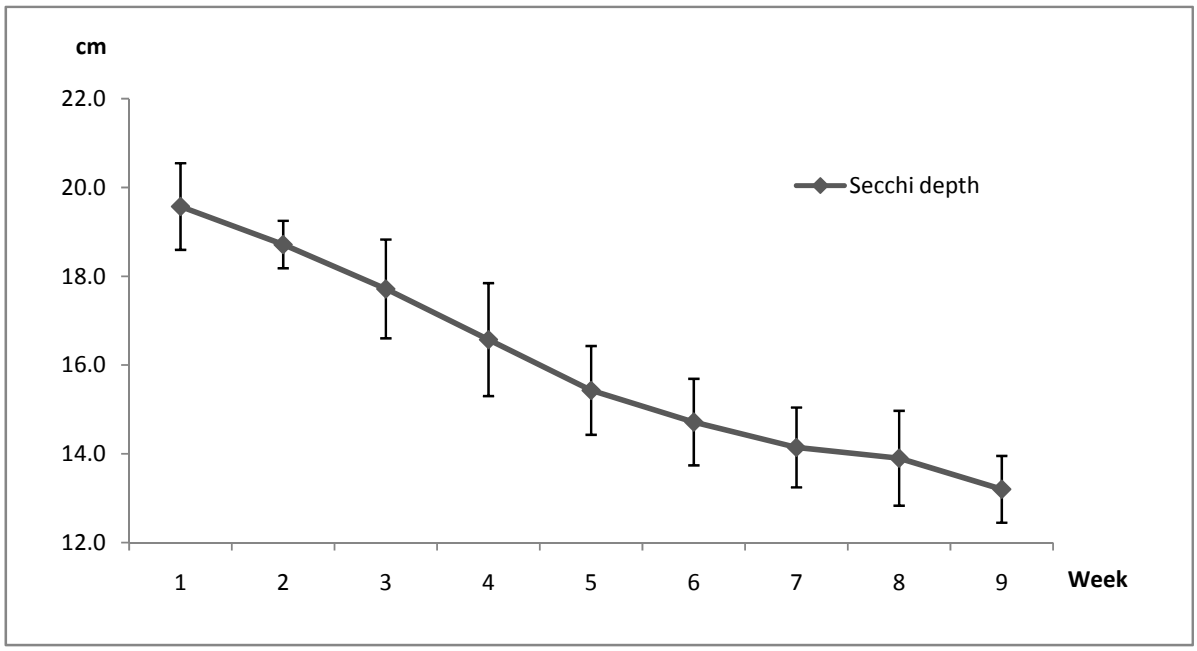

Figure 17: Water transparency in Trial 2

In contrast to temperature and pH values, water transparencies of this trial were low and had a tendency to decrease linearly (Figure 16). The highest Secchi value was 19.6 cm, at the beginning of the trial. This parameter decreased rapidly until the lowest value was

reached at the end of the trial (13.2 cm). Decreasing Secchi values were associated with water of a red, milky colour (Figure 17).

4.2.1.5 Total ammonium-N, ammonia

The concentrations of ammonia-N and phosphate-P in the water during the trial are shown in Figure 18. Ammonia-N concentration increased from 0.24 mg/l at the commencing of the trial to 0.51 mg/l in the third week. After that it increased steadily to reach a peak (1.04 mg/l) at the fourth sampling. It then decreased rapidly again in the sixth and seventh week to return to the same concentration as at the beginning of the trial (0.27 - 0.29 mg/l). In the following weeks, the ammonia-N concentration fluctuated around 0.42-0.71 mg/l.

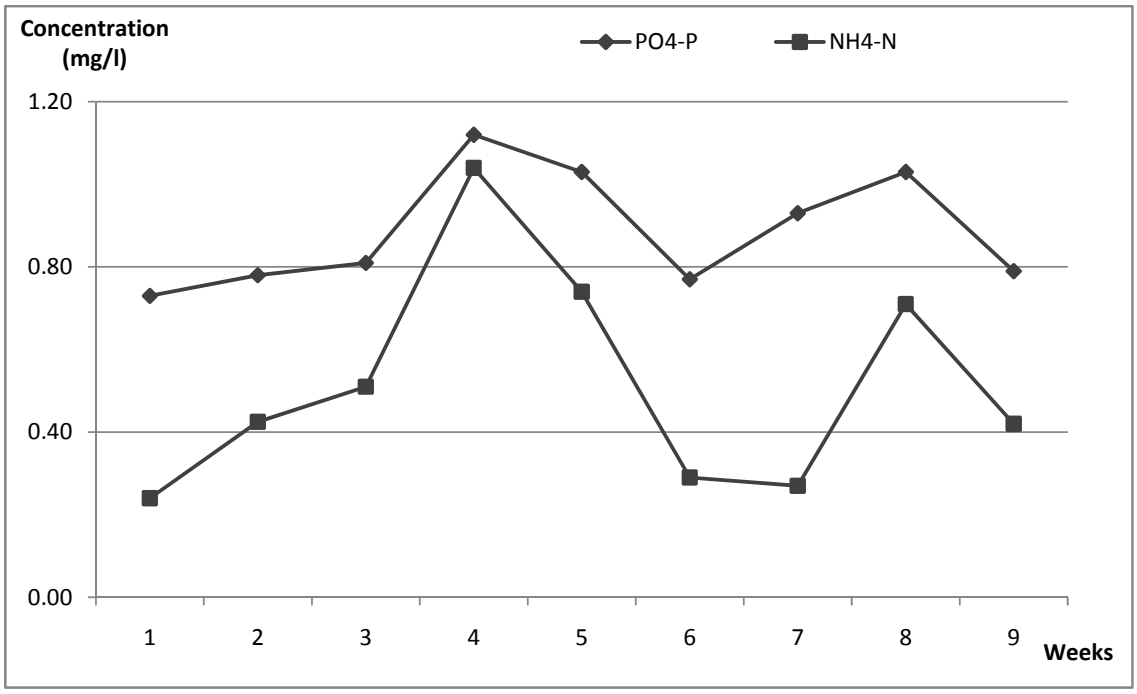

Figure 18: Ammonia-N and phosphate-P of water in Trial 2

4.2.1.6 Phosphorus

Like the ammonia-N concentration, phosphate-P also increased after stocking the fish. It was 0.73 mg/l at the start and rose to 1.12 mg/l at the fourth measurement. After that, water phosphate-P concentration decreased to 0.77 mg/l by the sixth week and reached a second peak of 1.03 mg/l in the eighth week (Figure 18).

4.2.1.7 Nitrate and nitrite

Both the nitrate-N and nitrite-N in this trial were very low at the beginning of the trial. The nitrate-N concentration was only 0.11 ± 0.04 mg/l in the first week, but increased rapidly to 0.24 ± 0.05 mg/l in the second week and dramatically to 0.82 ± 0.06 mg/l within the next two weeks. The highest nitrate-N concentration was recorded during the fourth week with 0.93 ± 0.06 mg/l. From this time on, nitrate-N concentration decreased to 0.6 ± 0.05 mg/l before reaching a second peak of 0.82 ± 0.04 mg/l in the seventh week followed by a decline to 0.74 ± 0.03 mg/l (Figure 19).

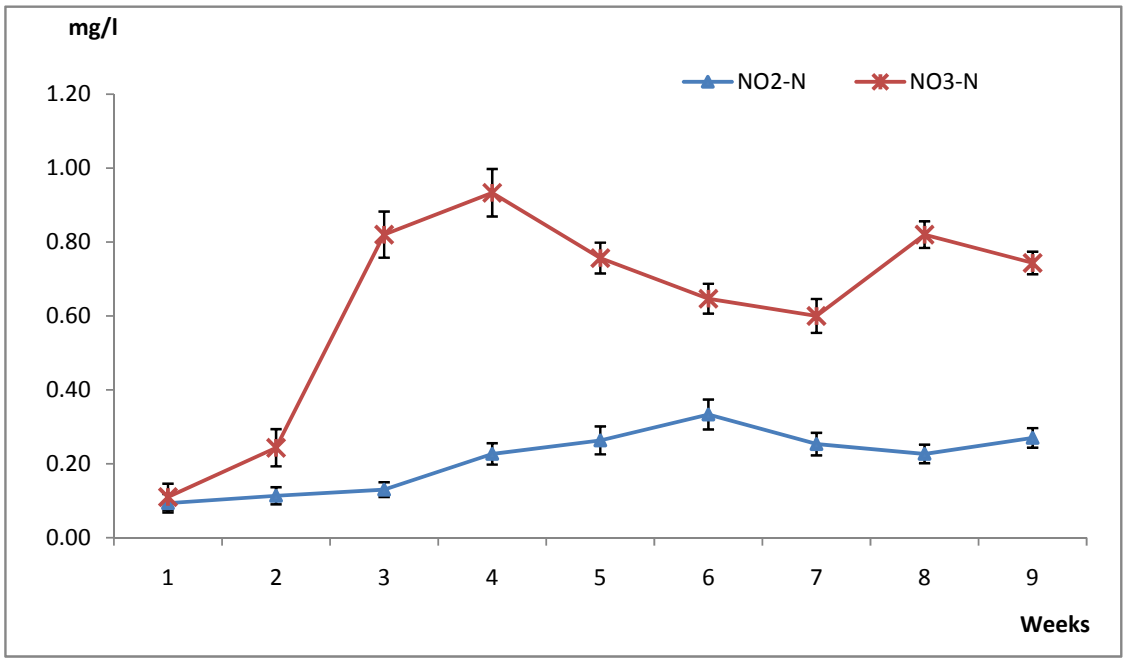

Figure 19: Nitrate-N and nitrite-N concentrations of pond water in Trial 2

Nitrite-N was always lower than nitrate-N. Initial water nitrite-N was only 0.09 ± 0.03 mg/l. Thereafter, it rose to 0.13 ± 0.02 mg/l in the second week. It increased regularly to a peak of 0.33 mg/l in the sixth week. After that, nitrite-N slightly decreased and stayed at 0.25 ± 0.03 mg/l and 0.23 ± 0.03 mg/l during the seventh and eighth weeks respectively. At the last sampling, nitrite-N was 0.27 ± 0.03 mg/l. However, this level of nitrite-N in the trial did not affect the growth and survival rates of the common carp because it was still in the acceptable range.

4.2.2 Feed acceptance, fish behaviour, and survival rate

Observations in both trials (Trial 1 and Trial 2) showed that all the feed sank immediately after being dropped into water. Fish pursued the feed actively and ate almost all of it before it reached the bottom of the tank aquarium. I made similar observations in the field trial, indicating that all the feeds were palatable and attractive to fish. The feeding rate of five times maintenance requirement was still lower than *ad libitum*. Thus, nutrient leaching was minimized since the feed was in the water for such a short time before being eaten.

Table 23: Survival rate of fish in Trial 2 (mean ± standard deviation)

		Control	F4.1	F4.2	F4.3
Replicate	1	100.0	100.0	95.6	97.8
	2	97.8	100.0	97.8	100.0
	3	100.0	100.0	100.0	100.0
Average		99.3 ± 0.7	100.0	97.8 ± 1.3	99.3 ± 0.7

All the nets remained intact until the end of the trial. No fish died during the trial but at the end of the trial when the fish were harvested some fish were missing according to our calculation. Therefore they were considered as dead. Accordingly, the lowest survival rate was recorded amongst fish fed diet F2.2 at approximately 97.8% (2 missing fish). The control group and F2.3 showed the same survival rate of 99.8%. In conclusion, the survival rates in this trial were relatively high and no significant difference was found between various feeding groups in the trial (Table 23).

4.2.3 Growth of fish- differences in growth of fish between laboratory and field conditions

4.2.3.1 Growth and feed intake of fish in trials

4.2.3.1.1 Growth and feed intake in Trial 1

Growth of fish

Figure 20 shows the fish growth in the laboratory trial (Trial 1). From the second week of the trial, fish in the control fed group had already shown superior growth to the experimental feed group (F1.1, F1.2, and F1.3) (Figure 19). At that time, the biomass gain of fish in the control group was 11.5 ± 0.4 g while that of fish of F1.1 to F1.3 were 10.0 ± 0.2, 10.5 ± 0.3, and 9.8 ± 0.2 g respectively. The differences in biomass gain between the control group and the others increased during the trial and after five weeks the control group was already significantly different from the experimental groups. At the end the trial the average weight of the fish fed on the control feed was 43.3 ± 3.7 g, notably higher than the fish in groups F1.1, F1.2, and F1.3 which reached only 34.8 ± 1.5, 30 ± 2.3, and 25.6 ± 1.1 g respectively. Similar results for weight gain were recorded: fish in the control group gained 34.2 g whereas fish in F1.1, F1.2, and F1.3 gained only 26.4 ± 1.6, 20.7 ± 2.0, and 17.9 ± 1.1 g respectively.

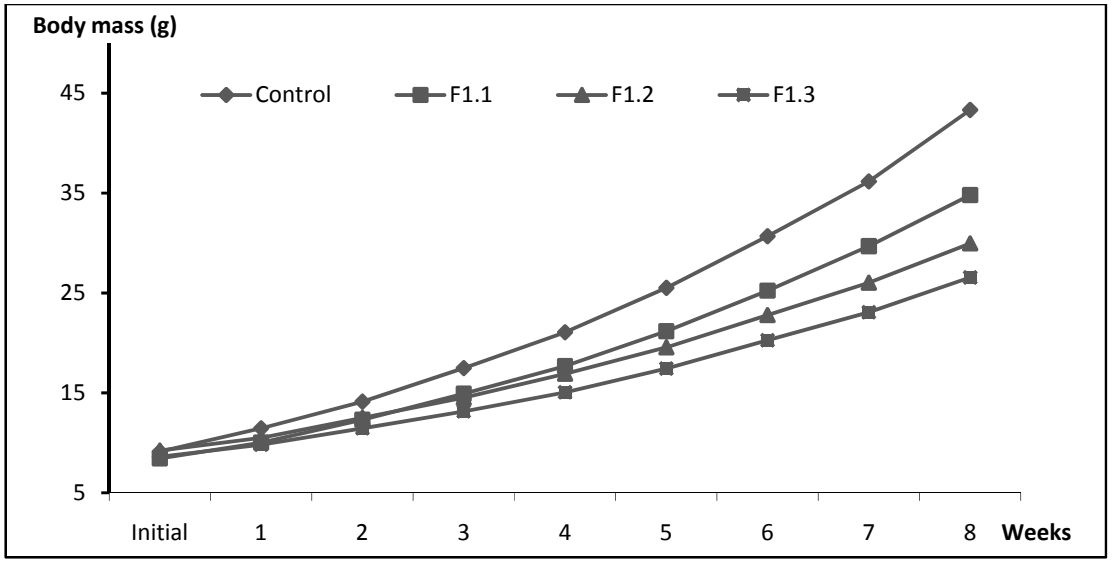

Figure 20: Growth of fish in Trial 1

Among the experimental diets, F1.1 was the best feed in terms of weight gain. Despite their lower final weight than the control feeding group, the fish in group F1.1 had significantly better growth rate and weight gain than those in the F1.2 and F1.3 groups. The specific growth rate followed a similar trend. The control feed in Trial 1 gave the highest SGR (2.8 ± 0.1%); followed by feed F1.1 (2.5± 0.1%) and F1.2 (2.1 ± 0.1%) while feed F1.3 gave the lowest (2.0 ± 0.1%). There were significant differences between the control feed and feeds F1.2 and F1.3 and between F1.1 and feeds F1.2 and F1.3 but not between the control and F1.1 or between F1.2 and F1.3 (Table 24).

Table 24: Weight gain and specific growth rate of fish in Trial 1

	Control	F1.1	F1.2	F1.3
IW (g)	9.1 ± 0.5	8.4 ± 0.2	9.2 ± 0.2	8.6 ± 0.1
WG (g)	34.2 ± 3.5^{a}	26.4 ± 1.6^{bc}	20.7 ± 2.0^{c}	17.9 ± 1.1^{d}
WG (%)	375.4 ± 33.2^{a}	312.6 ± 34^{b}	224.3 ± 16.2^{c}	208.6 ± 15.3^{c}
SGR (%.day^{-1})	2.8 ± 0.1^{a}	2.5 ± 0.1^{a}	2.1 ± 0.1^{b}	2.0 ± 0.1^{b}
FI (g)	191 ± 8.2^{a}	166.9 ± 3.6^{bc}	160.4 ± 3.7^{bc}	147.5 ± 1.9^{c}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. IW: Initial weight; WG: weight gain; SGR: specific growth rate; FI: feed intake.

Feed intake

The control feed seemed to be the most attractive feed because the fish ate significantly more of it than the other feeds (191 ± 8.2 g). Feed intake generally decreased according to the amount of fish meal in the diets. Feeds F1.2 and F1.2 had similar levels of intake while F1.3 had the lowest with only 147.5 ± 1.9 g (Table 23).

4.2.3.1.2 Growth of fish in Trial 2 (field trial)

Growth of fish and feed intake

In contrast to laboratory trials where the control feed gave the best growth rates, the control diet in this trial which contained the highest proportion of fish meal did not show better results than the experimental groups (Figure 20).

Figure 22 shows that all the feeding groups had similar growth rates within the first three weeks. However, they started showing differences by the fourth week and at the end of the trial significant differences had emerged between F2.1 and other feeding groups. Feed F2.1 appeared to be the best group in the trial. At the end of the trial, fish in this feeding group gained 151.1 ± 7.1 g, which was significantly more than the other experimental diets as well as the control (120.7 ± 10.9 g). In contrast to the growth of fish fed diet F2.1, the fish fed diet F2.2 grew least with a final weight gain of only 110.4 ± 7.1 g, which was less than the control feed and feed F2.3 (112.5 ± 7.2 g). However, no significant difference in growth was found between feeds F2.2 and F2.3 or between F2.1 and the control.

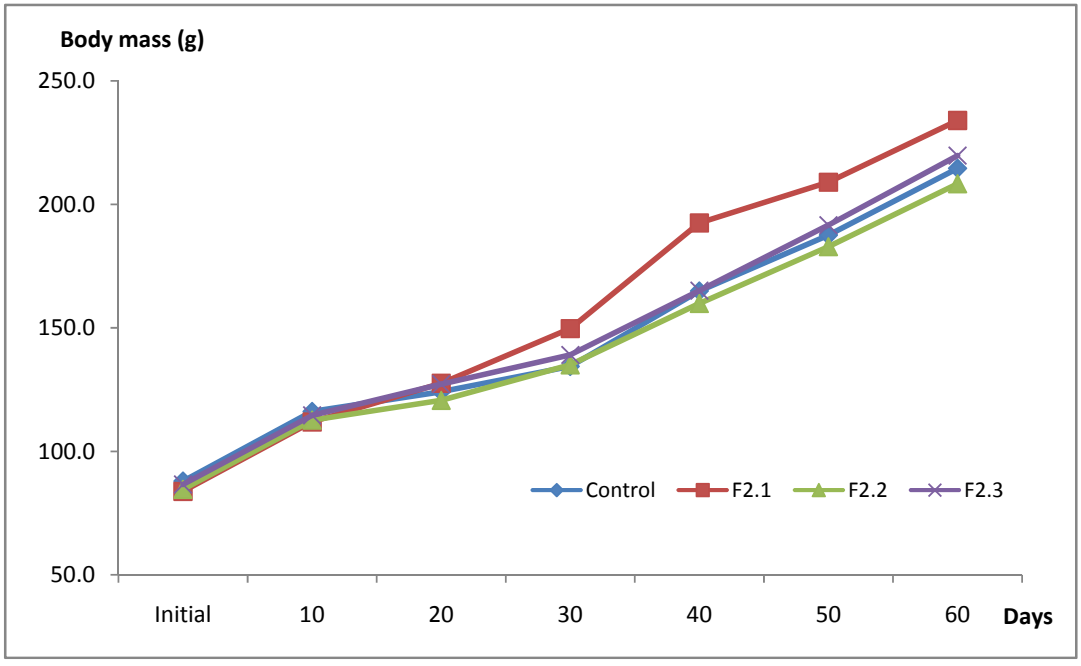

Figure 21: Growth of fish in Trial 2 (pond trial)

In general fish in this field trial did not grow as fast as fish in Trial 1. The highest SGR in the field trial was 2.1 ± 0.1% achieved by group F2.1 which was similar to the SGR of

feeding group F1.3 in Trial 1 (2.0 ± 0.1%). The feeding group F2.2 and F2.3 differed significantly from the group F2.1 as well as from the control feed (Table 25).

Feed intake in Trial 2 did not differ significantly between control and experimental groups. However, the feed intake of F2.1 was the highest (12.9 ± 0.1 kg). Feed F2.3 and control had the same feed intake whereas feed F2.2 had the lowest (11.7 ± 0.2).

Table 25: Weight gain and specific growth rate of fish in Trial 2

	Control	F2.1	F2.2	F2.3
IW (g)	86.6 ± 4.2	82.5 ± 8.2	84.6 ± 4.6	89.2 ± 5.3
WG (g)	120.7 ± 10.9^{b}	151.1 ± 7.1^{a}	110.4 ± 7.1^{b}	112.5 ± 7.2^{b}
WG (%)	139.3 ± 7.1^{b}	184.9 ± 25.9^{a}	131.0 ± 14.4^{b}	126.2 ± 5.9^{b}
SGR (%.day^{-1})	1.9 ± 0.1^{ab}	2.1 ± 0.1^{a}	1.8 ± 0.1^{b}	1.8 ± 0.1^{b}
FI (kg)	12.3 ± 0.6^{ab}	12.9 ± 0.1^{a}	11.7 ± 0.2^{b}	12.3 ± 0.5^{ab}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. IW: Initial weight; WG: weight gain; SGR: specific growth rate; FI: feed intake.

4.2.3 Nutrient utilization of fish in the laboratory and pond trial (Trial 1 and Trial 2)

4.2.3.1 Feed utilization in Trial 1

Utilization parameters reflect the capacity of fish to utilize the nutrients in their feed for their growth. Thus, a high rate of feed utilization usually leads to high growth. Table 26 shows some parameters of feed utilization of the fish in the two trials. In general, most feed utilization parameters of the control feed group were higher than those from the test diets and those of Trial 1 were higher than those of Trial 2. The PPV of the group F1.1 was significantly lower than that of the control group but it was considerably higher than that of F1.2 and F1.3 groups.

Table 26: Feed utilization parameters in Trial 1

	Control	F1.1	F1.2	F1.3
PER	3.0 ± 0.2^{a}	2.6 ± 0.1^{b}	2.2 ± 0.1^{c}	2.0 ± 0.1^{c}
PPV (%)	42.4 ± 3.7^{a}	36.4 ± 1.0^{b}	31.8 ± 0.5^{c}	26.5 ± 1.3^{c}
ANLU (%)	89.8 ± 9.9^{a}	60.3 ± 6.2^{b}	58.1 ± 3.1^{b}	48.2 ± 16.9^{b}
ER (%)	32.7 ± 3.2^{a}	25.3 ± 1.4^{b}	21.9 ± 0.9^{b}	18.7 ± 3.9^{b}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. PER Protein Efficiency Ratio, PPV: Protein Productive Value (%), ANLU: Apparent Net Lipid Utilization (%), ER: Energy Retention (%).

Similar trends were noted for ANLU and ER where the results show the ANLU for the control feed decreased as the proportion of fish meal in the diets decreased. Although no significant differences between the experimental diets could be shown, they are significantly different from the control. These results indicate that fish utilized the control feed more efficiently than the test feeds (Table 26).

4.2.3.2 Feed utilization in Trial 2

In this trial the control feed was not the best feed whereas the feed F2.1 showed the highest results of all utilization parameters. The result of ANLU of F2.1 group was 80.0% which meant fish could utilize lipid from the feed F2.1 more effectively than from the others, including the control group and this was significant. PER results were 2.2 ± 0.1, higher than the control feed (2.0 ± 0.2), F2.2 (1.9 ± 0.1) and F2.3 (1.9 ± 0.2). Similar observations were made in PPV and ER parameters. Fish could convert 35.6 ± 1.4% of protein and 17.8 ± 2.8% of energy in feed F2.1 into body protein and energy respectively. Next was the control group which achieved a PPV of 32.2 ± 6.8% and an ER of 14.2 ± 2.4%. Feeding group F2.3 was the worst which attained only 14.5 ± 2.1% of ER. However, the PPV of this group was 33.9 ± 1.7%, still higher than that of the control feeding group (Table 27).

Table 27: Feed utilization parameters in Trial 2

	Control	F2.1	F2.2	F2.3
PER	2.0 ± 0.2	2.2 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
PPV (%)	32.2 ± 6.8	35.6 ± 1.4	35.3 ± 5.4	33.9 ± 1.7
ANLU (%)	58.0 ± 7.1^{b}	80.0 ± 6.6^{a}	55.1 ± 3.9^{b}	53.5 ± 5.2^{b}
ER (%)	14.2 ± 2.4	17.8 ± 2.8	14.8 ± 3.4	14.5 ± 2.1

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. PER Protein Efficiency Ratio, PPV: Protein Productive Value (%), ANLU: Apparent Net Lipid Utilization (%), ER: Energy Retention (%)

4.2.4 Body condition parameters

Table 28: Condition factor, hepato-somatic index and intestine-somatic index of fish in Trial 1

	Control	F1.1	F1.2	F1.3
CF	3.3 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.3 ± 0.1
HSI	1.8 ± 0.2	1.5 ± 0.3	1.6 ± 0.1	1.6 ± 0.3
ISI	4.4 ± 0.2	4.6 ± 0.3	4.7 ± 0.4	5.1 ± 0.2

Note: Value = mean ± standard deviation.; CF: Condition factor, HSI: Hepato-somatic index, ISI: Intestine-somatic index.

Table 28 shows the results of condition factor (CF), hepato-somatic index (HSI), and intestine- somatic index (ISI) of Trial 1. According to this table, all the feeding groups gave similar results of CF. The group F1.2 had CF of 3.4 ± 0.1, slightly higher than the others but not significant. In this trial, F1.2 and F1.3 had the same value of hepato-somatic index (1.6), which was higher than that of the feed F1.1 (1.5 ± 0.3) but lower than the control group (1.8 ± 0.2). In contrast, the control feed delivered the lowest result of ISI (4.4 ± 0.2), followed by F1.1 (4.6 ± 0.3), and F1.2 (4.7 ± 0.4). The highest ISI in Trial 1 belonged to the feeding group F1.3 (5.1 ± 0.2). However, no significant difference in CF, HSI and ISI could be found between feeding groups (Table 28).

In term of body condition parameters, fish in Trial 2 showed a similar trend compared to Trial 1. However, most of the results achieved in Trial 2 were lower than those of Trial 1. For example, the highest CF in Trial 2 achieved by group F2.1 and F2.3 was 2.9 while that of Trial 1 was 3.4. Besides, the highest ISI in Trial 2 was 3.1 ± 0.4 (F2.1), still lower than the lowest in Trial 1. The highest HSI in Trial 2 was 2.0 ± 0.1 (control group), followed by F2.1 and F2.2. The feeding group F2.3 gave the lowest result of HSI with 1.4 ± 0.4. This was the lowest HSI in both trials (Trial 1 and Trial 2). However, all the results of body condition parameters indicate that diets in the study did not influence CF, HSI, as well as ISI of fish (Table 29).

Table 29: Condition factor, hepato-somatic index and intestine-somatic index of fish in Trial 2

	Control	F2.1	F2.2	F2.3
CF	2.8 ± 0.4	2.9 ± 0.1	2.8 ± 0.1	2.9 ± 0.2
HSI	2.0 ± 0.1	1.8 ± 0.2	1.6 ± 0.2	1.4 ± 0.4
ISI	2.7 ± 0.2	3.1 ± 0.4	2.8 ± 0.2	2.7 ± 0.3

Value = mean ± standard deviation. CF: Condition factor, HSI: Hepato-somatic index, ISI: Intestine-somatic index.

4.2.5 Body chemical composition

Body chemical compositions of fish in Trial 1 were analyzed individually whereas those of Trial 2 were done for groups of fish in each aquarium. The values presented here were the average of three replicates with the same treatment (Figure 22).

The results of the analysis of body chemical composition show that the water content of fish bodies did not differ significantly between the control group and the test diet groups. In Trial 1, the lowest water content belonged to the control group which had 74.9%, and the highest water content was 76.7% in the F1.1 group. In Trial 2, there was slightly lower water content in fish carcasses in comparison with that of Trial 1, ranging from 73.2% in group F2.1 to 75.6 in group F2.3. However, in both trials, initial fish had slightly higher content of crude protein, crude ash, and less crude fat than fish at the end of the trials.

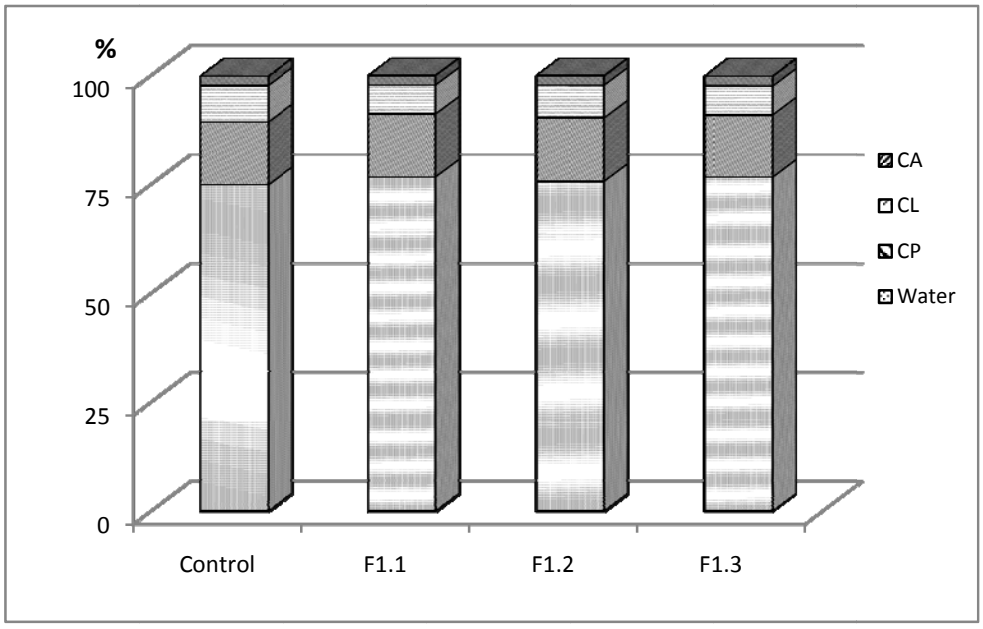

Figure 22: Fish chemical compositions in Trial 1

In Trial 1, in dry matter, initial fish consisted of 71.9% CP, 12.8 of CL, and 16.5% of CA. At the end of the trials, overall chemical composition of all experimental fish showed that final fish contained lower crude protein, lower crude ash but higher crude lipid. Control fish showed the lowest concentration of crude protein and crude ash but the crude lipid content of this group was the highest with 8.4% (33.3 ± 4.0% of DM). The highest CP content was 14.5% of FM (59.9% of DM) in group F1.2. However, the highest CP in DM was achieved by group F1.1 with 62.1 ± 2.7%. The lowest CL content belonged to group F1.3 which contained 27.8 ± 6.6% of CL of DM. This group accounted for 61.4 ± 3.6 and 9.7 ± 1.0% of CP and CA respectively (in dry matter).

Similar trends were observed in Trial 2 where initial fish contained 63.9% of CP, 14.5% of CA, and 17.6% of CL of dry matter while final fish comprised 59.3 ± 2.5, 11.3 ± 0.8, and 27.2 ± 2.6% of dry matter respectively for CP, CA, and CL. Final fish in group F2.1, which had the highest growth rate of Trial 2, contained the lowest proportion of protein with 56.1 ± 1.1% of DM (14.9% of FM), but the highest of lipid with 30.9 ± 1.4% of DM (8.6% of FM). Inversely, the group F2.2 with the lowest growth rate group had the highest proportion of protein (61.3 ± 3.4% of DM). However, the ash content of this group did not havethe highest proportion (11.7 ± 2.1% of DM) whereas lipid was 25.4 ± 2.3% of DM, the lowest crude lipid in fish carcass in this trial. Similar lipid content was recorded

for feeding group F2.3 with 25.5% of DM. This group had the highest ash content in DM with 12.2 ± 1.9% although it was not the highest in FM (2.7%). The control fish in this trial had 61.1 ± 0.7, 27.1 ± 2.9, and 10.3 ± 3.2% of CP, CL and CA respectively (Figure 23). However, analysis of fish body composition in the two trials did not show any significant difference in CP, CL as well as CA between feeding groups, indicating that diets did not influence body composition of experimental fish in both Trials 1 and 2.

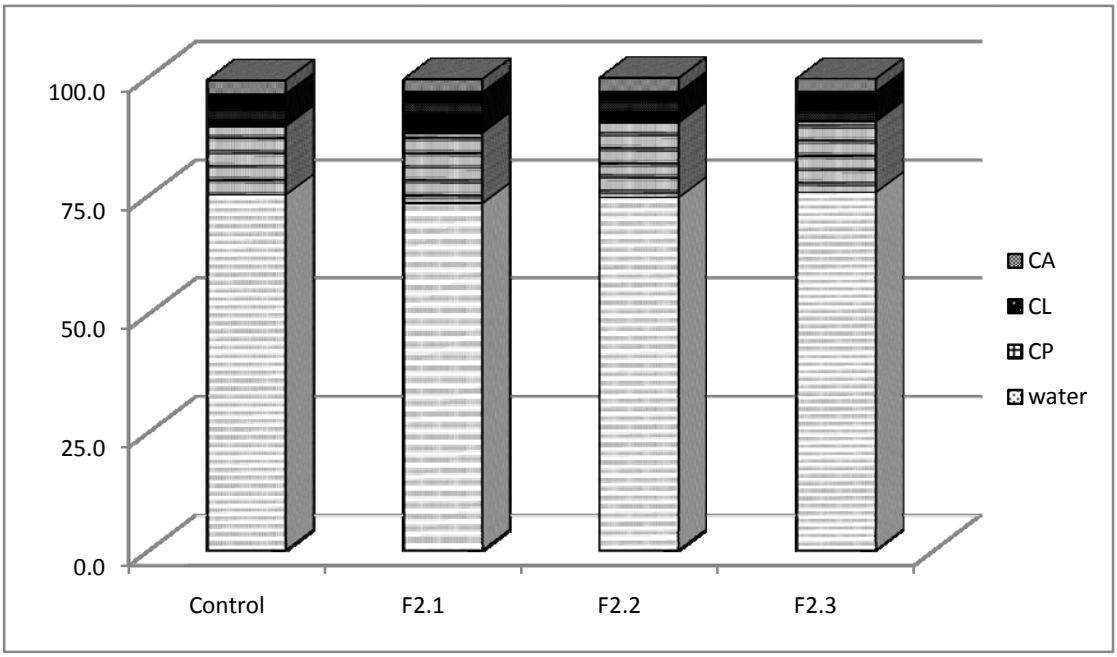

Figure 23: Fish chemical composition in Trial 2

4.2.6 Feed conversion ratio and cost efficiency

4.2.6.1 Feed conversion ratio (FCR)

Table 30: Feed conversion ratio and cost efficiency (x 1000 VND) in Trial 1 and Trial 2

Trial 1	Control	F1.1	F1.2	F1.3
FCR	1.1 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
Cost of feed	20.6	12.3	9.9	7.7
Cost efficiency	23.1	15.6	15.5	12.9
Trial 2	Control	F2.1	F2.2	F2.3
FCR	1.5 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Cost of feed	10.3	8.7	9.1	9.2
Cost efficiency	15.3	11.9	14.2	14.6

*1 euro = 20000VND

FCR is also one of the most important factors for evaluating feed utilization efficiency and cost efficiency. Data from Trial 1 show that feed affected FCR results. The lowest FCR was recorded in the control group with 1.1 ± 0.1 followed by F1.1 (1.3 ± 0.1), F1.2 (1.6 ± 0.1), and F1.3 with the highest FCR (1.7 ± 0.1). There was no significant difference between the FCR of the control group and feed F1.1 but there was a significant difference between the FCR of groups F1.2 and F1.3. Results for FCR in this trial indicated that a lower proportion of fish meal in the diets could lead to a high feed conversion ratio (Table 30).

Nevertheless, the results of FCR in Trial 2 were different from those of Trial 1. In the pond trial (Trial 2), the FCR of the fish fed the experimental feed was not significantly different from those of the fish in the laboratory trials. In contrast, the FCR of the control feed in the field trial was much higher than that of Trial 1. Feeding group F2.1 achieved

the lowest FCR (1.4 ± 0.1) while the control feed gave a higher result of FCR (1.5 ± 0.1), and both F2.2 and F2.3 gave the same FCR of 1.6 ± 0.1.

4.2.6.2 Cost of feed and cost efficiency

Cost efficiency was calculated from the cost of feed in relation to the FCR of the feed (Table 30). To minimize the effects from market changes, the same prices of feed ingredients at the start of Trial 1 were used for both Trials 1 and 4. Estimated cost of feed was calculated on the ingredient prices only; the other costs such as labour, processing, and other costs were excluded. However, some changes in ingredients led to changes in the cost of feed.

In Table 30, the cost for F2.1 was slightly higher than that of F1.3 although feed F2.1 was derived from the ingredients of F1.3. This change resulted from some small changes in ingredients to adapt to local conditions, in which vitamins and minerals were reduced but di-calcium phosphate and anti-fungus were added to protect the feed from humid conditions.

The control feed in Trial 2 was much cheaper than the control feed in Trial 1 (Table 30) because wheat meal was unavailable at that time due to the world food crisis and it was replaced by polished rice bran, which was cheaper. However, the price of the control feed was still higher than the other feed due to the high proportion of fish meal in the diet. The estimated cost for the control feed was 20600 VND/kg, much higher than the cost of F1.1 (15500 VND/kg) and double that of F1.2 (9900 VND/kg). Feed F1.3 had the lowest estimated cost, only 7400 VND/kg.

However, as can be seen from the results, the control feed was not that efficient in both trials. In Trial 1, 1.1 kg of feed was needed, equivalent to 23100 VND, to generate 1 kg of live fish. At the same cost, if F1.3 was applied, almost 2 kg of fish could be produced. The other feeds (F1.1 and F1.2) showed lower cost efficiencies in comparison with that of the control (15600 and 15500 VND/kg of fish respectively). Although the cost of the control feed in Trial 4 was lower, the cost efficiency of this group was still higher than the others. The control feed required 15300 VND to produce 1 kg fish while the other produced with only 11900, 14200, and 14600 VND for 1 kg fish respectively (Table 30).

4.3 Comparison of different protein and carbohydrate ingredients, and testing the suitability of sweet potato leaves (SPL) as an ingredient in diets for common carp (Trial 3)

As mentioned in the methods and materials part, this trial was set up to compare different sources of protein and carbohydrate. Another purpose was an investigation into the possibility of supplementing fish feed with sweet potato leaves (SPL). A fish meal-based diet was used as a control feed for comparison.

4.3.1 Feed acceptance

Similar to observations in previous trials, fish in Trial 3 consumed all the feed offered immediately when it was dropped into the aquaria. The amount of feed offered can be considered as the amount of feed intake. There was no visible difference in feed acceptance or any abnormal feeding activity of fish during the trial, although some feed contained a high proportion of SPL (F3.5, to F3.7).

4.3.2 Fish growth

Growth results in Figure 24 and Table 31 show that fish fed on fish meal-based protein diet (control feed) grew best, significantly higher than on other feeds (F3.1 to F3.7). At the end of the trial, fish in the control group reached an average weight of 14.5 g, while fish on feed F3.1to F3.4 reached 12.1, 11.7, 10.1, and 11.9 g respectively. There was virtually no difference between fish fed on F3.1 and F3.2. In other words, different sources of carbohydrate like cassava and maize in this trial did not affect fish growth significantly. However, fish in the group F3.3 fed on soy bean cake, grew significant less than fish fed on full fat soybean as a protein source (Feed F3.4).

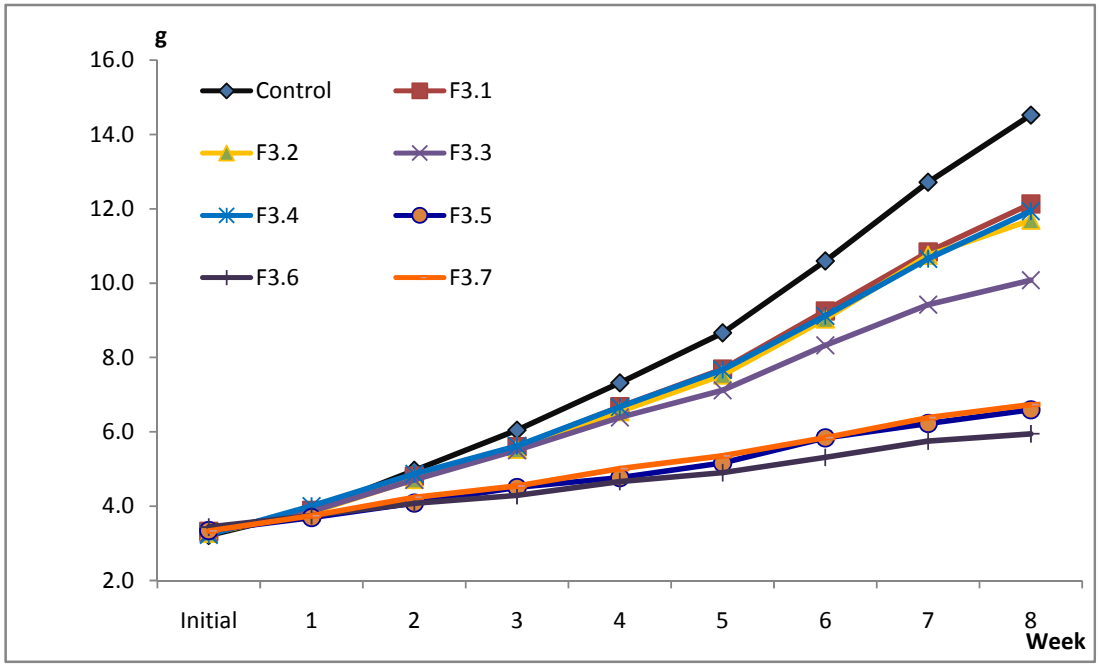

Figure 24: Growth of fish in Trial 3

Fish fed on SPL-containing feed grew visibly less than those on other feeds. At the end of the trial, they achieved only 6.7 g on feed F2.7, 6.6 g on feed F3.5, and feed F3.6 gave the worst final weight of only 6.0 g.

4.3.3 Fish body chemical composition

The average results of fish body composition in Trial 3 are shown in Figure 25. The crude protein of fish fed on experimental feed was obviously higher than fish fed on the control feed. Fish in feeding group F3.6 had the highest content of CP with 67.1% of DM (13.9% of FM), followed by fish in group F3.5 and F3.3 with 63.7% and 63.1% of DM respectively which is equivalent to 13.6% and 14.1% of FM (Figure 25). Fish from groups F3.5 and F3.6 had the lowest concentration of CL and highest content of body water. When fish contain a high proportion of fat, they contain less water and vice versa. Except for fish groups fed on diets containing SPL, fish in the trial had the same level of lipids, ranging from 31.5 to 36.1% of DM (7.1 to 8.8% of FM). The lipid content of SPL groups was significantly lower than those on other diets. Similarly, there was a significant difference in the proportions of CA between SPL-containing groups and the others.

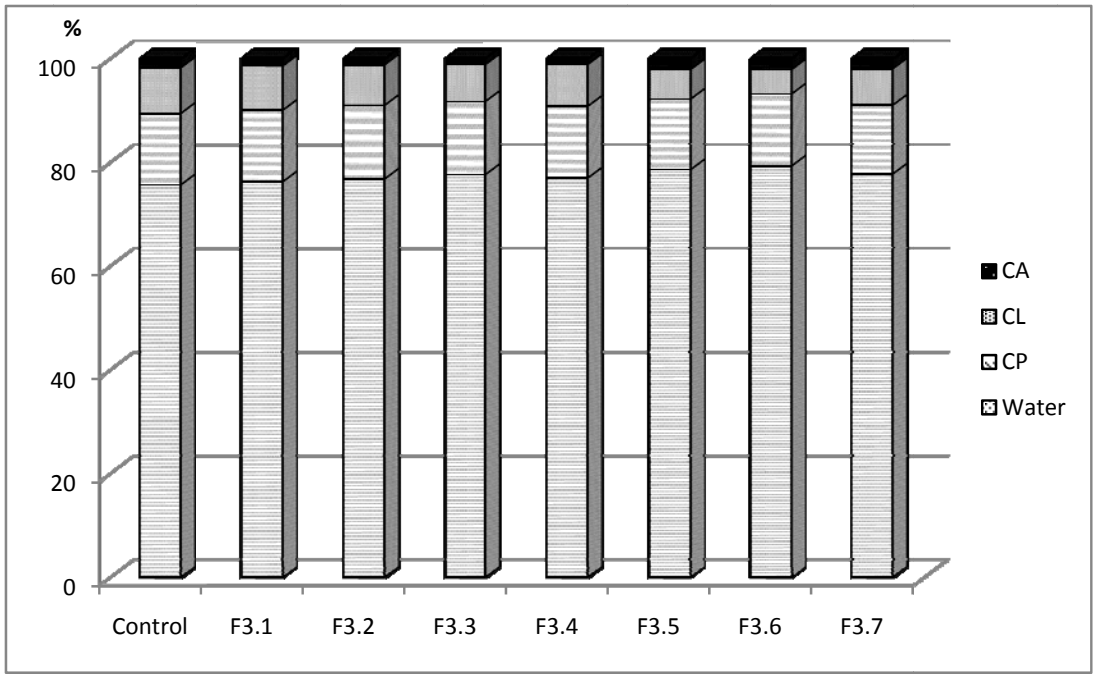

Figure 25: Fish body composition in Trial 3

Table 31: Weight gain and growth of fish in Trial 3

	IW (g)	WG (g)	WG (%)	SGR (%/day)
Control	3.2 ± 0.2	11.3 ± 0.5^{a}	352.8 ± 24.2^{a}	2.70 ± 0.09^{a}
F3.1	3.3 ± 0.1	8.8 ± 0.9^{b}	266.2 ± 35.3^{b}	2.31 ± 0.18^{b}
F3.2	3.3 ± 0.1	8.4 ± 0.7^{b}	258.0 ± 25.3b^{c}	2.27 ± 0.13^{b}
F3.3	3.3 ± 0.3	6.8 ± 0.4^{c}	207.3 ± 8.1^{c}	2.01 ± 0.05^{b}
F3.4	3.2 ± 0.1	8.7 ± 0.1^{b}	270.1 ± 8.8^{b}	2.34 ± 0.05^{b}
F3.5	3.3 ± 0.1	3.2 ± 0.1^{d}	97.3 ± 6.7^{d}	1.21 ± 0.06^{c}
F3.6	3.4 ± 0.1	2.5 ± 0.1^{d}	73.0 ± 2.7^{d}	0.98 ± 0.03^{c}
F3.7	3.3 ± 0.2	3.4 ± 0.8^{d}	102.7 ± 28.0^{d}	1.25 ± 0.25^{c}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. IW: Initial weight; WG: weight gain; SGR: specific growth rate.

The SPL groups had higher CA content in comparison with the others. F3.6 was observed to be the worst feed again for its high proportion of CA (11.3% of DM). The results of CA in feeding groups F3.1 to F3.4 did not differ from the control feed group. However, the total body composition slightly exceeded 100% of DM (100.2-100.6%), indicating either analytical manipulation errors or feed remaining in the intestine.

4.3.4 Feed utilization parameters

4.3.4.1 Feed intake

Table 32 shows obvious differences in feed intake between non-SPL and SPL-inclusion groups. All the SPL groups had significantly lower feed intake, ranging from 57.4 ± 1.1 to 60.2 ± 1.8 g. Group F3.1 growing at the lowest rate also showed the lowest feed intake. The control feed had the highest feed intake. However, it was not significantly different from that of feed F3.1, F3.2 and feed F3.4.

Table 32: Feed intake, specific growth rate and feed conversion ratio in Trial 3

	FI (g)	FCR
Control	82.0 ± 1.4^{a}	1.47 ± 0.06^{a}
F3.1	76.1 ± 3.1^{ab}	1.77 ± 0.12^{a}
F3.2	75.1 ± 3.8^{ab}	1.77 ± 0.12^{a}
F3.3	72.2 ± 4.7^{b}	2.13 ± 0.06^{a}
F3.4	75.8 ± 1.4^{ab}	1.73 ± 0.06^{a}
F3.5	59.1 ± 1.4^{c}	3.63 ± 0.15^{bc}
F3.6	57.4 ± 1.1^{c}	4.57 ± 0.21^{c}
F3.7	60.2 ± 1.8^{c}	3.67 ± 0.85^{bc}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. FI: feed intake; SGR: specific growth rate; FCR: feed conversion ratio.

4.3.4.2 Specific growth rate (SGR)

As with the results for final weight and weight gain, there was a great difference between SPL- containing feed groups and non-SPL feed groups. Specific growth rate on the control feed was the highest in the trial (2.70 ± 0.09%) and was significantly different from other groups. Although fish fed diet F3.3 grew faster, as proved by higher final body weight and weight gain, their SGR was not lower than that of fish on F3.4 and on the other non-SPL feed. Results in SGR of Feed F3.1 and F3.2 were similar, indicating that carbohydrate source had no effect on SGR. Fish in the SPL groups showed the lowest growth performances with SGR values of 1.21 ± 0.06, 0.98 ± 0.03 and 1.25 ± 0.25% respectively for the feeding groups F3.5, F3.6, and F3.7. These values were extremely low compared to that of the control group.

4.3.4.3 Feed conversion ratio and body condition factors

Table 33: Feed utilization in Trial 3

	PER	PVV %	ANLU %	ER %
Control	2.10 ± 0.11^{a}	28.82 ± 1.29^{a}	68.91 ± 6.93^{a}	18.74 ± 1.18^{a}
F3.1	1.92 ± 0.12^{ab}	26.29 ± 1.29^{ab}	46.91 ± 5.75^{b}	14.72 ± 1.23^{b}
F3.2	1.82 ± 0.12^{ab}	25.97 ± 1.48^{ab}	42.39 ± 5.28^{bc}	13.73 ± 1.16^{bc}
F3.3	1.67 ± 0.06^{b}	23.50 ± 0.19^{b}	34.21 ± 5.42^{cd}	11.32 ± 1.18^{c}
F3.4	1.90 ± 0.04^{ab}	26.14 ± 0.86^{ab}	46.14 ± 2.75^{bc}	14.44 ± 0.33^{b}
F3.5	0.92 ± 0.03^{c}	12.19 ± 0.32^{c}	14.32 ± 1.92^{ef}	5.19 ± 0.46^{d}
F3.6	0.78 ± 0.03^{c}	10.80 ± 0.87^{c}	8.14 ± 0.68^{g}	3.56 ± 0.37^{d}
F3.7	1.00 ± 0.23^{c}	12.45 ± 1.87^{c}	22.01 ± 2.53^{de}	6.10 ± 0.85^{d}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. PER Protein Efficiency Ratio, PPV: Protein Productive Value (%), ANLU: Apparent Net Lipid Utilization (%), ER: Energy Retention (%)

The results of FCR in Trial 3 were shown in Table 32. FCRs in this trial were considerably higher in comparison with the results in the previous trial. The lowest FCR value was recorded by the best growth group in the control feeding group (1.47). This

result was not different from any FCR of the non-SPL-containing feed groups (F3.1 to F3.4), but differed from those of SPL- containing feed groups (F3.5 to F3.7). The highest FCR (4.57) was that of feeding group F3.6 which also had the slowest growth rate.

4.3.4.4 Protein utilization (PPV and PER)

PPV and PER results did not repeat the exception from the rule achieved by SGR. The control feed group showed better results of feed utilization than the others. Fish from the control group converted approximately 29% of CP from the feed into body protein (PPV), followed by the groups F3.1 (26.29 ± 1.29%), F3.2 (25.97 ± 1.48%), and F3.4 (26.14 ± 0.86%). The lowest PPV values belonged to the group fed on the diets containing SPL. Feed F3.6 could utilize only 10.8% of crude protein efficiently, which was almost equivalent to one third of the control feed. The same results were observed for PER. Control feed obtained the highest value of PER (2.10 ± 0.11%), but it was not significantly different from feeds F3.1, F3.2, and F3.4 which attained 1.92 ± 0.12, 1.82 ± 0.12, and 1.90 ± 0.04% respectively. However, these results were significantly higher than the results from feed F3.3 and the SPL-containing feed (F3.5 to F3.7). These feeds had extremely low PPV and PER, while feed F3.6 was the lowest in terms of both PPV and PER with only 0.78 ± 0.03% and 10.80 ± 0.87% respectively (Table 33).

4.3.4.4 Lipid utilization (ANLU)

The same trends were observed in the utilization of lipid and energy. The control feed group was the best in lipid utilization with retention of 68.9 ± 6.93% for ANLU, followed by F3.1 (46.91 ±5.75%), F3.2 (42.39 ± 5.28%), and F3.4 (46.14 ± 2.75%). ANLU of the feed F3.3 was 34.21 ± 5.42%, significantly lower than that of the control feed, but considerably higher than the ANLUs from SPL groups (F3.5 to F3.7). The ANLU of feeding group F3.6 was the lowest (8.14 ± 0.68%), especially when compared to the control diet. Results indicated that the composition of the feed strongly influenced how fish utilized it (Table 33).

4.3.4.5 Energy retention (ER)

As with other parameters, results of ER were low compared to protein or lipid retention. The highest proportion of energy retention belonged to the control group with 18.7 ±

1.18%, significantly higher than the others. Feeds F3.1 and F3.4 had the same level of ER, 14.72 ± 1.23, and 14.44 ± 0.33% respectively, which were higher than F3.3 (11.32 ± 1.18) but not significantly higher than that of the feed F3.2 (13.73 ± 1.16). Energy retention of SPL-containing feeds (F3.5 to F3.7) were exceedingly low, 5.19 ± 0.46, 3.56 ± 0.37, 6.10 ± 0.85% respectively.

4.3.4.6 Fish body condition parameters

Table 34: Condition factors and hepato-intestine somatic index in Trial 3

	CF	HSI (%)	ISI (%)
Control	3.39 ± 0.03	2.16 ± 0.26^{a}	6.41 ± 0.32^{a}
F3.1	3.70 ± 0.08	1.79 ± 0.28^{abc}	7.86 ± 0.06^{bc}
F3.2	3.42 ± 0.25	1.72 ± 0.17^{abc}	6.72 ± 0.35^{a}
F3.3	3.80 ± 0.18	1.85 ± 0.16^{ab}	7.50 ± 0.24^{ab}
F3.4	3.67 ± 0.16	1.80 ± 0.16^{abc}	6.86 ± 0.53^{ab}
F3.5	3.36 ± 0.26	1.56 ± 0.08^{bc}	7.44 ± 0.61^{abc}
F3.6	3.36 ± 0.10	1.32 ± 0.10^{c}	7.47 ± 0.40^{abc}
F3.7	3.51 ± 0.34	1.61 ± 0.11^{bc}	7.99 ± 0.41^{c}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$.

Although there were some differences in growth and FCR, the body weight of fish grew in proportion to their standard body length. No significant difference was found in condition factors (CF) between control group and test groups as well as within the test groups. The value of CF in this trial ranged from 3.36 (in the groups F3.5 and F3.6) to 3.80 (in the group F3.3). Although fish in SPL-inclusion groups grew extremely slow with high FCR, the CF of these groups did not differ from those of the other feeding groups, including the control feed group. The lowest CF value was 3.36; belonging to feed F3.6 and the highest was 3.8 reached by fish in the group F3.3. These results seem to be similar to those of the previous trial (Table 34).

HSI is the liver weight against the body weight of fish. Results in the table showed that the control feed had the highest value of HSI (2.16 ± 0.26) in the trial. It was significantly higher than the HSI of feeds F3.5 to F3.7. The HSI of group F3.6 was the lowest (1.32 ± 0.10). This was even lower than the other feeds which contained the same proportion of SPL such as F3.5 and F3.7. The other feeds varied in range from 1.72 ± 0.17 in diet F3.2 to 1.85 ± 0.16 in F3.3, but there was no significant difference within these groups (Table 34).

The ISI of the control group was the lowest (6.41 ± 0.32). It was not significantly different from that of F3.2 but it was markedly different from all the others. The ISI value of F3.7 was the highest at approximately 7.99 ± 0.41, followed by feed F3.1 (7.86 ± 0.06), F3.3 (7.50 ± 0.24) and F3.6 (7.47 ± 0.40).

4.3.4.6 Digestibility in Trial 3

Results indicated a great difference in ADC between SPL feed and non-SPL feed (Table 35). Non-SPL feed had high digestibility of both protein and lipid among which feed F3.3 had the highest protein digestibility at approximately 87% of ADC, followed by F3.4 with nearly 80%. Feeds F3.1 and F3.2 showed lower results of P-ADC compared to that of the control feed (75.22%). Although F3.3 brought about the highest P-ADC, lipid digestibility (L-ADC) of this feed was the lowest among non-SPL feeds with only 79.81%, while the others had 86.75% to 88.45% and the control feed had 85.48% of L-ADC. Findings for E-ADC were the opposite. Here feed F3.4 gave rather low E-ADC (44.18%) against its high L-ADC (88.45%). The highest E-ADC belonged to the control feed with 75.27%, followed by feed F3.2 (68.68%), and feed F3.3 (63.24%).

SPL-containing feed had low digestibility all round - protein, lipid and energy. Protein digestibility of SPL feeds ranged from 60.47% in group F3.5 to 64.54% in group F3.7. Less difference could be recognized in digestibility of protein and lipid between SPL feeds. The highest L-ADC was feed F3.6 (78.23%) and the lowest was feed F3.5 (71.04%) which also had the lowest L-ADC in the whole of Trial 3. However, Table 35 shows that SPL has very low digestibility of energy, especially in feed F2.7 with only 32.49% of E-ADC. The other SPL had higher results for E-ADC (56.10% and 42.50%

respectively for F3.5 and F3.6) but they were still much lower than those of non-SPL feeds.

Table 35: Apparent digestibility coefficient of feed in Trial 3

	P-ADC	L-ADC	E-ADC
Control	75.22	85.48	75.27
F3.1	72.41	88.08	59.97
F3.2	74.50	86.75	68.68
F3.3	86.91	79.81	63.24
F3.4	79.97	88.45	44.18
F3.5	60.47	71.04	56.10
F3.6	61.32	78.23	42.50
F3.7	64.54	77.59	32.49

P-ADC: Protein Apparent Digestibility Coefficient; L-ADC: Lipid Apparent Digestibility Coefficient; E-ADC: Energy Apparent Digestibility Coefficient

4.4 Replacement of fish meal by earthworm meal (Trial 4)

4.4.1 Feed acceptance and feed intake

All the feed given to fish in the aquaria was eaten immediately. The feed sank quickly ensuring minimal nutrient loss due to a very short soak time. No abnormal fish activity was observed during the whole duration of the trial.

There was no difference between feed intake within the feeding groups, ranging from 121.5 ± 1.6g in the control group to 124.3 ± 1.9g in the F4.2 group (Table 36). This means that the substitution of earthworm in the diets did not influence feed intake.

4.4.2 Fish growth performance

In general, fish usually grew fastest when they were fed diets containing fish due to its rich and balanced nutrients as proved by the results from Trials 1, 3 and 4. However, in this trial, the control group who were fed the diet containing fish meal did not show better growth than fish fed experimental diets in which some or all of the fish meal had been replaced by other ingredients. The weight and weight gain of fish in the control feed group were lower than those of fish in the F4.2 group. Fish in group F4.2 showed the highest weight gain as well as growth rate. Figure 26 show that fish development appeared to be similar in all groups. However, results at the end of the trial showed that the fish in the F4.2 group reached 145.2 g, significantly higher than the 132.2, 135.3, 136.2 g of the control group and F4.1 and F4.3 groups respectively. Although replacement of fish meal by 30 and 100% earthworm had hardly any significant effect on fish growth, 70% replacement of fish meal resulted in significantly higher growth ratzes than the control after 8 weeks.

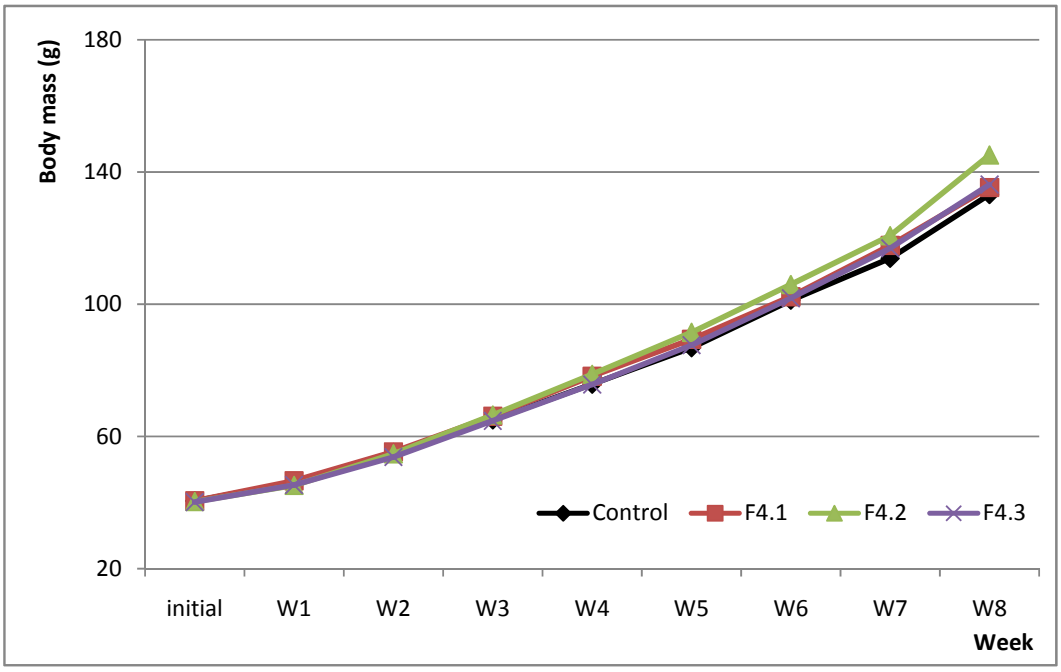

Figure 26: Development of fish in Trial 4

Table 36: Growth of fish in Trial 4

	IW (g)	WG (g)	WG (%)	SGR %
Control	8.1 ± 0.1	18.5 ± 1.1[b]	229.1 ± 11.4[b]	2.13 ± 0.06[b]
F4.1	8.1 ± 0.0	19.0 ± 0.4[b]	233.9 ± 5.2[b]	2.15 ± 0.03[b]
F4.2	8.1 ± 0.2	21.0 ± 0.3[a]	260.4 ± 2.2[a]	2.29 ± 0.01[a]
F4.3	8.0 ± 0.1	19.2 ± 0.5[b]	239.4 ± 7.0[b]	2.18 ± 0.04[b]

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. IW: Initial weight; WG: weight gain; SGR: specific growth rate.

4.4.2.1 Specific growth rate (SGR)

Despite fish consuming a similar amount of feed, they showed differences in final body mass as well as growth rate (Table 37). These were expressed more clearly in the SGR results. The SGR of the control group was the lowest (2.13%), lower than F4.1 (2.15 ± 0.03) and F4.3 (2.18 ± 0.04), and significantly lower than F2.2 (2.29 ± 0.01).

4.4.2.2 Feed conversion ratio (FCR)

Table 37: Feed intake and feed conversion ratio in Trial 4

	FI (g)	FCR
Control	121.5 ± 1.6	1.32 ± 0.08
F4.1	123.7 ± 1.5	1.26 ± 0.05
F4.2	124.3 ± 1.9	1.22 ± 0.02
F4.3	121.6 ± 1.7	1.27 ± 0.02

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. FI: feed intake; FCR: feed conversion ratio

FCR in this trial was considered as low. The highest FCR belonged to the control group, followed by F4.3 (1.27 ± 0.02), F4.1 (1.26 ± 0.05) while F4.2 was the lowest (1.22 ±

0.02). There was no significant difference between the control feed group and test groups in feed conversion ratio (Table 37).

4.4.4 Feed utilization

4.4.4.1 Protein utilization (PER and PVV)

The SGR of group F4.2 was higher than those of the other groups, and the PER and PVV of the HSI group were also higher. PER of the feeding group F4.2 was 2.80 ± 0.05, followed by feeds F4.1, F4.3, and the control feed had the lowest result of PER with 2.59 ± 0.16. There were no significant differences in PER but there were in PPV. F.3.2 showed the highest retention of protein with approximately 32%, which was significantly higher in comparison with the control feed group and from the other groups (Table 38). There was no difference in PPV level within these feeding groups. F4.1, F4.3, and the control had the same level of PPV with 28.57%, 27.52%, and 27.97% respectively.

4.4.4.2 Lipid utilization (ANLU)

Results in Table 38 show that fish utilized lipid best in the control diet (69.59%), followed by F4.2 (64.66 ± 3.22%), F4.1 (60.22 ± 4.60), and F4.3 (50.50 ± 4.89). There was no statistically significant difference of ANLU within test diets F4.1 to F4.3, but a significant one between the control feed and F4.3, an indicator that whole fish meal replacement (100%) influenced the ability of carp to utilize lipid.

4.4.4.3 Energy retention (ER)

The control group also obtained significantly higher results of energy retention than the F4.3 group (23.03% against 17.98%). However, this is not significantly higher than F4.1 and F4.2 (20.95% and 22.48% respectively). The ER of the feeding group F4.3 was the lowest (17.98 ± 1.07%) (Table 38).

Table 38: Protein, lipid, and energy retention in Trial 4

	PER %	PPV %	ANLU %	ER %
Control	2.59 ± 0.16^{a}	27.97 ± 1.22^{b}	69.59 ± 8.23^{a}	23.03 ± 2.64^{a}
F4.1	2.72 ± 0.10^{a}	28.57 ± 1.37^{b}	60.22 ± 4.60^{ab}	20.95 ± 0.94^{ab}
F4.2	2.80 ± 0.05^{a}	31.83 ± 1.39^{a}	64.66 ± 3.22^{ab}	22.48 ± 0.53^{a}
F4.3	2.66 ± 0.03^{a}	27.52 ± 0.39^{b}	50.50 ± 4.89^{b}	17.98 ± 1.07^{b}

Mean ± standard deviation; Value in the same row not sharing the same superscripts differ significantly at $P < 0.05$. SGR: Specific Growth Rate; PER Protein Efficiency Ratio, PPV: Protein Productive Value (%), ANLU: Apparent Net Lipid Utilization (%), ER: Energy Retention (%)

4.4.5 Body condition parameters

Similar results of other parameters such as CF, hepato-somatic index as well as intestine somatic index (HSI and ISI) were recorded. Control feed showed the highest results of CF and HSI (3.41 ± 0.13 and 2.08 ± 0.19 respectively). Conversely, feed F4.2 had the lowest values of CF and HSI. Although the control group provided the highest HSI, its ISI value was not the highest. The highest was recorded for the F4.3 group (6.73). However, there was no significant difference either between the control feed group and the test groups, or within the test groups.

Table 39: Condition factor, hepato-somatic index, and intestine-somatic index in Trial 4

	CF	HSI %	ISI %
Control	3.41 ± 0.13	2.08 ± 0.19	6.36 ± 0.55
F4.1	3.34 ± 0.02	1.87 ± 0.05	6.22 ± 0.14
F4.2	3.22 ± 0.13	1.83 ± 0.01	6.32 ± 0.11
F4.3	3.19 ± 0.05	1.93 ± 0.24	6.73 ± 0.65

Mean ± standard deviation; CF: Condition factor, HSI: Hepato-somatic index, ISI: Intestine-somatic index.

4.4.6 Body chemical composition

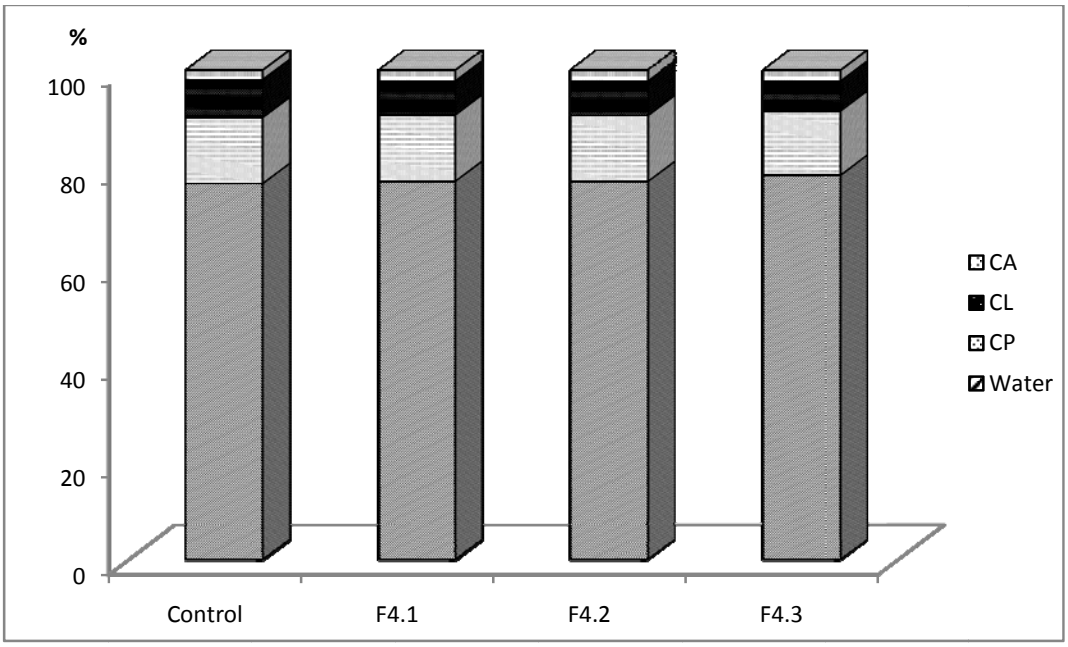

Figure 27: Chemical composition of fish at the end of trial 4

As in the previous trial, initial fish in this trial were high in CP, ash, and low in CL. On average, initial fish contained 78.6% water, 17.4% CA, and 28.6% CL. Water content of final fish ranged from 76.8 to 78.5%, slightly lower than that of initial fish. But CLs of final fish were higher than those of initial fish. Fish in the control feeding group had the highest proportion of CL with 7.8% (33.7% of DM), followed by feed group F4.2 with 7.1% (31.3% of DM). F4.3 was the lowest in body CL with only 28.6% of DM. Crude protein and crude ash of fish carcass in the trial fluctuated between 13.1 to 13.6% and from 2.0 to 2.2% of FM respectively (Figure 27).

4.4.7 Apparent digestibility co-efficient

The ADCs of some major nutrients and energy are given in Table 40. In general, digestibility of all nutrients was high. The apparent digestibility of protein of the control feed was 78.2% which represented the lowest protein digestibility in the trial. The digestibility of feed was increased when the proportion of earthworm was stepped up. Thus feed F4.3 had the highest P-ADC (84.3%), followed by feed F4.2 (82.4%) and then F4.1 (78.5%). In contrast, the apparent digestibility of lipid was inversely proportional to the amount of earthworm in the feed. The results in table 40 showed that the control feed

had the highest lipid digestibility (86.5%), followed by feed F4.1 (83.7%), F4.2 (80.3%) and F4.3 (72.8%). Energy digestibility was almost the same when the control feed, feed F4.2, and F4.3 had similar E-ADC (varying from 67.4 to 67.7%). Feed F4.1 gave the highest E-ADC (68.1%).

Table 40: Protein, lipid and energy digestibility of feeds in Trial 4

	P-ADC %	L-ADC %	E-ADC %
Control	78.2	86.5	67.4
F4.1	78.5	83.7	68.1
F4.2	82.5	80.3	67.7
F4.3	84.3	72.8	67.6

P-ADC: Protein Appearance Digestibility co-efficiency; L-ADC: Lipid Appearance Digestibility co-efficiency; E-ADC: Energy Appearance Digestibility co-efficiency

5. Discussion

5.1 Discussion on the nutritional value of local ingredients

5.1.1 Status of fish and crop production in Yen Chau

Farmers in Yen Chau rely on crop production not only as a source of food but also as a source of cash income (Steinbronn, 2009, MoFi, 1999). For many households, crop production, especially maize production is even considered as the main source of income (Keil et al., 2008, Phuong et al., 2003). However, a number of studies and observations have pointed out that the current situation in the region has many constraints which could lead to an unsustainable development of the system (Steinbronn et al., 2005a, Steinbronn, 2009). The report by Viet (2008) illustrates one of these key problems. In the harvesting season of 2008, thousands of local farming families went poor and hungry despite good harvests of maize, cassava and other crops because low market demand and resulting low prices meant that they could not cover the cost of production. Farmers suffered from a chronic shortage of ready cash for basic needs because their entire budget had been invested in plant cultivation. (Viet, 2008). In this situation, if a small proportion of these products had been processed and used as animal feed, for example pigs, poultry, and fish, farmers could have obtained their protein requirements from these animals. They could have benefited from their animals not only as food security but also as cash income by selling them in local markets where demand for these products is ever increasing.

Current fish production in the region is low. Fish production in the current system cannot meet local demand (Steinbronn,(2009)). For example, total fish production in Son La cannot supply more than 4 kg per capita per year (MoFi, 1999 and GSO, 2009b) and although aquaculture production in Yen Chau at 6 kg per capita per year was higher than the average for the whole province due to high water surface area (data from Yen Chau Statistics Office, 2005, unpublished), it was much lower than the average for the nation (MoFi, 2005, MoFi, 1999, GSO, 2009a). Nevertheless, demand for animal proteins is increasing due to the rising standards of living amongst some sectors of the population as well as an overall population growth in the region.

Local traders estimate that 200 to 350 kg of fish is sold in the local market per day, but where once Yen Chau was a fish exporter, now its market is dependent on imported fish (Steinbronn, 2009). Personal observation in this market (based on the number of traders selling fish from the lowlands to local fish traders in Yen Chau market) indicates that two thirds of aquatic products on sale in Yen Chau are not local but originated in lowland regions such as Ha Tay and Hung Yen provinces. This situation puts more pressure on the need for the development of aquaculture systems in the region. The demand could be almost met if fish productivity increased to 5 to 6 tons.ha^{-1}.year^{-1}, the average productivity of a small-scale aquaculture system described by Luu (1992), instead of the current productivity of from 1.5 to 1.6 tons.ha^{-1}.year^{-1} (Steinbronn, 2009).

Many factors need to be changed in order to improve the system in Yen Chau. Application of supplemental feed such as maize, cassava and natural food enhancement is one feasible option (Steinbronn, 2009). Availability and low price of feed materials makes it possible to develop compound feed for fish in the region. With a total of 220 hectares water surface (data from Yen Chau Statistics Office in 2005, unpublished), aquaculture in Yen Chau has the potential to produce about one thousand tons of fish if productivity reaches 5 tons/ha/year which would require approximately 1.5 to 2 thousand tons of feed annually. The amount of feed materials will not considerably influence the total crop production in the region even if the whole province uses maize and cassava for fish culture.

5.1.2 Yen Chau feed ingredients and their nutritional values

5.1.1.1 Quality and selection feed ingredients

In general, Yen Chau has high potential for the production of low cost feed based on availability of local ingredients. Analytical results showed that the quality of feed ingredients in Yen Chau is similar or even better than those from other regions, where full fat soybean and soybean cake are good samples. Protein of full fat soybean meal (steamed) in the study was 43%. This is much higher than the ones reported by Wilson (1992) -38% of DM- or in the reports of Shiau et al. (1990), and Cheng and Hardy (2003a) - 40.2 to 40.4%. The same result was found with soybean cake, a by-product from

oil extraction; soybean cake from Vietnam contained 58.6% of crude protein which was much higher than the 42.2% reported by Shiau (1990) and 52.9% reported by Nyirenda (2000).

In the current study, the proteins in full fat soybean and soybean cake were also found to have excellent amino acid composition. Full fat soybean in the study was found to contain 1.67, 0.57, and 0.53% of DM for threonine, methionine, and tryptophan respectively while Cheng and Hardy (2003a) reported only 1.47, 0.55, and 0.32% of DM respectively for these amino acids. Due to fat extraction, amino acid composition of soybean cake was even higher than that of full fat soybean.

Another advantage of using full fat soybean is that it is rich in lipid content, so that whereas other ingredients need an oil supplement to meet fish requirements, full fat soybean does not not. Therefore, instead of using large amount of fish meal and soybean cake together with extra oil supplementation, farmers could use full fat soybean directly so that they do not waste money on transportation and oil extraction. Furthermore, full fat soybean meal is a good source of some essential fatty acids such as linoleic and linolenic acids as well as phospholipids (Lim et al., 1992). Therefore, selection of full fat soybean or soybean cake should be considered carefully according to their benefit and cost. Soybean cake is cheaper, especially in comparison with its nutritive values (protein) but fish will perform better on full fat soybean. Therefore, it will be a waste of money if farmers use soybean cake with extra oil which normally costs more than full fat soybean in total. However, soybean cake is recommended when another more economic source of oil is available.

Selection of feed ingredients is an important initial step for formulating fish feed, especially in Yen Chau. To achieve a good feed, farmers in Yen Chau must be very careful selecting feed ingredients, particularly the expensive imported ingredients like fish meal. Analytical results show that some fish meals were very low in quality (protein, lipids), which farmers naturally could not have recognised. Too high a proportion of CA in the first fish meal indicated that inorganic materials had been added to that fish meal. Fish meal in Yen Chau so far is used only to supplement poultry feed. However, its quality is so poor that it should not be used for making fish feed as well as feed for

poultry or other animals. Probably, little attention on quality together with low demand for fish meal have made it less available in the region from both quality and quantity aspects.

Another fish meal purchased from a market in Hanoi which was used in the laboratory had an apparently very good chemical composition with more than 73% of CP and low CA. However, this suggested that the feed had been adulterated as such a high level of crude protein was extremely unlikely for a normal fish meal. In addition, the sum of all the components of this sample exceeded 104% which was taken as indicators of either error in analysis or adulteration of ingredients. Although the analytical methods used in this study cannot distinguish protein nitrogen from non-nitrogen protein, compositions of other samples seemed to be very accurately analysed. Therefore adulteration was the most likely scenario, particularly in Vietnam where using urea in storing aquatic products is fairly common (Anh, 2007, Hung, 2007). If this suspicion is true, fish growth in the study was definitely influenced by that fish meal.

Sweet potato leaves in the study had very high levels of crude protein, higher than those in the reports from Malavanh (2006), Dung (2002), An et al. (2003), and almost twice those in the report by Dongmeza (2009). This leaf also had an excellent profile of amino acids. Amino acids of sweet potato leaves were only lower than those of soybean meal but higher than those of all the other plant materials. Lysine, which is typically the most deficient amino acid in plant materials, was 0.89% of DM, twice that of maize and 8 times higher than that of cassava. Total amounts of cystine and methionine were the same as those of soybean. The other essential amino acids such as threonine, tyrosine, and phenylalanine were relatively high compared to those of cassava, maize and rice bran.

In Son La, maize and cassava are the most abundant feedstuffs in terms of both quantity and quality. Every year, Son La province produces approximately 450000 tonnes of maize and 270000 tonnes of cassava in which Yen Chau is one of the leading contributors (Keil et al., 2008). Analytical results indicated a low proportion of protein, lipid, and ash which were taken as an indicator of high carbohydrate content, especially for cassava meal. The study of Phonekhampheng et al. (2008) showed that total carbohydrate of cassava root meal could exceed 90% of DM. Maize starch accounts for 72 to 73% of DM (FAO,

1992). However, these carbohydrate-rich ingredients could be utilized better by omnivorous fish, such as common carp (Chiou, 1985, Fagbenro, 1999), Indian carp (Erfanullah and Jafri, 1998) and channel catfish (Tucker et al., 1990) than by carnivorous fish. In the present study, it appears that maize from Yen Chau was important not only as a source of carbohydrate but also as a good supplemental protein source because it contained a relatively high amount of crude protein (approximately 11% of DM). This result was similar to that found by Fagbenro (1999) and consistent with the study done by Bui Huy (1987) which reported that maize in northern Vietnam is similar in quality to that from other countries. The crude protein ranged from 8.4 to 12.9% of DM and its digestibility was quite high, lying in a range from 87.5 to 91.1%. Maize is limited as a feed ingredient owing to the deficiency of some essential amino acids. However, some of the normally most deficient amino acids such as lysine, cystine and methionine were rich in Yen Chau maize in comparison with those from previous reports. The lysine content of maize in present study accounted for 0.42% of DM (equivalent to 3.9 mg/g protein), higher than the1.7 to 2.8 mg/g protein reported by Bui et al. (1987). The sulphur-containing amino acids, methionine and cystine, were high as well. Bui (1987) found that methionine and cystine of maize in northern Vietnam ranged from 1.75 to 2.20% of the crude protein content, while Keeney (1970) stated that these amino acids constituted 3.08 to 3.33% of the crude protein. The current study found that 3.94% of the crude protein consisted of cystine and methionine. The gross energy of maize was (20.9 kJ/g) also higher than the 18.5 kJ/g found by Phonekhampheng et al. (2008).

Other materials such as wheat gluten, meat and bone meal also had excellent chemical compositions. The protein content of these materials was higher than that of soybean and comparable to that of fish meal, especially the protein of wheat gluten. This sample was not determined for amino acid composition but the reports from Storebakken et al. (2000) show that this material had very good amino acid composition with a high rate of amino acids absorption, from 94 to 100%. Although the lysine content of gluten was low, it almost covered salmon requirements while the other essential amino acids requirements were met even with the highest ratio of gluten replacement (50% protein inclusion). The capability of this ingredient to replace for fish meal was confirmed by the feeding trial on Atlantic salmon which showed a high performance of fish with 20% of wheat gluten in

the diets (Helland et al., 2006). The authors claim that substitution of wheat gluten could be increased to about 30% if some essential amino acids are supplied. The fact is that these materials are not currently available in Yen Chau local market, but when people pay more attention to the quality of feed materials and the demand exists, they will soon start to appear. Nevertheless, the need for these rich protein materials in compound feed is small compared to the need for other ingredients which are cheap and available at the local market. The results of the present study indicate that local feed ingredients in Yen Chau have excellent potential to be used in supplemental feed for common carp.

5.1.3 Trials on low cost feed and the necessity of low cost feed for aquaculture in Yen Chau

5.1.3.1 Water quality in the trial

The field trial was started at the beginning of the summer season in Hanoi, Vietnam. It was also the season for fish stocking after a long cold winter. Northern Vietnam at this time has characterised as a typical tropic climate, warm and humid with high rainfall. Temperature and heavy rain in this season therefore sometimes influence aquatic animals through changes of water quality.

However, most water quality parameters such as pH and temperature in the trial were in the acceptable range for fish culture (Boyd, 1982). Among all monitored parameters, only ammonia and nitrite-N can harm fish but the concentrations of ammonia as well as nitrite-N in the trial were still within acceptable range. Although ammonia and nitrite-N concentration increased continuously, the highest concentrations were only 0.2 and 0.32 mg/l respectively. These levels were not critical for common carp (Boyd, 1982).

However, many water parameters were not at optimal level for carp including water temperature. Suitable temperatures for carp range from 20 to 28°C (Horváth et al., 1992). The weekly measured temperature in the trial was not too high compared to the optimal temperature, but daily temperature in trial 4 often exceeded 30°C. High temperatures could lead to a higher metabolic rate so that fish require higher levels of dissolved oxygen in the water and waste energy on increased physical activity. High temperature can also stimulate the metabolism of micro-organisms, which include natural food for fish. But

other water quality parameters such as Secchi depth and water colour indicated that primary production was not high. The milky colour of water indicated high amounts of silt in the water instead of plankton. This was caused either by high precipitation or by carp digging for food in the net bottoms as well as tilapia in the pond. Silt can reduce water transparency and obstruct light for the photosynthesis of phytoplankton. The highest Secchi depth was only 20 cm which is considered as low. This transparency was lower than that in many reports but similar to observation of pond water colour in Yen Chau in rainy seasons (Steinbronn, 2009). Transparency was worst in the last month of the trial when Secchi depth decreased to 13 cm. Silt and detritus were washed into the pond due to heavy rainfall at that time.

Furthermore, other water parameters such as total ammonia-N and phosphate were not optimal for phytoplankton. Analysis showed that the amount of ammonia-N and phosphate were very low for the requirements for phytoplankton growth. The increase of ammonia-N and phosphate in the trial could be explained by the application of manure into the pond from the beginning of the trial. Manure was released slowly from manure bags at the pond corners. Ammonia-N and phosphate were released into the pond and maximum diffusion was achieved after 4 weeks. After the peak was reached, ammonia-N and phosphate decreased to 0.27 mg/l and 0.77 mg/l respectively. They then increased to 0.71 and 1.03 mg/l due to the accumulation of organic materials by the rain, but decreased quickly afterwards. The decrease of ammonia-N in the fourth and sixth measurements could be caused by dilution as a result of the heavy rains during this period.

pH values in the afternoon were always higher than in the morning due to the photosynthesis of plankton which consumes CO_2 and releases O_2 into water during daytime. The results of 24 hours measurement showed that the highest pH was 9.6 which exceeded the critical for carp, but during this time dissolved oxygen was in the optimal range that may reduce risk. The lowest dissolved oxygen was 2.4 mg/l at early morning (3-4 a.m.) indicating that little photosynthesis activity had happened or primary productivity of body water was not so high.

The increases of ammonia-N during the fourth and eighth week were understandable. Both of these peaks were achieved after the application of organic manure. There had also

been rain in the previous week so that small organic particles and nutrients around the pond could have been washed into the pond. The temperature increased after the rain which could have stimulated many oxidation reduction reactions which resulted in high ammonia levels in the water. The highest value of nitrate-N was 2 days after heavy rain, which could be explained by a large amount of organic material getting washed into the pond due to the rain. Basically, accumulation of waste can lead to high levels of total ammonia-N due to the oxidation - reduction reaction which releases nitrogen in water in the form of ammonia (Boyd, 1982).

5.1.3.2 Density and fertilizer

The aquaculture system in Yen Chau is normally practiced with a low density, approximately 1 fish per m^2 (Steinbronn, 2009). However, in this system, common carp is not the major fish species, therefore actual common carp density is even lower, leading to less competition within common carp. In the current study, 45 common carp were stocked in a hapa net of 9 m^2, equivalent to 5 fish per m^2. This density was not too high compared to intensive aquaculture but much higher than in semi-intensive and extensive systems. In order to obtain reasonable growth of fish, supplemental feed was offered. However, in this study observation indicated that benthos in the pond bottom was very limited. There was almost no benthos found in the net bottom after the second week. If carp were reared with lower density, they would probably perform better due to greater availability of natural feed. Availability of natural feed was almost certainly affected by the tilapia present which was at a stage requiring mostly natural feed, although artificial feed was applied.

Fertilization management

In this trial organic fertilizers were applied to the pond to maintain the growth of natural feed. The first application of fertilizer was made at the beginning of the trial and another in the middle of the trial (6^{th} week). The amount of natural feed available to the fish was not quantified in this trial but the effect of fertilization could be observed through water parameters such as nitrate-N, ammonia-N and phosphate-P. According to the results on these parameters, the first fertilization changed the quality of pond water. Ammonia-N, phosphate-P, nitrate-N increased steadily from the beginning of the trial. This change

accompanied a change in the colour of the water to green, indicating the development of plankton. The second application did not show immediate effects. Pig manure was added at the sixth week but its effect was expressed only from week 7 through phosphate-P and from week 8 through ammonia-N concentration. The ammonia-N concentration even decreased between the 6^{th} 7^{th} weeks. In general, rain can wash away or bring more organic matter into the ponds which leads to a corresponding decrease or increase of ammonia-N. In our case, the maximal concentrations of these parameters produced by the pig manure were still lower than those caused by the other fertilisers that had been added previously. Water in one pond was also influenced by water in the next pond, but since these two ponds were originally one pond and the same managements were applied to both ponds, there was assumed to be no difference between the two.

Growth of fish through feed made from local materials

The field trial (Trial 2) was set up at beginning of 2008 when the world food crisis was occurring. All imported feed ingredients became very expensive and some were extremely scarce by that time, for example wheat meal and fish meal. Therefore ingredients for the control had to be changed; polished wheat bran was used instead of wheat flour. Proportions of other ingredients were adjusted to balance the content of lipid and protein in the feed. These modifications could have led to changes in some fatty acid and amino acid composition which may have affected the growth of fish in the control group.

In spite of being fed similar feed, SGR of fish in the group F2.1 was (2.1 ± 0.2%) slightly higher than that of F1.3 in Trial 1 (2.0 ± 0.1%). Also, for fish in the group F2.1, weight gain was higher and other feed utilization of other feeds was more efficient than those of F1.3. Data showed that feed utilization in the field trial was higher than that in the laboratory by approximately 30%. Lipid retention (ANLU) especially in Trial 2 was very high in comparison with that of Trial 1. Only energy retention of Trial 2 was lower than that of Trial 1. The fact is that only one replicate of this group was lower; the other two had better results of ER, varying from 19.2 to 19.9%, higher as compared to that of F1.3.

In general, fish fed on low cost feed grew well. All parameters such as SGR, PPV ANLU, and ER showed that feed was utilized at relatively high levels although they were still

lower than those in some previous studies. For example, Nandeesha et al. (2002) claimed that common carp can achieved a growth rate above 4%.day^{-1}. But these authors conducted the experiment with very small common carp (0.31 ± 0.07 g). Another study, Ali and Mohammed (2002), who carried out an experiment with similar initial size (~11 g) of common carp also reported a high specific growth rate of fish, approximately 2.6 to 3.0%.day^{-1} but fish in that study were raised at a lower density (2.5 fish m^{-2}). Furthermore, common carp was not the majority species in the pond, but at the low density of 0.2 common carp m^{-2}. Moreover, fish were fed at higher feeding rates. In similar conditions, growth rate of fish in the current study was higher than that of many others. Growth rate was higher than that of tilapia or Indian carp in the study of Asgah and Bedawi (Asgah and Bedawi, 1984) in which common carp fed on low-cost feed could gain only 0.1 g.day^{-1} in spite of 43% of CP in feed. Magdy (2006) reported that SGR of Nile Tilapia (*Oreochromis niloticus*, L) fed on sources of plant meal supplemented with Mojave yucca (*Yucca schidigera*) fluctuated between 0.9 to 1.2% day^{-1}. Another study, Latif et al. (2008) reported that Rohu (*Labeo rohita)* fed on low-cost diets with similar protein composition (30%) could gain only 0.28 to 0.42% day^{-1}. Rothuis et al. (1998) pointed out that common carp stocked in a rice field integrated system gained 0.17 to 0.37% day^{-1}. However, the most important is the comparison with that of the current system in Yen Chau. It is evident that fish in the current study grow far better than that in the poly-culture system in Yen Chau described by Steinbronn (2009).

Fish in the current study were not expected to have the best growth rate since protein content was lower than the optimum level of 35% (Jauncey, 1981) to 38% (Ogino et al., 1970). It is also usually accepted that fish fed on local ingredient feed grow less than fish fed on the control feed while a number of nutritionists have claimed that fish meal is the most suitable protein source for fish growth due to its good balance of amino acids (Fagbenro, 1996, Degani et al., 1997, Degani, 2002). Usually, the higher proportion of fish meal in the diet (control feed) the better the growth performance of the fish (Boonyaratpalin et al., 1998, Asraf Mohamed et al., 2007). However, our data show that feed utilization in the study was relatively high. Cho et al. (2001) report that the protein efficiency ratio of common carp changed from 1.74 to 1.92 depending on the feed allowance and protein – energy ratio (protein fluctuates from 35 to 45% and GE/P from

8.4 to 10.8), while protein retention (PPV) was in the range 22.5 to 26.5%, but these results are lower in comparison with those of the current study.

Growth of experimental fish can even be significantly improved if some disadvantages are minimized. As mentioned above, the tilapia fingerlings living in the pond could have influenced fish growth. The number of tilapia might be cut down but the total biomass increased remarkably, especially in the second month. Assuming that the survival rate of tilapia was only 60%, the number of fish in the middle of the trial was approximately 25000 fingerlings. At this time, tilapia might consume most of the available natural food. Furthermore, the high density of tilapia could be the reason for the milky colour and low Secchi depth of the pond water. If water quality could be improved (perhaps through manure application and water management), fish growth would be enhanced.

Concerning the change of ingredients of the control feed in Trial 2, the composition of polished wheat bran was normally better than that of wheat bran, especially in some essential amino acids like valine, tyrosine, threonine and lysine (NRC, 1993). Consequently, composition of the amino acids of the control feed in Trial 2 should be superior to that of the control feed in Trial 1. However, non-digestible soluble compounds in the form of pentosans may inhibit the digestibility of wheat bran (Rakowska et al., 1989), and a high proportion of fibre in the diet could be a cause of growth reduction in animals (Anderson, 1991). Plant protein-based feed can also be poorer in some amino acids due to the low essential amino acid composition of its ingredients.

In the current study, the proportions of most essential amino acids in local ingredient feed (F1.1 to F1.3) were not much lower than those in the control (Table 16). Only phenylalanine (/tyrosine) and valine were lower while the others were similar or even higher than those in the control feed, for example leucine and histidine. The results suggested that essential amino acid content was not the most important factor in indicating the superiority of diets. However, although local ingredient feeds were not poor in amino acid composition, the energy cost of these feeds may be high due to influence of high fibre content on digestibility (Erfanullah and Jafri, 1998). Dongmeza et al. (2009) observed that the metabolic cost of poor digestible feed was higher than that of more digestible ones. Kaushik (1995) found that fish growth performances differ greatly

according to variety of protein sources even if they have high apparent digestibility (> 87%). The different compositions of essential amino acids and their availability in the diets could be the reason for this. Generally, animal proteins give better fish growth than plant proteins.

To sum up, fish can utilize local ingredient feeds quite well, especially under pond conditions where natural food is also available, resulting in better growth performance of fish in comparison with the system currently in use in Yen Chau. This shows there is great opportunity for improving fish production through development of local feed.

Fish composition

In the current study, the sum of chemical composition (CP, CL and CA) in both trials (trial 1 and 4) was close to 100% of the total DM, indicating that these analyses were done to a high degree of precision and that the data was reliable. Initial tests on fish showed lower results for fat but high protein and ash contents. The differences in the body chemical composition between initial and final fish could be the results of various culture conditions. Initial fish were kept at high density in a tank or net and fed at the maintenance feeding level, which did not provide any nutrients for growth.

Deficiencies of nutrients can lead to inadequate growth or compromised liver and intestine formation. However, such were not observed in either trial (Trial 1 and 2). Moreover, no significant difference was found when the fish chemical composition was analysed, indicating that the body mass of fish increased regularly with total body length.

Cost efficiency and the role of local feed in the development of fish production

Cost efficiency is the most important factor that farmers always consider since it relates not only to their liquidity but also directly to their income. Phuong et al. (2007) report that feed cost accounted for 74 to 93% of total production cost in some fish farms in south Vietnam, and that feed cost is more problematic for traditional system farmers than intensive system farmers because poor farmers can only afford the low investment characteristic of traditional farming rather than high cost cultivation. As discussed above, fish meal plays a very important role either in feed nutrition or in economic terms. However, due to its high cost, increasing the level of fish meal in the diets proportionally

increases the cost of feed. It was still used for the control feed of Trial 2 but imported wheat meal was replaced by cheaper ingredients. The cost of this feed was still the highest in comparison with that made from local material feeds.

The FCR parameter is also very important even though it does not relate directly to the benefit of farmers, but indirectly through feed cost efficiency. Fish fed on diets lower in fish meal gave lower growth rate, resulting in higher FCR and vice versa. Relation between FCR and cost efficiency is visually presented in Figures 26 and 27. In the current study, low cost diets show better efficiency when they could lead to lower cost of feed as well as lower cost per unit of fresh fish weight produced. In contrast to the decrease of FCR, cost efficiency increased linearly. The feed cost per unit of fish produced was least in feeding group F1.3 (12900 VND) while the control feed had the highest cost of feed (18300 VND) as well as the cost per unit of fish produced (23100 VND). As can be seen in Figures 28 and 29, only 1.4 kg of feed F2.1 was needed to produce 1 kg of live fish but 1.7 kg of feed F1.3 to produce same amount of fish. These levels of FCR are in the average range for semi-intensive systems in Vietnam (Hung, 2004).

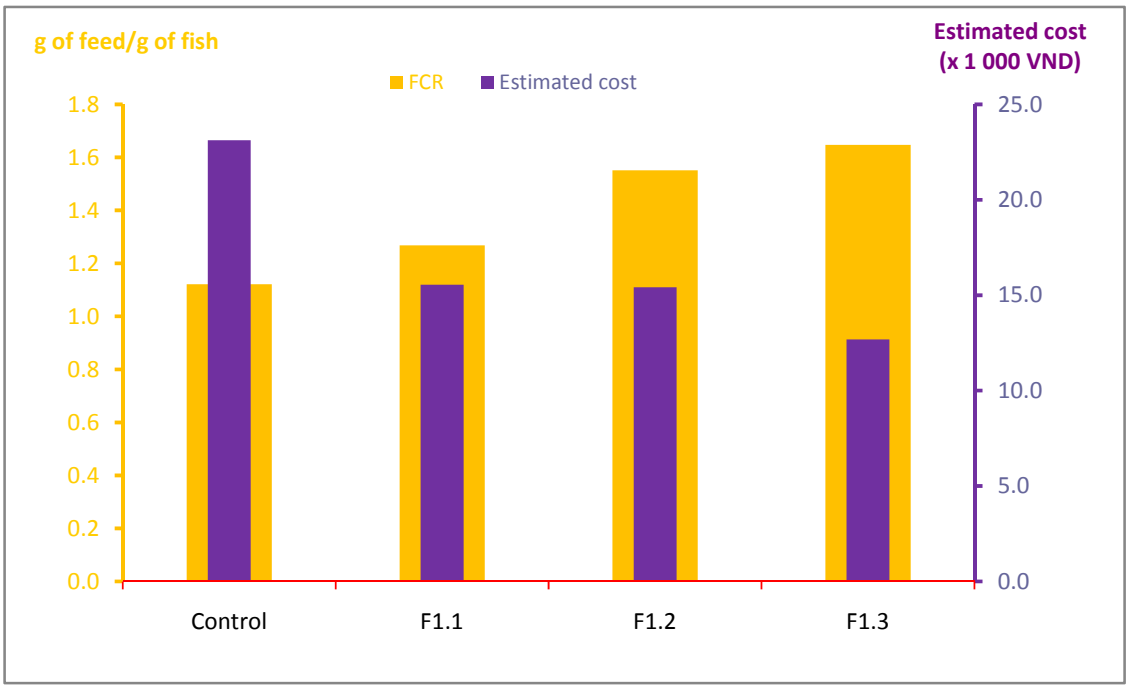

Figure 28: FCR and estimated feed cost per kg of product of the experiment

The lower FCRs in the field trial compared to those of the laboratory trial reflected the association between supplemental feed and natural food. The results indicated that fish in

the field trial either utilized feed more effectively than that under field conditions or contribution of natural feed had positively influenced fish growth and feed utilization. Supplemental feed is economical in the balance between quality of feed and natural endogenous food within the pond (Tacon and De Silva, 1997). Tacon and De Silva (1997) also found that a sub-optimal supplementary feed is less utilized with decreasing apparent efficiency as the natural food availability falls. Similarly, Akiyama (1993) pointed out that the use of sub-optimal quality feed resulted in satisfied yield compared to that obtained by high quality feed. Thus, if fish are cultured in lower density under better water management to improve the availability of natural food, fish can perform even better.

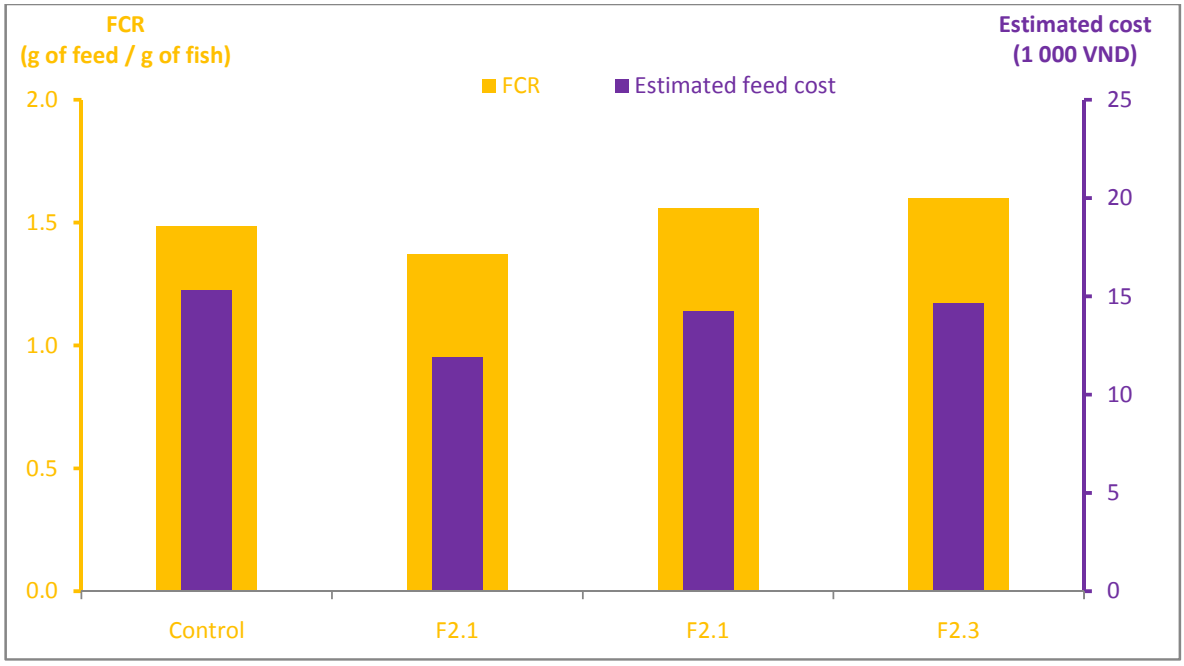

Figure 29: FCR and estimated feed cost in the Trial 2

In the context of Yen Chau, there are then possibilities for the development of low cost feed in the region. So far, disease and limited fresh leaf availability make it difficult to expand aquaculture through herbivorous grass carp (Steinbronn, 2009), forcing farmers to enhance fish production through improving pond inputs. However, the high price of fish meal and commercial feed make it impossible for poor farmers to use these materials (Chong, 1993). Thus, this expensive feed may be more suitable in the production of high value fish species. Although manufactured feed gave the highest gross return, its net return was the lowest (Phuong et al., 2007). On the other hand, low cost feed showed

satisfactory growth of fish and enhancement of benefit from fish culture. Furthermore, when we compare the cost of feed in this study with the actual price of fish at the local market; it is evident that farmers can achieve great benefits because these costs are far lower than the price of fish (from 50000 to 70000 VND/kg). Chong (1993) claimed that it is relatively cheaper for farmers to prepare fish feed themselves than to buy commercial feed. Similarly, Luu (1993) showed that prices may be 15-20% less if products are purchased locally and in large amounts. Therefore, if farmers use the materials which were produced themselves, the cost of feed can be even cheaper. Future aquaculture development can strongly contribute to alleviating poverty and assisting rural development.

5.2 Comparison of different protein and carbohydrate sources

Increasing demand, high costs and the uncertain availability of fish meal make looking for alternative protein sources a priority. A number of plant proteins have been tested for fish meal replacement, for instance, soybeans, ground nuts. To date, there are no publications on the use of grain for fish feed formulation in Yen Chau.

5.2.1 Comparison between full fat soybean and soybean cake

Full fat soybean seems to be superior to soybean cake - a by-product of soybean oil production. Protein content of soybean cake is naturally higher than that of full fat soybean due to oil removal. However, the amino acid composition of soybean cake is not better than that of full fat soybean meal as can be seen if concentrations of amino acids are converted to the proportion of protein. Table 42 shows that concentrations of many amino acids in soybean cake are obviously lower than those in full fat soybean meal, for instance methionine, leucine, isoleucine, lysine etc. The quality of protein in soybean probably changes during oil extraction owing to the high temperatures and chemicals used in the extraction process which influence amino acid composition (Lim and Akiyama, 1992, Snyder et al., 1987, Ljøkjel et al., 2000).

Consequently, fish fed on soybean cake did not give better growth than full fat soybean. Fish fed on full fat soybean-incorporated feed reached 11.9 g which was significant higher than fish fed on soybean cake (10.1 g). The low growth rate of fish in the group F3.3 could have been affected by crude protein level in the feed. Although both of them were calculated for the same amount of crude protein, actually Feed F3.3 contained only 28.3% of crude protein while feed F3.4 and the control feed contained respectively 2% and 4.5% more than that. If the feed F3.3 contained the same crude protein as the feed F3.4, the difference of growth between two groups would be smaller.

Table 41: Essential amino acid composition of full fat soybean and soybean cake (% of CP)

	Full fat soybean	Soybean cake
Threonine	3.82	3.05
Valine	3.96	1.09
Cystine	1.49	3.63
Methionine	1.30	1.14
Isoleucine	3.52	3.33
Leucine	7.00	6.18
Tyrosine	3.16	2.68
Phenylalanine	4.39	4.04
Histidine	3.46	2.51
Lysine	5.35	5.05
Arginine	7.05	5.77

Moreover, the growth of fish on these feeds could have been affected by lipid quality. More than half of the total lipids in the feed F3.3 were substituted by extra sunflower oil while F3.4 lipids originated from soybean. Many reports state that soybean is rich in

phospholipid polyunsaturated fatty acids such as linoleic (18:2n-6) and linolenic (18:3n-3) (Regost et al., 2003, Lim and Akiyama, 1992). In contrast, these fatty acids are limited or unavailable in sunflower oil (Lim and Akiyama, 1992). Both linoleic and linolenic fatty acids are essential for carp (Takeuchi and Watanabe, 1977, Watanabe et al., 1975). Grisdale-Helland et al. (2002) and Sargent et al. (1999) suggest that composition of fatty acids in the diets not only affects fish growth but also influences lipid utilization as well as the body fatty acid composition of fish. This suggestion received support from our results which show statistically significant differences in both ANLU and ER between F3.3 and F3.4 feeding groups.

However, cost efficiency is more important for farmers. Since soybean cake costs less than full fat soybean, farmers have to consider carefully the benefit of using full fat soybean or using soybean cake with additional oil supplement.

5.2.1 Comparison between maize and cassava as carbohydrate sources

The intensification of fish culture needs not only improvement of nutritional input but also consideration of its economical benefits because many feedstuffs continually increase in price. Carbohydrate-containing feedstuffs are available at low prices (Krogdahl et al., 2005, Ufodike and Matty, 1983, Tan et al., 2006). Therefore, using inexpensive locally-available ingredients seems to be a feasible option to improve fish production. Although no level of carbohydrate requirement has been given (Wilson, 1994), adequate levels of non-protein and energy sources in the diets can optimize economically the utilization of protein (Fagbenro, 1999, Mohapatra et al., 2003, Wilson, 1994). The formulation of cheap diets consisting of locally available feedstuff, especially carbohydrate-rich ingredients, could significantly reduce costs in carp production (Keshavanath et al., 2002, Fagbenro, 1999). On the other hand, carp have the ability to utilize higher levels of carbohydrate than carnivorous fish, particular in comparison with cold water fish (Keshavanath et al., 2002, Wilson, 1994). Forneris et al. (1993) reported that fish utilize carbohydrate especially better in the form of gelatines. In another study, Krogdahl et al. (2005) found that the presence of α-amylase, the most important enzyme for carbohydrate digestion in the liver, intestine, and bile of common carp was far higher than that in other fish species,

such as goldfish, sea bream and trout. Vietnam in general and Yen Chau in particular have a variety of feed ingredients which are relatively cheap and rich in carbohydrate and energy, such as maize and cassava (Steinbronn, 2009). However, so far not little has been published on the use of these materials for the improvement of fish production systems in thse regions.

Feed F3.1 and F3.2 in Trial 3 were formulated to compare two different carbohydrate sources - maize and cassava - in the growth and feed utilization of common carp. Carbohydrate in cassava and maize consists of 72 to 73% of starch. The other portions can be sugar (glucose, fructose) which take about 3% of chemical composition (FAO, 1992). The results in Trial 3 showed that fish fed on a cassava carbohydrate diet grew at a rate similar to those fed on the maize substitution diet. Although all parameters such as SGR, PPV, ANLU and ER seem to be lower in the cassava groups, they were not statistically different from that those of maize inclusion diets. Despite the lower growth rate in comparison to the control group, fish fed on cassava and maize performed better than those fed on many other feeds in Trials 1 and 2 such as F1.2, F1.3, F2.2. or in comparison with earlier reports of Keshavanath et al. (2002) who report that, as in our study, sources of carbohydrate do not alter feed conversion ratio.

Many authors have claimed that digestibility of carbohydrate diets varies depending on different factors such as sources, levels of incorporation and fish species (Krogdahl et al., 2005, Wilson, 1994). However, digestibility coefficients in the present study indicate that both of the two feeds seemed to be well digested and equally utilized for all proteins lipid and energy in line with the earlier investigations of Fagbenro (1999), who claimed that the digestibility of protein, and the energy of fish was similar for many different cereal grains such as maize, rice bran, wheat middling etc. although their composition and proportion were not uniform. However, nutrient efficiencies were slightly higher in the fish fed on a maize inclusion diet.

The utilization of cassava seems to be not so different from maize when these materials are incorporated with other highly digestible protein ingredients, but it is poorly utilized when combined with poor quality digestible materials, such as sweet potato leaves in this study. All the sweet potato leaf inclusion feeds showed lower digestibility coefficients. It

was even worse in feed F3.6 which had no full fat soybean or maize powder but had a high incorporation rate of cassava. Although our statistics do not show any impact of the ingredients on fish growth, differences between these groups seemed to increase until the end of the trial. If the trial had lasted for a longer period of time, significant differences might have been proven.

Many researchers claim that the proportion of carbohydrate in the diets affects carcass chemical composition (Ufodike and Matty, 1983), but this was not observed in this study. All fish seem to be very similar in protein, lipid, and ash composition. Feeding high dietary carbohydrate can lead to increased liver weight of animals (Hilton et al., 1982, Walton, 1986, Hutchins et al., 1998) but observation in the current study could not find much change of this factor. However, the proportion of the intestine of fish fed on cassava inclusion diet was similar to that of the control group but appreciably higher than that of maize-containing diets.

Growth and feed utilization then do not differ, but the costs of these materials do. In the local market cassava is half the price of maize. Therefore, the incorporation of cassava seems to be more cost-effective than using maize. Farmers in the region can also improve the digestibility of these materials by simple pre-processing such as steaming, especially for cassava, because it not only enhances the availability of nutrients but also reduces possible anti-nutrient factors in cassava (Erfanullah and Jafri, 1998, Wilson, 1994, Krogdahl et al., 2005).

5.2.1 Sweet potato leaves and the use of sweet potato leaves

Sweet potato is a traditional plant in many regions (Giang et al., 2004), used as food for humans and other animals such as pigs, poultry, and fish (Keutgen et al., 2008, Backer et al., 1980). It is also an important income source for farmers (Luu, 1992). This multi-use plant has a wide environmental growth range and adapts to many different soil types including harsh conditions where many plants may not grow (Ramirez, 1992, Abidin et al., 2005). It is widely grown in tropical, subtropical and temperate regions in 111 countries, 90% of which are developing countries, and is ranked as the seventh most

important plant after wheat, rice, maize, potato, barley, and cassava (Keutgen et al., 2008).

Sweet potato can be planted in combination with other plants such as maize, cassava and benefit farmers not only as cash income, but also in terms of soil protection (Aladesanwa et al., 2008). Because it lies and climbs on the soil surface, sweet potato is a soil cover which protects soil from erosion, reduces nitrate-N leaching (Islam et al., 1994), and reduces water evaporation as well as protecting other plants and animals living on the soil surface (Akobundu, 1984, Ogbonna et al., 2007). It seems sensible to develop this plant in Yen Chau where erosion of steep soil is becoming one of the biggest constraints to the whole system of production (Steinbronn, 2009, Friederichsen et al., 2000). Benefits to farmers should be balanced with other sustainable environmental factors including soil protection. Thus, if this plant is developed in Yen Chau region, it could supply humans and animals with food. However, in spite of its importance, so far, few investigations into the nutrition of sweet potato leaves as well as its use in the region especially for fish have been published.

Sweet potato could have great potential in Vietnam ((Phuc, 2000). All parts including the leaves can be used as human and animal feed, fresh, dried or otherwise processed. In the current study, sweet potato leaves (SPL) had a high CP content (32%), more than many other plant leaves such as grass, cassava leaves, banana leaves (Dongmeza et al., 2005, Dongmeza, 2009), and peanut leaves (Mario and Miguel Ángel, 2008). The analyzed value in this study was also higher than the protein content of SPL in many previous reports (Dongmeza, 2009, Ty et al., 2007, Dongmeza et al., 2005, Adewolu, 2008, Antia et al., 2006). Analytical results show that this material contained large amounts of neutral and acid detergent fibre (NDF, ADF), 37.4 and 24.6% respectively. These fibres and the energy content of SPL in this study were similar to those in the study of Dongmeza et al. (2005). Although the fibre content of sweet potato is relatively low compared to that from other plants (Ishida et al., 2000, An et al., 2003, Woolfe, 1992), the leaves contained quite high amounst of indigestible crude fibre, ranging from 7.2 (Antia et al., 2006) to 8.3 (Adewolu, 2008).

The amino acid content of SPL in the present study were higher than those of Keutgen et al. (2008). Most of them were, however, lower than those reported by An et al. (2003). Lysine, leucine, methionine especially seem to be very limited in this material. Due to these low concentrations, the amino acid content of the whole feed (F3.5 to F3.7) was low and most of the amino acids in these feeds were too low for fish requirements. It was, however, interesting that the sweet potato-containing feed had higher levels of amino acid than those of the control.

The common problem in using alternative protein is the palatability of the feed (Rodríguez-Serna et al., 1996, Drew et al., 2007). In the present study, fish ate all the feed applied quickly, indicating that palatability of feed was not influenced by SPL. However, fish fed on SPL feed grew significantly slower than those fed on the other feeds. Not only the growth rates but all other parameters such as PER, PPV, ANLU as well as ER of SPL groups were lower than those from the control and non-SPL feeding groups. The values of PPV in this trial ranged from 23.5 (in the group F3) to 28.8 (in the control group) while fish in the last trial converted up to 42% of protein (control feeding group). The combination of the two worst ingredients, SPL and cassava powder, made it the worst feed. Fish in this group grew least. The results are consistent with the study of De Melenaere and Feldman (1960) who claimed that the nutritional quality of a protein depends not only on its amino acid composition and specific amino acid requirement but also on the availability of the amino acids.

The high amount of indigestible fibre in SPL could also have affected feed utilization as well as fish growth. In addition, fish in this trial were smaller than in previous trials and therefore less able to cope with a high fibre diet. The availability of nutrients for fish may be lowered if the material is dried rather than in fresh (Dongmeza, 2009). Some dried plant leaves even have negative digestibility in grass carp. Dongmeza suggests that energy lost as heat due to metabolism is higher in fibre-rich feeds. Feed utilization by the fish in our trial seemed to be less efficient than in tilapia (Adewolu, 2008). Other authors also reported that tilapia can digest and utilize peanut leaves (Mario and Miguel Ángel, 2008), cassava (Keong and Leong, 1989).

The low growth rate in the trial could also have been influenced by the presence of anti-nutrient factors. Although analysis of results showed that there was tannin in the leaves, much could be deactivated when it was dried. Francis et al. (2001a) and Siddhuraju and Becker (2001) claim that reduction of anti-nutrients by different processing techniques can enhance feed palatability. However, a large number of other anti-nutrient factors were not determined. Almazan (1996) reports that SPL contained high amount of tannic acids, oxalic acids, phytic acids. Tannic acid concentrations in untreated SPL varied from species to species and ranged from 590 to 1050mg/g. Oxalic acids were from 300 to 490mg/g and phytic acids were from 280 to 380mg/g. Tannic acids and oxalic acids could be reduced up to 70% by blanching. However, blanching can reduce only small amounts of phytic acids in SPL (10-40%). Antia et al. (2006) claim that SPL contain also about 30mg/100g cyanide. SPL in this study were not treated except for drying. Thus, high amount of anti-nutrients presented in the feed could be an explanation for the low growth rates of fish. Cooked SPL is recommended for better digestibility in fish.

In general, deficient diets can lead to changes in body proportions reflected in change of CF and imbalanced diets can cause significant changes in HSI as low feed quality leads fish to produce longer (heavier) intestines to increase nutrient absorption. But in this experimen fish showed no significant change; data in Table 34 indicate that different feeds in the experiment did not influence CF, HSI and ISI of fish. In other words, body condition of fish in the experimental groups was similar to fish in the control group.

The result is also consistent with other studies indicating that fish can utilize protein, lipids and energy from fish meal much better than from plant ingredients. (e.g. Morales et al., 1994; Sudaryono et al., 1999).

5.2.2 Earthworm, a potentially economical source of protein

5.2.2.1 Earthworm chemical composition

Earthworms have been used as human medicine for a thousand years (Titov et al., 2006) and were recorded as a potentially economical source of protein since the beginning of the 1980s by Hartenstein (1981a). In the current study, earthworms appeared to be one of the best protein sources among all collected feed ingredients. Chemical composition results

showed that the protein content of earthworms was much higher than that of all plant sources and many animal protein sources such as termite meal (Sogbesan et al., 2008a, Sogbesan et al., 2008b) or even higher than the protein content of many fish meals. It was even higher than the average range for earthworms (from 50 to 59%) given by Tacon et al. (1983) or from 50 to 67% in the studies reported by Tacon and Jackson (1985), Hilton (1983), and by Stafford and Tacon (1984).

Though there are many studies on the use of earthworms, to date there are few available references on their amino acid composition. Pokarzhevskii et a. (1997) determined amino acid composition for several different earthworm species, but *Perionyx excavatus*, used in our study, seems to be superior. Most amino acids of earthworms in the present study were higher than those in many previous reports. Lysine, the most deficient amino acid, is one example. Except for that of *L. terrestris* in the study by Pokarzhevskii et al. (1997) (7.2%), earthworms in the current study consist of 4.7% lysine, which is similar to the lysine level of *N. caliginosus and E. nordenskioldi* but higher than that of all the other species in Pokarzhevskii et al. (1997) and *Metaphire canifornica* (Xiang et al., 2006). Earthworms in the present study were also rich in methionine. Concentration of methionine in earthworms *(P.excavatus)* was 1.3% while it was reported as less than 1% for most earthworm species (Pokarzhevskii et al., 1997). Our *P. excavatus* especially contained a high amount of cystine, 0.9%, while it was only 0.2% for *L. terrestris* and 1.6% for *L. rubellus* (Pokarzhevskii et al., 1997). The results of amino acid composition of earthworms in our study differed from those in the study of Hilton (1983) who implied that the amino acid composition of earthworms was imbalanced, leading to reduction of feed intake and growth of fish. However, the protein content of earthworms can be different from species to species (Dynes, 2003), and the composition of amino acids in the current study indicates that most amino acids, especially the essential amino acids of earthworm meal in the study were higher than those in fish meal. This excellent nutritive profile makes it very likely that the inclusion of earthworms in fish diets will produce a high growth rate.

5.2.2.2 Trial on replacement of fish meal by earthworms

The high proportion of protein in earthworms resulted in a high content of almost all amino acids in the diets, and data showed that amino acid composition of the feed in Trial 4 increased proportionally with the level of earthworm inclusion (Table 21). However, despite the fact that earthworms have such a beneficial composition of amino acids, the content of many amino acids in the feed was still low. Except for leucine, all amino acids were below requirement levels for common carp (NRC, 1993). This could be because the proportions of ingredients in the feed in the current study were calculated to give a lower level of protein. Protein in the feed was less than the optimal level for carp (38%; NRC, 1993a). Therefore, if the feed were formulated to give a higher level of crude protein, the amino acid composition of the feed would be improved. However, some amino acids such as cystine/methionine, phenylalanine/trypsine, and lysine would not be met even if the protein level of the feed reached the requirement level of carp. Thus it is difficult to meet fish requirements using earthworms as the unique protein source. In order to reach maximum growth, earthworms should be used in combination with other protein sources.

In the current study, fish in the group F4.2 grew best with the highest growth rate, SGR as well as the highest protein conversion (PPV), in accordance with many previous reports that earthworms can be an excellent alternative protein source to replace fish meal. Basically, better feed ingredients give better performance. Earthworms have a better quality of protein, resulting in higher growth rate of fish as well as more efficiency of feed utilization than those of the control. Similarly, the results of PER in all treatments (F4.1 to F4.3) were higher than in the control feed. Only the PPV of F4.3 was slightly lower than the control whereas the other groups gave better results of PPV compared to that of the control. This result is consistent with the results of digestibility co-efficiency; the higher proportion of earthworms in the diet gave the higher protein digestibility co-efficiency.

However, growth rate of fish in the present study was lower than that in the study of Nandeesha et al. (1988). The highest SGR in the current study was 2.3% day^{-1} while the lowest SGR achieved in trial by Nandeesha was 4.8%.day^{-1}. The difference may be because of the different initial sizes of fish. Nandeesha started with very small common carp (0.9 g) while initial fish in the current study were 8 g. Another possible factor is the feeding rate. Nandeesha et al. fed fish with 5% of body mass instead of metabolic body

mass as in the current study. The different formulae used for calculation led to a big difference in the amount of feed, especially when the fish were small. Moreover, feed in the study by Nandeesha had higher protein content: 31-32% of CP, while current study feed contained less than 28% of CP.

Theoretically, feed F4.3 which contained a higher ratio of amino acids should show a higher growth rate of fish than the other groups. But fish growth results show that fish in the F4.3 group grew significantly slower than those on feed F3.2. In addition, the digestibility of feed increased proportionally with the level of earthworm in the diets, suggesting that the low growth rate of fish was not only due to the amount and quality of the protein.

As in earlier studies (Nandeesha et al., 1988, Tacon et al., 1983, Stafford and Tacon, 1984) our study showed that total replacement by earthworms of fish meal did not bring the highest growth even though earthworms hold a better nutritive profile. In the trial by Nandeesha et al. (1988), the superior feed was the feed including 25% earthworms. However, those results could have been strongly influenced by the addition of 5% of sardine oil in the diet since Espe et al. (2006) report that a few percent of fish oil in the diet can improve palatability leading to an increase in feed intake or in fish growth. In our study, fish did not grow best in the total replacement group (F4.3) but in the group F4.2 with 70% of crude protein replacement. This level is equivalent to 20% dietary dried matter. Therefore, our results accord with earlier studies (Tacon et al., 1983, Hilton, 1983, Nandeesha et al., 1988) that total replacement of earthworms in the diets could lead to a reduction of fish growth. Tacon et al. (1983) found that the best growth of fish was achieved with below 30% earthworm replacement.

Why should total earthworm replacement cause lower growth rates? Tacon et al. (1983), Andrews and Kukulinsky (1975), Edwards and Lofty (1977) suggest that the yellow fluid in earthworms could make feed unpalatable to fish when the feed contains a large amount of earthworms, and consequently decrease feed intake. However, other reports state that earthworms make excellent fish bait, indicating high attractiveness and palatability. In fact, fish in the current study ate all their feed instantly during the whole trial. The appetite of the carp fed on different diets was uniform at the beginning. On the other hand

report of Nandeesha et al. (1988) claimed that earthworm should be cooked before being incorporated into feed pellets. Generally, yellow fluid which is mainly protein (Kauschke et al., 2007) can be deactivated by heat treatment. Nevertheless, total replacement of earthworms for fish meal did not achieve better results than the feed with 25% earthworm inclusion. This indicates that fish growth is influenced not just by yellow liquid but also other factors such as lipid content or mineral composition.

The results show that the lipid composition of earthworms could strongly affect fish growth. To date, little is known of the lipid composition of earthworms, especially *P. excavatus*. Paoletti et al. (2003) report that two earthworms *Andiorrhinus kuru* and *Andiorrhinu motto* appear to be insufficient in triacylglycerols which are necessary for many different cells in energy storing, resulting in a low proportion of fatty acids in earthworms. In contrast, Holmstrup et al. (2007) show that *Dendrobaena octaedra* contain high amounts of long-chain unsaturated fatty acids (20:n and 18:n). Thus the composition of fatty acids in earthworms seems to be very complex and different from species to species. However, in the current study, increased level of earthworm incorporation in diets led to proportional reduction of L-ADC and E-ADC. Moreover, results of lipid retention (ANLU) showed that full replacement achieved the lowest lipid utilization of feeding group F4.3. As mentioned above, other lipid sources such as fish oil need to be added to earthworm to improve fish growth (Nandeesha et al., 1988).

Other factors which influence fish growth are the vitamins and minerals in earthworm flesh. Earthworms seem to be deficient in vitamin A. Dayna et al. (1998) report that mineral concentrations in earthworms (*Lumbricus terrestris*) are much higher than in other invertebrates, particular concentrations of iron and manganese, and indeed, earthworms prefer to live in waste materials in which the presence of heavy metals, detergents and parasites is high. There are high concentrations of many chemicals accumulated in earthworms living in polluted soil such as fluoride (Vogel et al., 1991), iron, zinc, lead, cadmium (Stafford and Tacon, 1984, Khwairakpam et al., 2009, Paoletti et al., 2003). These materials can contaminate diets with high levels of earthworm incorporation and so impair fish performance (Stafford and Tacon, 1984). However, the quality of earthworms is also strongly influenced by the living environment, especially by the quality of soil or organic matter in the soil (Xiang et al., 2006).

6. Conclusions and outlook

The current study evaluated the possible feed ingredients available in Yen Chau district, Son La province, Vietnam. Compound feeds were tested under laboratory as well as field conditions. The carbohydrate and protein in feed ingredients were investigated. The study was conducted in order to meet the requirements of farmers as regards supplemental feed to develop fish production which has become increasingly important as farmers tend towards the adoption of polyculture which includes more omnivorous carp in the system.

Local feed ingredients in the study showed high potential for formulating fish feed. Using maize and cassava in Yen Chau seems to be the most feasible due to their availability and cheapness. Besides fish meal, many other ingredients can be used as a source of protein such as full fat soybean meal, earthworms, meat meal, as well as soybean cake. All of these materials have good nutritive profiles of protein, lipid, ash, and energy content as well.

Sweet potato leaves, in spite of having an excellent chemical composition, did not give good growth of fish. Fish can digest this material but they seem to suffer from nutrient deficiency. Probably high energy is lost during digestion and metabolism. Further studies on sweet potato leaves should be continued, especially the comparison between fresh and dried sweet potato leaves on fish growth. Study of the anti-nutrients in this material is also recommended.

Another source of protein, earthworms, showed good quality high protein and amino acid composition in comparison with many conventional protein sources. However, in accordance with previous studies, earthworms show better results at a low level of inclusion in the diets. Replacement of 70% of protein (or 22% of diet dried matter) by *P. excavatus* can significantly improve fish growth, but they should not be used as a unique protein source in the diet. Earthworm powder as a dietary supplement in local low cost feed should be further studied under local conditions. Further studies are also needed on the digestion and energy metabolism of earthworms by fish.

Both of trial 1 and 2 showed that the local feeds were well digested and utilized by omnivorous common carp. Although local feed ingredients did not give the best growth of

fish, they gave the best results in terms of economic return. Local ingredient feed gave the lowest cost of feed as well as the lowest cost of fresh fish produced. The field trial (trial 2) fish performance seemed to be better than in the laboratory because the fish received a certain amount of natural food. Thus the fish utilizing feed more efficiently resulted in the reduction of feed conversion ratio as well as the cost of feed per unit of fish produced. Transferring knowledge about low-cost feed to the local farmer together with basic techniques of producing, storing, and effective use of fish feed could help farmers to improve production of fish in the region. Furthermore, development of low cost fish feed could enhance the livelihood of small-scale farmers as well as contributing to poverty alleviation in the rural areas of Vietnam. The information provided in this study especially could be used for further studies on the production and use of supplemental feed under local conditions by poor farmers.

7. Abstract

Cyprinids play very important role in aquaculture, especially in Asia and Pacific regions. Vietnam is not an exception. Although aquaculture in Vietnam increased dramatically recently, its high value products such as catfish, tiger prawn seem to be unaffordable for the people in rural areas, especially for the mountainous ethnic minority in the North who rely on herbivorous and omnivorous fish such as grass carp, Indians carp, and common carp for their livelihood.

Yen Chau is a district of Cyprinids play very important role in aquaculture, especially in Asia and Pacific regions. Vietnam is not an exception. Although aquaculture in Vietnam increased dramatically recently, its high value products such as catfish, tiger prawn seem to be unaffordable for the people in rural areas, especially for the Son La province, located in mountainous region in North-Western Vietnam. In this district, Black Thai ethnic minority accounts for approximately 54% population. Besides producing huge amount of grain and tuber crop such as maize and cassava for selling, aquaculture in a small poly-culture pond system is also a very typical activity which supplies an important nutritional source for human consumption and income generation as well.

However, even though most of farmers in Yen Chau own at least one pond each, the demand on the local market cannot be covered (Steinbronn, 2009). Recently, unknown diseases causing high mortalities could be the reason of the decrease of grass carp production. To avoid risk from losing grass carp, farmers are tending to change the composition of the fish species stocked in favour of e.g. common carp and mud carp without having an appropriate feed base for these species. So far, no high quality feed was applied into the pond system. Therefore, the study entitled “Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources” was conducted to not only contribute to an improvement of income and food security in the region but also meet the appeal of improvement of scientific knowledge on cyprinids which is inadequate with their great contribution.

In the study, most of the possible feed ingredients were collected and analyzed for nutritive and non-nutritive values. Based on the results of the analysis a number of diets were formulated for 30% of CP and 10% of CL then tested by common carp. In total, four

experiments were designed. The first was conducted in laboratory in which common carp were fed by local pelleted feed with 25, 50, and 75% protein derived from local ingredients. Results of this trial were confirmed by the trial 2 which was implemented under pond condition at Hanoi University of Agriculture. Trial 3 was conducted to compare different protein (full fat soybean meal and soybean cake) and carbohydrate sources (maize and cassava powder). Furthermore, sweet potato leaves were preliminarily investigated in fish feed inclusion. The last trial, trial 4, was carried out to evaluate the use of earthworm for common carp.

Trial 1, 3 and 4 were designed for a recirculation system in which five common carp were stocked in each aquarium of 40 l with 3 replicates. Water flow through the aquaria was maintained at 6-7 litters per minute. Water temperature, DO and pH were kept at 25-27°C, above 4 mg/l and around 7.0-8.0, respectively. The photoperiod was set up at 12 hrs light:12 hrs dark. Fish were fed daily five times metabolic body mass requirement for eight weeks. The trial 2 consisted of four feeding groups (3 test diets and 1 control), each having three replicates. In each net, 45 common carp were stocked, Fish were fed three times per day (8 h, 12 h, and 16 h) at a feeding rate of 5 times of maintenance requirement manually. Feed amount was adjusted every ten days after taking the weight of fish. The total trial lasted for 2 months.

Analytical results show that all of local materials have good nutritive values, similar or better that those in other regions. Besides, fish meal and many other ingredients can be used as source of protein such as full fat soybean meal, soybean cake, meat meal, as well as earthworm powder. This earthworms *Perionyx excavatus* show a suitable quality for fish feed, high protein (71% of DM) and amino acids in comparison with many conventional protein sources, including fish meal. Replacement of 70% of protein (~ 20% of DM) by earthworms can significantly improve growth of fish. However, it is still unclear in full replacement fish meal by this material. Using maize and cassava in Yen Chau is also feasible because these materials not only are cheap but also are the most available. Among all plant materials, full fat soybean seems to be the best ingredient with high protein and lipid content. In contrast, fish seem to be deficient of nutrient by feeding sweet potato leaf inclusion diets. In conclusion, except for sweet potato, all of local ingredients can be used for fish feed formulation.

The local feeds were well digested, utilized by omnivorous common carp. Despite local feed ingredients did not give the best growth of fish, they gave the best results in term of economic return. Local ingredient feed gave the lowest cost of feed as well as the lowest cost of fresh fish produced. The field trial fish performance seems to be better than in the laboratory due to receiving certain amount of natural food. Thus, fish utilize feed more efficiently resulted in reduction of feed conversion ratio as well as cost of feed per unit of fish produced. Transfer knowledge about low-cost feed to the local farmer together with basal techniques of producing, storing, and effective using fish feed is an important issue which could help farmers to improve production of fish in the region as well as contribute to poverty alleviation in the rural areas in Vietnam.

8. Zusammenfassung

Karpfenartige Fische spielen eine wichtige Rolle in der Aquakultur, besonders in Asien und pazifischen Regionen. Vietnam ist davon nicht ausgenommen. Obwohl Aquakultur in Vietnam in der letzten Zeit sehr stark zugenommen hat, sind ihre wertvolleren Produkte wie z.B. Welse oder Tigershrimps nahezu unerschwinglich für die Bevölkerung in den ländlichen Gebieten. Dies gilt insbesondere für die Bewohner der bergigen Regionen im Norden, welche mehr auf herbivore und omnivore Fische wie z.B. Graskarpfen, Indische Karpfen und den gemeinen Karpfen angewiesen sind.

Yen Chau ist ein Distrikt der Son La Provinz im bergigen Nordwesten Vietnams. In dieser Region macht die Minderheit der "Black Thai" mit 54% den größten Anteil in der Bevölkerung aus. Neben der Produktion großer Mengen an Getreide, Mais und Maniok für den Verkauf, spielt die Produktion von Fischen in Polykultur in kleinen Teichen eine wichtige Rolle. In ihnen werden die hauptsächlich für den Eigenverzehr aber auch für den Verkauf auf lokalen Märkten gedachten Fische produziert.

Obwohl die meisten Kleinbauern mindestens einen Teich besitzen, kann in der jüngeren Zeit der Bedarf auf den Märkten nicht mehr gedeckt werden. Dies hat seine Ursache vermutlich in einer seit wenigen Jahren registrierten Graskarpfen-spezifischen Krankheit, durch die die Produktion merklich zurückging. Um den Verlust der Graskarpfen zu vermeiden, verändern die Kleinbauern oft die Artenzusammensetzung der Besatzfische in den Teichen und bevorzugen dabei Karpfen und "Mud Carp" ohne dabei jedoch das nötige Futter für diese Fischarten zu haben. Bis heute ist noch kein hochqualitatives Futter für diese Karpfenarten getestet wurden. Daher wurde diese Studie mit dem Titel: "Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources" durchgeführt um nicht nur einen Beitrag zu der Einkommens- und Ernährungssicherung in der Region zu leisten, sondern auch um das unzureichende Wissen über diese Karpfenarten zu erweitern.

In dieser Studie sind die meisten der möglichen Futtermittel gesammelt und auf ihre nutritiven und anti-nutritiven Eigenschaften untersucht worden. Den Ergebnissen entsprechend sind eine Reihe von Futtermitteln formuliert worden, die 30% Rohprotein und 10% Rohfett enthielten, welche anschliessend an Karpfen getestet wurden. Insgesamt

sind vier Experimente geplant und durchgeführt worden. Das erste fand im Labor statt und beinhaltete die Fütterung von Karpfen mit pelletiertem Futter. Dieses enthielt 25, 50 und 75% Rohprotein aus lokalen Zutaten. Die Ergebnisse des Experiments wurden im zweiten Experiment bestätigt. Dieses wurde unter Feldbedingungen an der Agrarwissenschaftlichen Universität von Hanoi durchgeführt.

In dem nächsten Versuch wurden verschiedene Proteinquellen (nicht entfettetes Sojamehl und Soja-Presskuchen) sowie Mais und Maniokpulver als Kohlenhydratquellen miteinander verglichen. Ausserdem wurden Süßkartoffelblätter zum ersten Mal als Fischfutterzusatz getestet. Das letzte Experiment wurde durchgeführt um den Einsatz von Regenwürmern als Futtermittel zu evaluieren.

Versuche 1, 3 und 4 wurden in einer Kreislaufanlage durchgeführt, in der pro 40 L Becken fünf Karpfen in drei Replikaten gehalten wurden. Die Wasserversorgung betrug zwischen sechs und sieben Liter pro Minute. Die Wassertemperatur, gelöster Sauerstoff und der pH-Wert betrugen 25-27°C, über 4 mg/L und zwischen 7.0-8.0. In diesem Labor war ein Licht-:Dunkelzyklus von 12:12 Stunden eingestellt. Die Futtermenge betrug das Fünffache des metabolischen Körpergewichts. Alle drei Experimente dauerten jeweils 8 Wochen.

Der 2. Versuch bestand aus vier Fütterungsgruppen (drei experimentelle und eine Kontrolldiät) mit je drei Replikaten. Pro Netz wurden 45 Karpfen eingesetzt und drei Mal täglich von Hand gefüttert (8:00, 12:00 und 16:00). Die Futtermenge betrug dabei wieder den fünffachen Erhaltungsbedarf. Nach je 10 Tagen wurden die Fische gewogen und die Futtermenge angepasst. Der Versuch dauerte zwei Monate.

Die analytischen Ergebnisse haben gezeigt, dass alle einheimischen Materialen einen hohen Nährwert aufwiesen, ähnlich denen oder besser als die aus anderen Regionen. Darüber hinaus können Fischmehl und viele andere Zutaten als Proteinquelle verwendet werden wie z.B nicht entfettetes Sojamehl, Sojapresskuchen, Fleischmehl oder Regenwurmpulver. Der Regenwurm *Perionyx excavatus* zeigt eine hervorragende Proteinqualität und -quantität (71% von Trockengewicht) und eine ebenfalls gute Aminosäurezusammensetzung im Vergleich zu vielen herkömmlichen Proteinquellen, einschließlich Fischmehl. Das Ersetzen von 70% Protein (entspricht 20% vom

Trockengewicht) durch Regenwurmprotein konnte das Wachstum der Fische erheblich verbessern. Allerdings ist es noch unklar ob das vollständige Ersetzen von Fischmehl durch dieses Material eine ähnliche Wirkung bringt. Das Verwenden von Mais und Maniok in Yen Chau ist ebenso plausibel, denn diese Materialen sind nicht nur günstig sondern auch meistens verfügbar. Unter den pflanzlichen Materialen scheinen Vollfettsojabohnen der beste Einsatzstoff zu sein mit hohem Protein- und Lipidgehalt. Im Gegensatz dazu erscheinen die Fische unter Fehlernährung zu leiden wenn Sie mit Süßkartoffelblättern gefüttert werden. Es lässt sich schlußfolgern, dass bis auf Süßkartoffel alle einheimische Zutaten für den Einsatz als Fischfutter verwendet werden können.

Die getesteten Futtermittel sind gut verdaulich und verwertbar von den omnivoren Karpfen. Obwohl diese Futterbestandteile nicht das beste Wachstum hervorgebracht haben, haben sie die besten wirtschaftlichen Ergebnisse ergeben. Sie sind die günstigsten Futtermittel einerseits und haben andererseits den geringsten Preis pro produzierter Einheit Fisch ergeben. Die Leistung der in dem Feldversuch produzierten Fische erscheint besser als die im Labor, wahrscheinlich wegen der zusätzlich verfügbaren natürlichen Nahrung in den Teichen. Aus diesem Grund konnten die Fische effizienter das Futter verwerten, welches zur Abnahme sowohl von der Futterverwertung als auch von den Futterkosten pro Fischproduktionseinheit geführt hat. Das Wissen über die Produktion von kostengünstigen Futtermitteln an Kleinbauern zu übertragen, zusammen mit grundlegenden Techniken in Herstellung, Lagerung und wirksamere Nutzung von Fischfuttern stellen ein wichtiges Thema dar, wodurch den Bauern geholfen werden könnte, um nicht nur die Fischproduktion in der Region zu verbessern, sondern auch die Armut in den ländlichen Gebieten Vietnams zu beseitigen.

9 References

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10. Appendix

Weight of fish in Trial 1 (g)

Feed	Fish					Sum	Average
	1	2	3	4	5		
6/12/2007							
Control	8.9	10.1	8.4	5.5	9.7	42.5	8.5
Control	10.8	8.3	9.1	10.3	8.4	46.8	9.4
Control	10.3	9.9	10.6	8.6	8.0	47.4	9.5
F1.1	7.5	8.5	9.1	8.5	8.1	41.8	8.4
F1.1	8.0	8.2	8.5	8.4	8.4	41.5	8.3
F1.1	7.9	8.7	8.7	10.0	8.0	43.3	8.7
F1.2	10.5	8.5	9.0	9.5	10.0	47.5	9.5
F1.2	10.0	8.8	9.0	8.4	9.3	45.5	9.1
F1.2	8.5	8.1	10.9	8.4	9.6	45.4	9.1
F1.3	8.1	9.1	8.0	8.0	9.4	42.7	8.5
F1.3	8.6	7.8	9.9	9.5	7.6	43.5	8.7
F1.3	8.0	10.8	7.9	8.3	8.0	43.0	8.6
6/19/2007							
Control	11.3	12.0	10.2	9.6	12.5	55.6	11.1
Control	10.2	12.9	10.5	11.7	11.6	56.8	11.4
Control	13.2	11.5	12.9	11.3	10.6	59.6	11.9
F1.1	8.5	10.3	9.9	11.4	9.9	49.9	10.0
F1.1	8.7	10.5	9.9	9.8	10.4	49.3	9.9
F1.1	9.4	10.4	9.7	11.6	10.0	51.1	10.2
F1.2	12.2	10.8	9.7	10.8	10.7	54.2	10.8
F1.2	10.9	9.7	10.8	11.7	9.6	52.7	10.5
F1.2	11.9	10.5	8.9	10.7	8.9	50.9	10.2
F1.3	9.7	8.7	11.3	10.0	9.0	48.8	9.8
F1.3	9.6	10.8	8.6	10.2	9.0	48.2	9.6
F1.3	9.4	9.6	12.7	9.5	8.9	50.2	10.0

6/27/2007							
Control	14.2	14.9	15.6	11.3	12.2	68.3	13.7
Control	16.3	12.7	13.4	13.4	14.0	69.7	13.9
Control	16.2	14.2	14.3	13.0	16.1	73.8	14.8
F1.1	12.5	13.6	11.4	13.1	13.3	64.0	12.8
F1.1	13.1	12.8	11.8	11.4	10.8	59.9	12.0
F1.1	12.8	13.8	11.7	10.9	11.6	60.7	12.1
F1.2	13.5	12.8	14.8	13.1	12.1	66.3	13.3
F1.2	10.4	11.4	12.8	13.5	13.1	61.1	12.2
F1.2	12.4	13.9	10.8	10.5	12.7	60.2	12.0
F1.3	11.5	13.3	12.7	10.6	10.0	58.1	11.6
F1.3	12.8	11.5	11.5	10.1	10.1	56.1	11.2
F1.3	11.6	10.8	15.3	9.7	10.4	57.7	11.5
7/3/2007							
Control	18.1	20.3	17.9	14.8	15.7	86.8	17.4
Control	17.0	17.0	15.7	18.8	15.7	84.2	16.8
Control	17.6	17.5	16.1	19.8	20.5	91.4	18.3
F1.1	13.4	16.2	17.4	15.6	15.7	78.2	15.6
F1.1	14.1	13.9	12.8	16.5	15.2	72.5	14.5
F1.1	14.0	16.1	16.1	12.8	14.0	73.0	14.6
F1.2	14.2	15.6	15.7	16.5	17.7	79.7	15.9
F1.2	11.7	15.2	15.3	13.6	15.7	71.5	14.3
F1.2	12.1	13.5	13.9	11.9	15.3	66.7	13.3
F1.3	13.0	15.3	12.4	12.0	12.6	65.3	13.1
F1.3	13.3	13.4	11.8	15.0	11.7	65.1	13.0
F1.3	10.5	11.8	18.2	12.3	13.9	66.8	13.4

7/10/2007							
Control	21.0	23.2	21.9	18.3	20.3	104.7	20.9
Control	18.3	20.7	19.6	22.6	19.8	101.0	20.2
Control	19.1	24.0	24.8	20.6	22.0	110.4	22.1
F1.1	21.3	18.4	18.4	15.5	18.9	92.4	18.5
F1.1	16.4	19.7	15.6	18.1	17.0	86.7	17.3
F1.1	16.5	17.0	19.1	14.6	18.9	86.1	17.2
F1.2	20.0	18.7	18.3	16.4	19.5	92.8	18.6
F1.2	15.8	13.4	16.9	17.5	18.6	82.3	16.5
F1.2	13.9	14.4	16.1	15.7	18.5	78.6	15.7
F1.3	17.6	15.0	14.2	14.1	14.1	75.0	15.0
F1.3	14.0	15.7	13.2	16.4	14.9	74.3	14.9
F1.3	13.2	21.4	16.4	11.9	13.9	76.7	15.3
7/17/2007							
Control	24.0	22.3	27.5	25.8	24.9	124.5	24.9
Control	24.5	24.0	25.7	21.8	26.6	122.6	24.5
Control	24.2	30.7	28.7	26.5	25.4	135.5	27.1
F1.1	22.6	22.0	17.2	22.6	25.5	109.9	22.0
F1.1	21.8	23.9	20.7	18.3	19.4	104.2	20.8
F1.1	23.1	17.2	20.7	22.7	19.8	103.5	20.7
F1.2	20.7	22.6	21.6	18.8	22.7	106.4	21.3
F1.2	15.1	21.8	20.4	18.2	19.3	94.9	19.0
F1.2	16.6	19.2	18.6	16.4	21.3	92.1	18.4
F1.3	17.0	16.6	20.6	16.9	17.0	88.3	17.7
F1.3	14.7	17.6	17.1	18.7	16.8	84.8	17.0
F1.3	25.1	19.1	15.1	13.5	15.8	88.5	17.7

7/24/2007							
Control	32.2	27.8	29.3	27.2	31.2	147.7	29.5
Control	31.0	29.1	32.1	30.2	25.2	147.6	29.5
Control	28.4	31.6	36.6	36.2	32.1	164.9	33.0
F1.1	29.8	26.8	25.9	20.5	27.7	130.7	26.1
F1.1	22.2	26.0	22.6	25.2	28.8	124.7	24.9
F1.1	27.4	27.3	20.0	24.8	23.5	123.0	24.6
F1.2	26.0	25.1	26.9	21.5	24.8	124.2	24.8
F1.2	21.8	23.8	17.9	20.7	26.2	110.3	22.1
F1.2	18.5	19.1	22.4	22.1	25.3	107.3	21.5
F1.3	20.0	19.9	19.3	19.5	24.0	102.7	20.5
F1.3	21.0	21.5	19.3	17.2	19.5	98.6	19.7
F1.3	15.8	16.6	18.4	22.8	29.1	102.8	20.6
F1.3	20.0	19.9	19.3	19.5	24.0	102.7	20.5
F1.3	21.0	21.5	19.3	17.2	19.5	98.6	19.7
F1.3	15.8	16.6	18.4	22.8	29.1	102.8	20.6
7/31/2007							
Control	34.9	30.5	38.5	34.1	35.8	173.7	34.7
Control	28.9	33.7	34.6	36.8	37.2	171.2	34.2
Control	35.1	37.2	38.2	40.8	46.3	197.7	39.5
F1.1	23.6	30.6	31.1	35.1	32.7	153.1	30.6
F1.1	25.9	27.0	29.8	33.8	30.6	147.0	29.4
F1.1	23.1	28.2	32.2	32.5	29.1	145.1	29.0
F1.2	24.3	28.6	30.6	27.6	29.5	140.7	28.1
F1.2	20.1	29.9	24.7	23.2	27.5	125.4	25.1
F1.2	22.0	25.6	21.9	24.7	30.1	124.2	24.8
F1.3	21.7	23.1	22.7	21.8	28.0	117.3	23.5
F1.3	21.7	23.1	19.2	24.0	23.4	111.5	22.3
F1.3	26.7	20.6	33.1	19.0	18.0	117.3	23.5

8/7/2007							
Control	47.4	41.5	40.4	44.3	32.8	206.3	41.3
Control	45.0	34.9	43.1	39.6	42.8	205.3	41.1
Control	42.0	48.5	56.6	46.6	44.3	238.1	47.6
F1.1	39.6	28.3	41.9	35.9	36.4	182.2	36.4
F1.1	34.3	39.9	35.7	32.3	29.8	171.9	34.4
F1.1	37.4	33.0	37.1	27.1	33.3	167.9	33.6
F1.2	35.2	34.9	32.7	32.0	28.2	162.9	32.6
F1.2	23.0	25.4	32.1	28.6	34.8	144.0	28.8
F1.2	24.8	28.9	24.9	29.4	34.5	142.5	28.5
F1.3	26.2	33.6	25.1	27.6	25.2	137.6	27.5
F1.3	27.0	24.2	27.5	26.4	21.9	127.0	25.4
F1.3	37.6	21.5	24.0	20.2	30.4	133.7	26.7

Length of fish in Trial 1 (length of fish appropriate with order of fish weight on 8/7/2007, mm)

	Fish				
Group	1	2	3	4	5
Control	11.5	11.2	10.5	11.2	10.1
Control	11.0	9.9	11.1	10.7	10.9
Control	10.4	11.5	12.4	11.2	11.0
F1.1	10.6	9.5	11.0	10.3	10.6
F1.1	10.1	10.7	10.1	10.2	9.8
F1.1	10.3	9.7	10.6	9.3	9.9
F1.2	10.3	9.6	10.2	9.7	9.6
F1.2	8.7	9.0	9.9	9.4	10.1
F1.2	8.6	9.7	9.1	9.5	10.1
F1.3	8.9	10.5	9.4	9.4	9.2
F1.3	9.4	9.1	9.1	9.4	8.8
F1.3	10.7	8.3	8.8	8.4	10.1

Length of fish intestine in Trial 1 (cm)

	Fish				
Group	1	2	3	4	5
Control	19.8	14.3	16.0	15.6	14.5
Control	14.2	14.7	12.1	15.5	13.1
Control	11.5	16.9	20.6	15.1	16.8
F1.1	18.1	11.1	13.9	16.0	13.5
F1.1	14.5	17.3	14.3	12.4	13.0
F1.1	16.5	13.1	14.9	11.5	14.4
F1.2	15.3	16.7	15.4	14.1	13.7
F1.2	12.5	11.0	14.1	15.2	15.1
F1.2	12.9	14.2	13.4	13.6	13.1
F1.3	14.9	15.4	12.7	15.1	12.1
F1.3	11.0	13.1	12.2	12.6	11.6
F1.3	19.9	11.0	12.8	13.2	13.5

Weight of fish intestine in Trial 1 (g)

	Fish				
Group	1	2	3	4	5
Control	2.31	1.64	1.87	2.20	1.41
Control	1.89	1.23	1.71	1.77	1.75
Control	1.74	2.05	3.51	1.99	1.92
F1.1	2.07	1.51	2.21	1.77	1.64
F1.1	1.49	1.89	1.70	1.40	1.19
F1.1	1.69	1.33	1.76	1.14	1.51
F1.2	1.66	1.64	1.65	1.53	1.35
F1.2	1.30	0.90	1.44	1.57	1.65
F1.2	1.32	1.17	1.03	1.40	1.46
F1.3	1.39	1.97	1.29	1.41	1.21
F1.3	1.22	1.15	1.35	1.30	1.09
F1.3	1.89	0.93	1.18	1.33	1.57

Weight of fish liver in Trial 1 (g)

	Fish				
Group	1	2	3	4	5
Control	1.14	0.76	0.99	1.03	0.59
Control	0.60	0.51	0.68	0.75	0.76
Control	0.67	0.83	1.14	0.73	0.83
F1.1	0.75	0.44	0.53	0.45	0.53
F1.1	0.46	0.53	0.67	0.55	0.40
F1.1	0.51	0.59	0.54	0.35	0.56
F1.2	0.58	0.50	0.68	0.59	0.59
F1.2	0.31	0.22	0.48	0.45	0.45
F1.2	0.45	0.38	0.44	0.43	0.68
F1.3	0.50	0.63	0.41	0.40	0.56
F1.3	0.45	0.32	0.44	0.29	0.28
F1.3	0.61	0.27	0.45	0.33	0.37

Trial 2

Weight of fish in Trial 2 (g)

Initial fish

Treatments	Control			F2.1			F2.2			F2.3		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
Average	95.3	85.6	86.6	75.4	82.4	91.5	81.6	86.5	90.8	80.7	82.4	89.9
Std	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

24/5/2008

Treatments	Control			F2.1			F2.2			F2.3		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
No of fish												
1	159.0	144.6	156.0	95.4	166.2	102.0	109.2	109.5	115.6	119.0	194.4	110.0
2	113.0	105.3	115.4	84.0	92.9	140.3	110.3	115.6	129.2	123.1	136.0	123.3
3	71.1	109.4	124.2	120.0	131.0	109.0	85.4	105.3	97.7	115.7	166.7	154.2
4	108.7	163.2	105.8	87.3	94.7	110.2	112.2	110.2	117.7	120.0	89.2	133.8
5	113.0	121.2	84.9	121.0	152.0	113.3	115.4	120.7	148.2	145.1	64.6	104.5
6	119.9	95.8	84.8	124.5	103.6	112.3	109.1	109.5	129.2	126.2	153.0	112.0
7	74.5	98.7	165.7	106.6	128.5	109.0	133.4	110.6	110.1	131.2	82.1	121.2
8	127.6	107.0	117.1	122.0	112.5	107.9	109.1	113.4	148.2	117.5	93.0	95.6
9	141.0	82.6	100.0	70.0	98.4	89.6	102.6	112.0	111.0	114.8	91.5	76.2
10	75.6	117.1	134.1	102.8	106.1	97.3	109.3	111.3	72.1	120.0	65.7	115.0
Sum	1103.5	1145.0	1188.0	1033.6	1185.9	1090.9	1096.0	1118.1	1179.1	1232.6	1136.2	1145.7
Average	110.3	114.5	118.8	103.4	118.6	109.1	109.6	111.8	117.9	123.3	113.6	114.6
Std	29.3	23.8	27.3	18.9	25.1	13.2	11.7	4.1	22.9	9.1	45.5	21.1

Treatments	Control			F2.1			F2.2			F2.3		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
No of fish												
1	132.5	188.8	105.9	157.0	154.5	128.3	134.4	133.5	147.7	125.1	127.8	80.0
2	130.5	145.3	175.8	167.1	136.5	107.1	106.8	132.2	104.3	147.9	130.4	176.2
3	162.0	161.0	152.3	162.9	149.9	120.0	92.8	119.1	154.4	99.6	105.3	126.3
4	143.4	97.6	136.9	150.4	130.8	163.0	103.0	157.3	80.3	158.2	94.5	106.6
5	127.5	112.6	141.0	105.5	162.5	138.3	136.7	111.9	153.2	130.9	162.1	123.4
6	128.0	119.3	247.4	190.2	151.0	105.4	106.2	119.2	142.0	196.0	137.7	131.8
7	100.7	103.5	140.4	147.7	177.5	111.8	142.9	103.7	143.5	150.0	182.9	123.6
8	113.2	183.3	120.8	185.7	138.1	112.2	121.4	89.9	168.9	122.8	162.7	107.8
9	119.9	126.2	69.2	131.5	139.0	145.3	131.0	137.1	99.7	159.3	100.5	99.2
10	73.3	125.9	156.3	132.1	135.1	76.9	121.5	188.9	150.7	182.4	111.9	89.1
11	131.4	86.9	97.9	134.5	139.4	113.0	151.8	76.7	168.2	125.7	105.5	120.7
12	131.4	144.0	144.2	113.5	135.3	170.0	151.7	88.9	103.1	116.4	103.2	123.3
13	93.3	97.5	128.2	112.7	149.7	128.9	107.5	75.7	105.9	174.9	191.7	158.5
14	92.9	117.7	160.2	111.7	75.6	102.8	83.7	118.8	81.7	130.2	99.7	205.6
15	107.0	146.0	135.2	114.5	125.5	128.8	124.3	98.9	115.4	87.5	119.2	181.9
16	107.1	120.2	143.1	111.7	154.6	87.9	118.0	82.5	124.3	100.6	78.7	150.8
17	130.1	137.8	134.6	96.5	181.0	115.8	93.5	118.4	88.9	127.8	73.6	169.7
18	103.2	112.5	98.5	120.8	73.2	133.5	98.4	113.6	126.6	71.5	135.6	77.6
19	117.3	111.7	97.4	109.3	121.2	83.8	115.5	69.7	122.8	104.4	93.1	148.9
20		147.4	98.2	80.4	126.2		94.2		80.0	134.5	96.5	74.1
21				108.9	118.4		73.1			133.4		83.4
Sum	2244.6	2585.0	2683.4	2744.5	2874.9	2272.8	2408.1	2135.7	2461.8	2779.0	2412.5	2658.3
Average	118.1	129.3	134.2	130.7	136.9	119.6	114.7	112.4	123.1	132.3	120.6	126.6
Std	20.6	27.4	37.5	29.4	26.6	24.5	21.8	29.9	29.4	31.1	33.0	37.4

Treatments	Control			F2.1			F2.2			F2.3		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
No of fish												
1	178.0	96.8	144.9	176.3	192.7	88.0	145.0	215.8	111.4	201.2	162.4	174.4
2	130.2	131.8	232.7	211.8	160.8	157.9	140.5	112.6	189.9	214.7	176.7	142.7
3	102.2	134.9	191.4	208.4	139.6	224.8	108.4	168.1	135.3	220.7	106.7	136.4
4	152.1	217.3	162.2	251.0	122.9	228.4	138.8	149.8	117.0	170.6	144.7	194.2
5	116.2	197.3	202.6	245.7	158.2	196.5	175.3	179.4	167.2	132.3	154.7	236.8
6	105.7	201.4	161.1	211.6	213.8	173.2	169.8	108.9	173.7	96.4	116.3	132.4
7	121.6	142.5	156.6	143.2	170.4	127.5	164.8	146.5	142.6	150.7	152.6	137.6
8	91.6	164.7	190.6	146.8	132.4	166.1	120.8	160.5	139.9	192.6	113.5	144.3
9	126.8	97.7	165.9	192.7	155.5	129.7	124.9	153.5	86.5	158.4	190.8	156.2
10	116.9	177.5	150.2	154.0	172.1	136.6	122.8	135.5	144.7	186.6	133.1	76.5
11	145.8	111.3	163.0	150.2	181.7	127.5	139.5	145.9	127.7	141.8	182.7	111.2
12	93.0	175.2	168.5	167.9	148.6	134.5	141.8	136.0	138.8	192.8	128.6	138.3
13	139.3	111.5	176.2	171.6	127.6	126.4	148.5	135.0	150.1	131.1	130.6	105.6
14	165.0	144.9	113.3	156.6	109.4	147.1	84.4	132.6	102.0	107.9	97.7	111.5
15	184.1	138.2	80.7	148.5	150.0	127.1	97.1	108.0	129.5	133.5	154.8	167.1
16	118.4	132.6	133.3	117.9	97.0	135.8	106.3	113.0	105.1	96.7	139.8	154.0
17	136.8	137.1	149.7	131.2	109.7	135.8	117.3	88.2	87.8	118.8	101.3	140.9
18	132.1	96.4	117.2	115.4	81.5	102.0	96.5	76.8	85.8	142.2	104.8	143.3
19	129.2	103.7	128.3	124.5	122.7	92.3	97.1	110.7	109.2	98.8	91.2	170.2
20	116.6	83.0	111.2	124.1	112.6	117.6	78.6	100.0	102.1	88.5	81.0	
21			129.1	102.9		86.8			115.4	81.0		
22			69.7			100.1			117.1			
23			88.3									
Sum	2601.5	2795.8	3386.7	3452.3	2859.2	3061.6	2518.2	2676.8	2778.6	3057.0	2663.7	2773.5
Average	130.1	139.8	147.2	164.4	143.0	139.2	125.9	133.8	126.3	145.6	133.2	146.0
Std	25.4	38.6	39.7	42.2	33.7	39.4	28.0	33.1	28.2	43.3	31.6	34.9

Treatments	Control			F2.1			F2.2			F2.3		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
No of fish												
1	176.3	225.2	352.5	275.8	273.7	306.1	176.5	147.5	190.1	227.5	282.6	291.7
2	161.9	222.2	275.5	241.8	224.6	263.9	158.0	185.5	205.4	218.3	225.6	217.9
3	182.7	201.4	197.8	248.0	276.3	159.8	160.8	158.6	168.0	188.9	152.6	168.7
4	203.4	228.2	135.9	293.4	182.3	152.5	214.7	186.7	207.4	176.7	218.5	168.6
5	148.6	252.4	190.4	277.6	213.7	266.9	163.0	175.3	185.2	250.9	156.6	161.7
6	174.9	196.1	185.4	246.1	200.5	159.7	187.3	155.3	210.6	179.5	216.9	145.1
7	130.8	202.1	118.4	193.2	187.1	224.2	138.5	121.8	180.1	216.4	149.2	128.8
8	165.8	145.8	128.1	218.4	175.3	318.7	179.9	173.5	149.1	162.4	127.5	149.5
9	164.5	158.3	191.1	199.6	178.9	189.2	134.6	164.4	161.6	109.8	201.5	158.5
10	179.1	98.7	183.5	193.6	182.2	159.1	168.2	106.4	153.7	93.0	224.4	202.8
11	171.0	123.8	164.5	175.3	200.2	182.4	163.9	186.8	204.2	242.2	133.9	205.5
12	144.7	154.8	207.8	159.0	180.5	250.0	155.4	136.0	164.4	151.8	131.8	144.5
13	160.0	161.7	137.4	191.4	141.0	146.9	131.4	133.1	179.7	182.3	170.5	129.0
14	157.2	137.7	180.4	170.6	189.3	144.8	112.7	91.0	152.8	236.9	145.3	173.2
15	148.7	160.4	189.5	164.2	166.4	166.5	130.8	122.9	167.0	208.5	166.4	167.6
16	114.9	167.6	129.2	174.5	181.4	227.9	136.1	123.1	143.3	153.9	100.6	156.6
17	150.2	99.9	153.3	152.7	133.7	175.3	139.3	148.3	135.1	193.2	116.6	172.2
18	136.9	109.3	94.4	141.3	137.9	153.0	113.3	94.4	121.9	185.1	121.6	146.7
19	127.8	112.9	143.8	156.6	126.4	115.8	88.9	141.8	126.6	166.1	154.0	158.3
20	138.5	89.8	159.0	95.2	144.5	163.6	82.9	101.4	137.6	108.7	103.6	108.2
Sum	3137.9	3248.1	3517.6	3968.2	3695.9	3926.3	2936.2	2853.7	3343.8	3652.2	3299.8	3355.2
Average	156.9	162.4	175.9	198.4	184.8	196.3	146.8	142.7	167.2	182.6	165.0	167.8
Std	21.5	48.8	57.5	51.7	40.7	57.7	32.6	30.8	27.6	44.6	48.4	39.3

Treatments	Control			F2.1			F2.2			F2.3		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
No of fish												
1	169.5	283.7	195.0	231.5	308.8	335.2	178.1	225.0	235.2	258.3	213.7	293.7
2	212.6	293.1	203.2	274.5	202.7	380.5	302.1	209.9	247.7	227.9	132.4	184.8
3	183.9	256.9	202.8	243.0	209.5	361.0	190.4	266.0	186.5	207.7	145.0	227.9
4	246.3	244.2	232.4	270.1	234.3	292.4	207.8	293.6	249.3	191.0	154.1	257.6
5	185.5	239.9	240.9	303.5	190.7	235.2	196.4	232.0	207.9	170.4	148.6	136.6
6	153.6	281.0	205.1	175.9	217.2	209.5	143.8	209.6	155.8	177.1	161.4	182.9
7	182.0	229.3	239.4	217.6	200.4	195.5	209.0	151.4	178.0	117.8	175.8	204.5
8	220.1	149.5	172.0	222.2	276.4	251.2	203.1	156.1	210.2	195.6	223.3	175.6
9	173.3	223.3	270.0	207.4	237.9	171.3	174.0	148.0	161.0	187.1	176.1	169.0
10	154.0	194.1	224.1	210.4	240.7	193.9	222.7	198.2	166.5	198.7	240.9	180.3
11	175.6	236.6	262.9	279.2	200.5	194.8	173.6	146.6	152.0	202.4	252.0	253.4
12	136.5	198.7	278.5	288.8	230.2	165.3	186.6	178.5	185.6	242.2	250.2	166.0
13	162.1	155.8	184.8	203.8	190.1	186.2	158.2	177.4	150.3	182.1	113.3	175.2
14	170.1	135.2	196.6	163.8	172.6	183.2	171.0	159.4	167.1	142.4	242.4	226.7
15	189.2	169.4	140.5	201.4	196.8	196.8	161.6	128.5	132.7	169.4	175.5	170.5
16	159.2	108.6	177.6	189.6	200.3	182.8	156.1	164.6	125.6	234.7	136.8	203.0
17	153.7	152.6	172.5	154.7	186.6	151.0	172.4	130.6	146.9	255.9	149.4	157.8
18	139.5	117.3	112.5	169.0	198.8	159.3	176.3	114.1	113.0	252.9	167.9	117.4
19	164.2	146.6	185.2	129.1	149.0	169.6	179.2	149.6	108.4	132.0	120.8	153.9
20	138.2	151.3	162.5	164.5	217.1	145.7	159.8	98.5		180.2	98.5	134.5
										219.9		
										118.8		
Sum	3468.8	3966.9	4058.4	4299.8	4260.5	4360.3	3722.1	3537.5	3279.6	4264.4	3478.2	3771.1
Average	173.4	198.3	202.9	215.0	213.0	218.0	186.1	176.9	172.6	193.8	173.9	188.6
Std	28.0	58.1	42.8	49.2	35.5	70.2	33.9	50.3	42.2	42.2	47.6	44.7

14/7/2008

Treatments	Control			F2.1			F2.2			F2.3		
Replicates	1	2	3	1	2	3	1	2	3	1	2	3
No of fish												
1	212.5	174.0	324.9	257.1	229.7	207.1	204.6	221.4	214.0	244.4	287.0	201.5
2	223.1	284.0	260.8	281.1	206.9	347.9	244.2	266.1	268.6	286.8	184.3	263.3
3	218.9	209.8	280.2	308.1	233.5	421.1	188.3	201.4	362.3	271.2	148.2	302.7
4	187.4	163.6	277.2	335.5	312.2	276.2	214.2	240.1	223.9	239.8	175.8	207.3
5	212.7	299.1	299.4	236.6	212.7	221.0	183.5	173.8	270.4	208.6	281.8	215.8
6	194.8	218.5	241.0	241.5	264.9	239.1	215.4	175.3	198.4	248.7	281.9	213.4
7	218.4	291.3	293.6	198.1	218.6	262.5	239.2	208.1	232.7	299.4	282.8	167.5
8	225.4	265.6	236.0	187.8	314.4	213.9	225.5	200.1	279.1	275.8	218.8	303.0
9	169.6	284.3	225.7	170.2	227.7	292.6	303.6	186.5	193.5	207.0	177.6	205.7
10	184.7	224.8	222.5	257.7	245.9	322.1	220.0	179.1	257.9	190.4	270.2	219.8
11	221.1	268.4	220.1	231.7	276.1	188.2	209.8	209.9	165.3	299.4	246.4	200.8
12	196.5	167.0	210.6	197.8	258.4	209.3	202.8	200.1	211.7	263.7	190.1	368.8
13	203.0	187.1	158.1	233.5	223.1	219.9	158.2	241.4	168.0	242.2	172.5	211.4
14	169.4	177.2	232.3	165.6	213.4	225.5	196.8	130.1	201.3	195.3	252.6	237.1
15	250.6	221.5	231.2	214.0	235.2	210.2	226.7	208.2	158.6	209.5	188.8	212.9
16	191.5	170.3	211.4	203.8	222.8	199.0	184.3	104.2	142.9	195.9	156.5	178.3
17	198.3	296.4	165.5	307.7	272.0	256.3	178.8	218.9	119.9	157.2	129.8	151.0
18	156.9	241.0	234.0	233.6	180.8	210.9	133.1	218.3	135.9	224.9	127.8	166.1
19	192.2	121.1	170.5	215.7	218.4	113.1	234.4	226.2	103.6	112.6	114.5	117.0
20	215.6		157.0	240.2	215.7	162.6	233.7		175.3	148.3	139.8	132.6
Sum	4042.7	4265.0	4651.9	4717.2	4782.5	4798.5	4197.1	3809.2	4083.0	4521.0	4027.3	4275.8
Average	202.1	224.5	232.6	235.9	239.1	239.9	209.9	200.5	204.1	226.1	201.4	213.8
Std	22.5	54.4	47.4	45.7	34.4	68.0	35.7	37.8	62.8	51.0	58.9	60.2

Weight of sacrificed fish in Trial 2 (g)

Treatments		Number of fish				
		1	2	3	4	5
Control	1	200.7	212.6	208.6	236.5	148.1
	2	164.2	197.9	274.8	167.2	160.6
	3	264.4	227.4	198.7	220.8	148.2
F2.1	1	328.9	194.2	228.9	211.4	235.5
	2	202.8	306.1	308.3	253.4	230.6
	3	270.8	234.4	221.1	110.9	159.4
F2.2	1	232.6	205.2	227.8	193.2	170.3
	2	191.8	165.6	177.6	123.9	99.3
	3	203.8	221.6	245.6	157.5	191.7
F2.3	1	230.6	270.6	255.8	226.2	196.8
	2	270.8	173.8	139.8	165.8	265.9
	3	190.1	201.3	285.8	199.5	110.4

Standard length of fish in Trial 2 (cm)

Treatments		Number of fish				
		1	2	3	4	5
Control	1	18.1	18.5	17.9	18.7	17.5
	2	18.9	19.7	22.2	17.1	19.3
	3	23.1	20.3	19.8	20.3	17.6
F2.1	1	24.4	18.9	17.8	20.1	20.4
	2	18.7	21.6	23.2	19.8	22.1
	3	20.4	19.6	19.5	16.3	17.3
F2.2	1	20.9	20.1	18.1	18.3	18.9
	2	18.9	19.5	18.2	16.8	15.6
	3	20.4	19.3	21	18.3	18.6
F2.3	1	19.9	21	21.5	18.5	18.2
	2	21.9	19.3	17.8	17.6	20
	3	19.2	20.2	20.3	19.6	14.4

Weight of fish liver in Trial 2 (g)

Treatments		Number of fish				
		1	2	3	4	5
Control	1	5.15	4.14	5.06	2.70	2.78
	2	3.53	2.01	6.38	3.42	2.70
	3	3.83	3.78	5.12	5.63	2.79
F2.1	1	3.64	4.31	2.54	3.81	6.53
	2	4.04	4.97	4.77	7.70	3.66
	3	4.00	3.97	3.90	1.93	2.12
F2.2	1	7.99	3.64	3.37	3.20	2.01
	2	4.02	2.68	1.81	1.63	1.85
	3	3.68	2.23	4.30	2.69	2.37
F2.3	1	2.46	4.79	4.78	3.11	3.05
	2	1.48	1.32	1.64	2.77	1.84
	3	5.32	2.91	2.55	2.32	2.74

Weight of fish intestine in Trial 2 (g)

Treatments		Number of fish				
		1	2	3	4	5
Control	1	6.10	3.92	8.12	5.88	4.02
	2	4.37	2.74	9.23	4.24	3.53
	3	6.39	6.40	3.83	8.06	4.46
F2.1	1	9.65	4.91	6.43	5.36	6.46
	2	6.42	6.91	11.58	10.83	8.60
	3	8.57	7.93	5.04	3.12	5.47
F2.2	1	6.91	6.94	6.73	5.47	5.52
	2	5.59	3.92	4.27	2.26	3.45
	3	3.77	5.51	7.77	3.51	5.97
F2.3	1	7.44	9.25	6.89	5.62	5.58
	2	7.20	3.09	3.79	4.61	5.54
	3	5.30	5.27	6.38	5.78	3.41

Length of fish intestine in trial 2 (cm)

Treatments		Number of fish				
		1	2	3	4	5
Control	1	31.5	29.1	22.0	33.6	21.0
	2	23.4	18.6	37.4	25.0	25.5
	3	30.5	32.4	23.0	34.4	26.0
F2.1	1	47.2	23.7	43.2	28.0	40.1
	2	29.7	34.7	33.0	40.0	47.0
	3	33.1	26.8	25.8	22.5	23.5
F2.2	1	36.7	36.5	34.5	27.3	26.0
	2	26.1	25.0	26.7	23.4	20.5
	3	29.3	28.3	37.1	30.5	24.0
F2.3	1	28.2	27.6	38.2	30.4	34.6
	2	32.5	23.8	27.6	31.5	39.6
	3	30.1	30.4	31.0	31.4	25.7

Weight of fish in Trial 3

Initial weight (g)

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	3.2	3.6	2.9	2.7	3.3	15.6	3.1	0.3
	2	3.4	3.3	3.3	3.3	3.7	17.1	3.4	0.2
	3	3.5	2.8	2.9	2.8	3.5	15.5	3.1	0.4
F3.1	1	3.8	2.9	3.1	3.0	3.8	16.5	3.3	0.4
	2	3.1	2.9	3.6	3.1	3.5	16.1	3.2	0.3
	3	2.9	3.3	3.5	3.8	3.8	17.3	3.5	0.4
F3.2	1	3.6	2.9	3.6	3.3	3.4	16.7	3.3	0.3
	2	3.0	3.3	3.2	3.1	3.4	15.9	3.2	0.2
	3	3.3	3.6	3.2	2.8	3.5	16.4	3.3	0.3
F3.3	1	3.1	3.7	3.0	2.9	3.4	16.1	3.2	0.3
	2	3.2	2.7	3.0	3.2	3.3	15.3	3.1	0.2
	3	3.6	3.2	3.8	3.5	3.7	17.9	3.6	0.2
F3.4	1	2.8	3.2	3.3	3.4	3.4	16.1	3.2	0.3
	2	3.3	2.9	3.4	3.4	3.6	16.6	3.3	0.3
	3	2.9	3.7	3.0	3.0	3.3	15.8	3.2	0.3
F3.5	1	3.6	3.1	3.9	3.2	3.4	17.2	3.4	0.3
	2	3.5	2.9	3.3	3.3	2.9	16.0	3.2	0.3
	3	2.7	3.5	3.7	3.8	3.3	17.0	3.4	0.4
F3.6	1	3.3	3.3	3.8	3.2	3.6	17.2	3.4	0.2
	2	3.8	3.4	3.1	3.1	3.6	16.9	3.4	0.3
	3	3.0	3.4	3.7	3.7	3.8	17.5	3.5	0.3
F3.7	1	3.6	2.8	3.4	3.5	3.1	16.3	3.3	0.3
	2	3.0	3.1	3.0	3.2	3.5	15.8	3.2	0.2
	3	3.3	3.7	3.6	3.7	3.6	17.9	3.6	0.2

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	3.2	3.6	2.9	2.7	3.3	15.6	3.1	0.3
	2	3.4	3.3	3.3	3.3	3.7	17.1	3.4	0.2
	3	3.5	2.8	2.9	2.8	3.5	15.5	3.1	0.4
F3.1	1	3.8	2.9	3.1	3.0	3.8	16.5	3.3	0.4
	2	3.1	2.9	3.6	3.1	3.5	16.1	3.2	0.3
	3	2.9	3.3	3.5	3.8	3.8	17.3	3.5	0.4
F3.2	1	3.6	2.9	3.6	3.3	3.4	16.7	3.3	0.3
	2	3.0	3.3	3.2	3.1	3.4	15.9	3.2	0.2
	3	3.3	3.6	3.2	2.8	3.5	16.4	3.3	0.3
F3.3	1	3.1	3.7	3.0	2.9	3.4	16.1	3.2	0.3
	2	3.2	2.7	3.0	3.2	3.3	15.3	3.1	0.2
	3	3.6	3.2	3.8	3.5	3.7	17.9	3.6	0.2
F3.4	1	2.8	3.2	3.3	3.4	3.4	16.1	3.2	0.3
	2	3.3	2.9	3.4	3.4	3.6	16.6	3.3	0.3
	3	2.9	3.7	3.0	3.0	3.3	15.8	3.2	0.3
F3.5	1	3.6	3.1	3.9	3.2	3.4	17.2	3.4	0.3
	2	3.5	2.9	3.3	3.3	2.9	16.0	3.2	0.3
	3	2.7	3.5	3.7	3.8	3.3	17.0	3.4	0.4
F3.6	1	3.3	3.3	3.8	3.2	3.6	17.2	3.4	0.2
	2	3.8	3.4	3.1	3.1	3.6	16.9	3.4	0.3
	3	3.0	3.4	3.7	3.7	3.8	17.5	3.5	0.3
F3.7	1	3.6	2.8	3.4	3.5	3.1	16.3	3.3	0.3
	2	3.0	3.1	3.0	3.2	3.5	15.8	3.2	0.2
	3	3.3	3.7	3.6	3.7	3.6	17.9	3.6	0.2

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	4.3	4.2	3.5	3.6	4.1	19.7	3.9	0.4
	2	3.7	4.3	4.0	3.8	4.3	20.1	4.0	0.3
	3	4.0	4.4	3.4	3.3	3.4	18.4	3.7	0.5
F3.1	1	4.3	4.3	3.2	3.2	3.9	18.9	3.8	0.5
	2	3.8	4.2	3.8	4.4	3.9	19.9	4.0	0.3
	3	3.7	3.2	4.0	4.5	4.1	19.5	3.9	0.5
F3.2	1	4.3	3.5	3.5	4.0	4.1	19.5	3.9	0.4
	2	3.8	3.7	3.7	3.5	3.7	18.4	3.7	0.1
	3	4.7	3.8	3.2	4.0	3.9	19.7	3.9	0.5
F3.3	1	3.4	3.9	3.6	4.1	3.5	18.5	3.7	0.3
	2	3.2	3.6	3.7	3.5	4.1	18.0	3.6	0.3
	3	3.8	4.4	4.4	4.5	4.5	21.4	4.3	0.3
F3.4	1	4.2	4.3	3.6	4.2	4.1	20.4	4.1	0.3
	2	3.9	4.3	3.7	4.3	3.9	20.1	4.0	0.3
	3	3.6	4.8	3.5	3.6	4.2	19.6	3.9	0.5
F3.5	1	4.0	3.4	3.5	3.6	3.8	18.3	3.7	0.3
	2	3.2	3.7	3.9	3.9	3.3	18.1	3.6	0.3
	3	3.5	4.3	4.2	4.0	3.1	19.1	3.8	0.5
F3.6	1	3.5	3.8	3.3	4.1	3.6	18.3	3.7	0.3
	2	3.5	4.2	3.3	3.6	4.1	18.7	3.7	0.4
	3	4.0	4.1	3.6	4.5	3.0	19.1	3.8	0.6
F3.7	1	3.2	3.9	3.4	4.0	4.1	18.7	3.7	0.4
	2	3.2	3.5	3.5	4.2	3.4	17.8	3.6	0.4
	3	3.8	3.5	4.0	4.1	4.1	19.6	3.9	0.2

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	5.3	5.1	4.5	4.7	5.4	25.0	5.0	0.4
	2	5.6	4.5	5.4	4.9	5.3	25.7	5.1	0.5
	3	3.9	4.4	5.6	4.6	5.3	23.8	4.8	0.7
F3.1	1	5.4	4.1	3.6	5.0	5.4	23.5	4.7	0.8
	2	5.6	4.6	4.9	5.0	4.9	25.0	5.0	0.4
	3	4.8	4.0	5.4	5.0	4.5	23.6	4.7	0.5
F3.2	1	4.1	5.5	5.0	4.3	5.3	24.2	4.8	0.6
	2	4.3	4.0	4.7	4.7	4.6	22.2	4.4	0.3
	3	4.7	4.7	4.8	4.0	6.2	24.4	4.9	0.8
F3.3	1	4.4	4.6	5.3	4.6	4.2	23.0	4.6	0.4
	2	4.3	3.2	4.1	5.2	4.8	21.7	4.3	0.8
	3	5.2	5.2	4.6	5.6	5.3	25.9	5.2	0.4
F3.4	1	5.2	5.3	5.4	4.2	5.2	25.3	5.1	0.5
	2	5.3	5.0	4.2	4.2	4.9	23.7	4.7	0.5
	3	4.2	4.5	6.0	3.8	5.6	24.0	4.8	0.9
F3.5	1	4.2	4.4	4.0	4.0	3.4	20.0	4.0	0.4
	2	4.4	3.4	4.0	4.3	4.0	20.2	4.0	0.4
	3	3.8	3.6	4.7	4.2	4.8	21.1	4.2	0.5
F3.6	1	3.6	4.2	3.7	3.6	4.6	19.6	3.9	0.5
	2	4.0	3.2	4.4	3.9	4.9	20.5	4.1	0.6
	3	4.6	4.8	4.2	4.1	3.3	21.0	4.2	0.6
F3.7	1	3.6	3.9	4.6	4.8	4.5	21.3	4.3	0.5
	2	4.0	5.0	3.6	4.1	3.8	20.4	4.1	0.5
	3	3.8	4.9	4.4	4.0	4.7	21.9	4.4	0.4

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	6.8	5.7	6.2	5.5	6.2	30.4	6.1	0.5
	2	6.7	6.0	6.6	6.5	5.3	31.0	6.2	0.6
	3	4.6	7.0	6.6	5.7	5.3	29.2	5.8	1.0
F3.1	1	5.2	6.7	4.1	5.8	6.4	28.1	5.6	1.0
	2	6.8	5.5	5.3	5.7	5.7	29.1	5.8	0.6
	3	5.6	5.1	6.1	4.8	5.3	26.8	5.4	0.5
F3.2	1	6.0	4.9	4.5	6.0	6.7	28.0	5.6	0.9
	2	4.6	5.4	5.4	5.6	5.0	26.0	5.2	0.4
	3	5.7	7.4	5.5	4.8	5.6	29.0	5.8	1.0
F3.3	1	5.5	5.3	5.2	6.2	5.6	27.8	5.6	0.4
	2	6.2	4.7	5.9	3.6	4.9	25.2	5.0	1.0
	3	5.3	6.6	5.9	6.1	5.7	29.6	5.9	0.5
F3.4	1	5.0	5.9	6.1	6.2	6.0	29.2	5.8	0.5
	2	4.3	6.1	5.6	4.9	6.1	26.9	5.4	0.8
	3	4.7	7.6	3.9	5.1	6.9	28.3	5.7	1.6
F3.5	1	4.7	3.5	4.5	4.4	4.2	21.3	4.3	0.5
	2	4.7	4.4	4.4	6.7	3.6	23.7	4.7	1.2
	3	5.2	5.1	4.1	4.3	3.9	22.5	4.5	0.6
F3.6	1	3.8	3.8	3.7	4.3	5.0	20.6	4.1	0.5
	2	4.1	3.3	5.4	4.7	4.3	21.8	4.4	0.7
	3	4.4	3.5	4.6	4.4	5.0	21.9	4.4	0.5
F3.7	1	4.3	3.9	4.9	5.3	4.9	23.3	4.7	0.6
	2	3.8	4.6	4.8	4.0	4.4	21.6	4.3	0.4
	3	4.2	4.2	4.7	5.1	5.1	23.3	4.7	0.5

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	7.1	6.8	8.5	6.7	7.6	36.6	7.3	0.7
	2	8.2	7.7	7.3	6.4	8.2	37.7	7.5	0.7
	3	7.2	5.6	8.2	8.1	6.4	35.5	7.1	1.1
F3.1	1	7.6	6.7	4.5	8.3	6.5	33.6	6.7	1.4
	2	8.3	6.5	6.8	6.4	7.3	35.1	7.0	0.8
	3	5.7	6.2	6.5	7.2	5.8	31.4	6.3	0.6
F3.2	1	5.4	6.7	6.8	5.7	7.9	32.5	6.5	1.0
	2	6.1	5.4	6.5	6.6	6.4	30.9	6.2	0.5
	3	6.7	8.6	6.9	5.9	6.7	34.7	6.9	1.0
F3.3	1	7.2	6.8	5.2	6.1	6.3	31.7	6.3	0.8
	2	7.0	5.7	4.0	7.4	5.4	29.5	5.9	1.4
	3	7.0	7.6	6.6	6.3	7.2	34.6	6.9	0.5
F3.4	1	6.8	7.1	7.5	5.8	7.0	34.2	6.8	0.7
	2	5.7	7.1	6.5	5.1	7.4	31.8	6.4	0.9
	3	8.5	4.7	9.7	5.9	5.3	34.0	6.8	2.2
F3.5	1	4.8	3.7	4.6	4.8	5.3	23.1	4.6	0.6
	2	4.9	3.7	5.2	5.0	5.2	23.9	4.8	0.6
	3	5.6	4.7	4.4	5.5	4.3	24.5	4.9	0.6
F3.6	1	4.0	5.5	4.3	4.9	3.9	22.5	4.5	0.6
	2	5.8	4.4	4.7	3.4	5.0	23.4	4.7	0.9
	3	4.8	4.0	4.9	4.9	5.4	24.0	4.8	0.5
F3.7	1	4.3	4.5	6.0	5.6	5.2	25.6	5.1	0.7
	2	4.2	4.4	5.4	4.8	5.1	24.0	4.8	0.5
	3	4.5	5.9	5.1	5.4	4.8	25.6	5.1	0.5

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	7.7	7.3	8.7	9.1	10.3	43.1	8.6	1.2
	2	9.0	9.8	8.6	7.3	10.0	44.7	8.9	1.1
	3	9.4	8.6	7.7	6.8	9.7	42.1	8.4	1.2
F3.1	1	5.5	7.4	8.9	9.8	8.6	40.3	8.1	1.7
	2	9.6	7.5	7.5	7.5	8.4	40.4	8.1	0.9
	3	6.9	6.5	5.4	7.8	8.1	34.6	6.9	1.1
F3.2	1	7.7	6.2	9.0	6.2	7.8	37.0	7.4	1.2
	2	7.7	7.4	7.6	6.1	7.2	36.0	7.2	0.6
	3	10.3	7.3	6.8	8.1	7.5	40.0	8.0	1.4
F3.3	1	5.7	6.9	8.1	7.1	6.8	34.5	6.9	0.9
	2	4.4	7.8	5.9	6.5	8.7	33.2	6.6	1.7
	3	7.3	7.7	8.6	8.3	7.2	39.0	7.8	0.6
F3.4	1	6.6	8.1	8.3	8.8	7.6	39.4	7.9	0.8
	2	5.7	7.4	8.8	8.2	6.6	36.7	7.3	1.2
	3	11.5	5.5	5.8	6.5	9.8	39.0	7.8	2.7
F3.5	1	3.9	5.1	5.2	5.8	5.0	25.0	5.0	0.7
	2	5.3	5.4	3.9	5.7	5.6	25.8	5.2	0.8
	3	6.2	6.0	4.7	4.6	5.1	26.6	5.3	0.7
F3.6	1	4.0	4.6	4.3	6.0	5.1	24.0	4.8	0.8
	2	6.2	4.8	5.1	5.1	3.5	24.7	4.9	1.0
	3	5.0	5.6	5.0	5.1	4.2	24.9	5.0	0.5
F3.7	1	6.5	4.5	4.9	6.1	5.6	27.6	5.5	0.8
	2	4.4	5.2	5.6	5.8	4.6	25.6	5.1	0.6
	3	6.3	5.4	5.7	5.1	4.7	27.2	5.4	0.6

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	9.5	10.7	8.7	11.0	13.4	53.3	10.7	1.8
	2	11.6	10.3	12.3	10.5	8.4	53.1	10.6	1.5
	3	11.3	8.4	12.4	9.6	11.0	52.6	10.5	1.5
F3.1	1	11.2	5.8	8.3	9.7	12.6	47.5	9.5	2.6
	2	8.6	9.0	12.1	9.1	10.5	49.2	9.8	1.4
	3	9.0	7.8	7.3	8.3	9.7	42.1	8.4	1.0
F3.2	1	10.7	9.3	7.5	7.2	9.5	44.2	8.8	1.5
	2	8.5	7.3	8.9	9.2	9.5	43.4	8.7	0.9
	3	9.0	8.2	12.3	9.7	8.7	47.9	9.6	1.6
F3.3	1	8.0	9.5	7.8	8.7	6.5	40.5	8.1	1.1
	2	6.8	5.1	9.0	7.5	10.6	39.0	7.8	2.1
	3	10.0	8.3	9.9	8.4	8.8	45.4	9.1	0.8
F3.4	1	10.2	9.8	10.1	7.9	8.8	46.9	9.4	1.0
	2	10.2	9.0	11.2	6.8	7.8	45.0	9.0	1.7
	3	13.3	6.3	6.6	7.2	11.5	44.9	9.0	3.2
F3.5	1	4.3	5.5	6.7	5.7	5.6	27.8	5.6	0.8
	2	6.4	6.1	4.2	6.2	5.9	28.9	5.8	0.9
	3	7.0	5.3	7.3	5.4	5.9	31.0	6.2	0.9
F3.6	1	4.9	5.1	6.6	4.3	5.6	26.4	5.3	0.9
	2	5.5	6.5	3.8	5.1	5.2	26.1	5.2	1.0
	3	5.5	5.3	4.6	6.0	5.7	27.2	5.4	0.5
F3.7	1	6.9	4.7	6.0	7.3	5.3	30.3	6.1	1.1
	2	5.0	6.3	6.3	5.0	5.8	28.3	5.7	0.7
	3	6.4	6.0	6.1	5.7	5.0	29.1	5.8	0.5

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	11.7	12.9	9.7	16.8	11.3	62.3	12.5	2.7
	2	12.8	10.5	12.9	14.5	13.3	63.8	12.8	1.5
	3	10.4	11.8	13.7	13.6	15.2	64.7	12.9	1.9
F3.1	1	7.7	15.1	13.2	6.2	11.6	53.9	10.8	3.7
	2	10.2	12.3	14.0	10.9	10.7	58.0	11.6	1.5
	3	8.6	11.2	11.7	9.8	9.5	50.8	10.2	1.3
F3.2	1	8.5	11.0	12.4	10.8	8.8	51.5	10.3	1.6
	2	10.9	10.2	12.8	10.1	8.6	52.5	10.5	1.5
	3	10.8	10.3	10.1	11.4	14.7	57.2	11.4	1.9
F3.3	1	10.4	10.7	7.3	8.9	8.9	46.1	9.2	1.4
	2	6.0	8.5	10.2	12.8	7.5	44.9	9.0	2.6
	3	11.1	9.2	9.4	9.2	11.5	50.3	10.1	1.1
F3.4	1	11.5	9.9	11.2	9.4	11.5	53.5	10.7	1.0
	2	10.1	13.7	7.6	9.2	12.4	53.0	10.6	2.4
	3	7.7	8.3	6.6	16.3	14.4	53.3	10.7	4.4
F3.5	1	4.6	6.1	5.9	5.9	7.3	29.8	6.0	1.0
	2	6.9	6.4	4.4	6.7	7.1	31.5	6.3	1.1
	3	7.7	6.1	5.5	7.2	5.6	32.1	6.4	1.0
F3.6	1	5.6	6.1	7.0	5.3	4.4	28.5	5.7	0.9
	2	5.9	5.7	5.6	6.9	4.1	28.2	5.6	1.0
	3	5.8	5.0	6.3	6.2	6.4	29.7	5.9	0.6
F3.7	1	8.0	6.6	8.0	5.8	5.0	33.3	6.7	1.4
	2	6.8	5.3	6.3	5.1	7.1	30.5	6.1	0.9
	3	7.6	6.3	5.2	6.1	6.7	31.9	6.4	0.8

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	14.4	12.7	10.8	12.8	19.0	69.6	13.9	3.1
	2	14.6	12.2	15.1	16.6	15.5	74.1	14.8	1.6
	3	13.2	12.3	15.8	15.6	17.4	74.2	14.8	2.1
F3.1	1	12.9	15.2	17.7	6.8	9.7	62.3	12.5	4.3
	2	12.1	11.3	11.8	13.2	15.0	63.4	12.7	1.5
	3	12.6	10.8	12.9	9.6	10.5	56.3	11.3	1.4
F3.2	1	11.6	12.0	9.2	9.4	13.1	55.3	11.1	1.7
	2	9.7	11.2	11.2	11.9	13.9	57.9	11.6	1.5
	3	11.6	11.0	16.4	11.1	12.2	62.2	12.4	2.2
F3.3	1	9.4	11.3	7.4	11.0	9.3	48.4	9.7	1.6
	2	6.5	8.1	9.1	10.7	14.2	48.5	9.7	2.9
	3	9.9	9.9	12.0	12.8	9.8	54.3	10.9	1.4
F3.4	1	13.2	12.6	12.7	10.8	10.3	59.5	11.9	1.3
	2	10.0	8.4	14.0	11.4	16.2	59.9	12.0	3.1
	3	6.3	9.0	9.0	16.6	18.9	59.7	11.9	5.4
F3.5	1	6.3	7.9	6.2	6.3	6.5	33.2	6.6	0.7
	2	7.1	6.6	7.1	7.5	4.5	32.7	6.5	1.2
	3	8.3	5.4	5.8	7.2	6.4	33.0	6.6	1.2
F3.6	1	5.6	4.5	7.3	6.4	5.8	29.6	5.9	1.1
	2	4.2	6.8	6.1	5.5	6.2	28.9	5.8	1.0
	3	6.0	6.4	5.2	6.5	6.6	30.7	6.1	0.6
F3.7	1	8.5	8.4	5.0	9.9	6.0	37.8	7.6	2.0
	2	5.5	5.6	6.3	7.3	7.0	31.6	6.3	0.8
	3	6.2	5.2	6.0	6.2	7.9	31.6	6.3	1.0

Standard length (cm)

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	7.5	7.2	7.0	6.9	8.4	37.0	7.4	0.6
	2	7.4	7.1	7.6	8.0	8.0	38.1	7.6	0.4
	3	7.3	7.3	7.5	7.7	8.1	37.9	7.6	0.3
F3.1	1	7.0	7.3	7.9	5.5	6.5	34.2	6.8	0.9
	2	6.8	6.8	7.1	6.8	7.5	35.0	7.0	0.3
	3	7.1	6.7	6.9	6.5	6.6	33.8	6.8	0.2
F3.2	1	7.8	7.2	6.5	6.5	7.4	35.4	7.1	0.6
	2	6.4	6.7	7.0	6.8	7.6	34.5	6.9	0.4
	3	7.1	6.7	7.5	6.7	7.0	35.0	7.0	0.3
F3.3	1	6.0	6.9	5.6	6.6	6.3	31.4	6.3	0.5
	2	5.7	5.9	6.1	6.4	7.1	31.2	6.2	0.5
	3	6.2	6.5	7.0	7.3	6.6	33.6	6.7	0.4
F3.4	1	7.0	7.1	7.1	7.0	6.7	34.9	7.0	0.2
	2	6.4	6.2	7.2	6.6	7.5	33.9	6.8	0.5
	3	5.6	6.2	6.2	7.6	7.9	33.5	6.7	1.0
F3.5	1	5.6	6.1	5.7	5.4	5.8	28.6	5.7	0.3
	2	6.0	5.8	5.8	6.0	5.0	28.6	5.7	0.4
	3	6.4	5.7	5.6	6.1	6.1	29.9	6.0	0.3
F3.6	1	5.6	5.1	6.1	5.9	5.5	28.2	5.6	0.4
	2	5.0	5.8	5.7	5.9	5.5	27.9	5.6	0.4
	3	5.4	5.5	5.5	5.7	6.0	28.1	5.6	0.2
F3.7	1	6.2	6.1	5.0	6.0	5.5	28.8	5.8	0.5
	2	5.5	5.6	5.7	6.0	6.0	28.8	5.8	0.2
	3	5.6	5.3	5.8	5.9	6.1	28.7	5.7	0.3

Length of intestine (cm)

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	14.5	13.6	12.5	10.7	14.5	65.8	13.2	1.6
	2	11.1	12.5	11.5	12.5	13.3	60.9	12.2	0.9
	3	14.2	12.7	11.2	12.5	14.7	65.3	13.1	1.4
F3.1	1	12.4	11.9	15.0	8.7	12.0	60.0	12.0	2.2
	2	12.0	11.8	10.9	12.8	12.7	60.2	12.0	0.8
	3	10.8	10.7	11.0	10.3	11.5	54.3	10.9	0.4
F3.2	1	10.5	12.6	9.5	11.2	11.2	55.0	11.0	1.1
	2	10.7	10.9	9.5	11.2	11.2	53.5	10.7	0.7
	3	10.4	9.5	15.6	11.3	10.5	57.3	11.5	2.4
F3.3	1	9.5	9.8	9.5	9.5	10.1	48.4	9.7	0.3
	2	8.6	7.3	10.9	10.7	13.5	51.0	10.2	2.4
	3	9.5	11.2	12.6	12.0	9.7	55.0	11.0	1.4
F3.4	1	12.7	11.1	12.0	10.9	9.5	56.2	11.2	1.2
	2	11.1	8.5	13.7	11.6	12.3	57.2	11.4	1.9
	3	7.5	10.5	9.8	14.0	12.4	54.2	10.8	2.5
F3.5	1	9.1	10.3	10.1	8.2	9.2	46.9	9.4	0.8
	2	10.4	9.5	9.5	11.7	7.4	48.5	9.7	1.6
	3	8.5	6.7	7.1	8.7	8.6	39.6	7.9	0.9
F3.6	1	8.1	8.6	9.3	10.1	7.5	43.6	8.7	1.0
	2	7.2	8.0	8.9	9.0	8.6	41.7	8.3	0.7
	3	8.5	8.8	8.0	8.5	8.7	42.5	8.5	0.3
F3.7	1	10.2	10.4	7.5	10.0	9.2	47.3	9.5	1.2
	2	9.0	10.3	8.6	9.3	11.1	48.3	9.7	1.0
	3	10.4	8.6	10.3	8.4	9.2	46.9	9.4	0.9

Weight of intestine

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	1.0	1.0	0.7	0.8	1.1	4.5	0.9	0.2
	2	0.8	0.8	0.9	1.0	1.0	4.5	0.9	0.1
	3	0.9	0.9	1.0	1.0	1.2	4.9	1.0	0.1
F3.1	1	1.0	1.1	1.4	0.5	0.8	4.9	1.0	0.3
	2	1.0	0.9	0.8	1.1	1.2	5.0	1.0	0.1
	3	1.1	0.8	0.9	0.7	1.0	4.4	0.9	0.1
F3.2	1	0.8	0.9	0.7	0.7	0.7	3.7	0.7	0.1
	2	0.7	0.8	0.8	0.8	1.0	4.1	0.8	0.1
	3	0.7	0.7	1.1	0.8	0.7	4.0	0.8	0.2
F3.3	1	0.7	1.0	0.5	0.8	0.7	3.7	0.7	0.2
	2	0.4	0.5	0.7	0.8	1.2	3.6	0.7	0.3
	3	0.7	0.7	0.9	1.1	0.8	4.2	0.8	0.1
F3.4	1	1.0	0.9	0.9	0.9	0.8	4.4	0.9	0.1
	2	0.7	0.5	1.0	0.9	1.2	4.2	0.8	0.3
	3	0.3	0.7	0.6	1.2	1.3	4.0	0.8	0.4
F3.5	1	0.4	0.8	0.5	0.4	0.5	2.6	0.5	0.1
	2	0.6	0.5	0.6	0.6	0.3	2.6	0.5	0.1
	3	0.5	0.4	0.3	0.5	0.5	2.2	0.4	0.1
F3.6	1	0.4	0.3	0.5	0.5	0.4	2.2	0.4	0.1
	2	0.3	0.5	0.4	0.4	0.4	2.1	0.4	0.1
	3	0.5	0.5	0.4	0.5	0.5	2.4	0.5	0.1
F3.7	1	0.7	0.6	0.4	0.5	0.6	2.8	0.6	0.1
	2	0.5	0.5	0.5	0.5	0.7	2.6	0.5	0.1
	3	0.5	0.4	0.6	0.5	0.6	2.5	0.5	0.1

Weight of liver (g)

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	0.3	0.4	0.3	0.3	0.4	1.7	0.3	0.1
	2	0.3	0.3	0.3	0.3	0.3	1.4	0.3	0.0
	3	0.3	0.2	0.3	0.3	0.4	1.6	0.3	0.1
F3.1	1	0.3	0.3	0.3	0.1	0.2	1.2	0.2	0.1
	2	0.2	0.3	0.2	0.3	0.3	1.3	0.3	0.1
	3	0.2	0.1	0.2	0.2	0.2	0.8	0.2	0.0
F3.2	1	0.2	0.2	0.2	0.2	0.2	1.0	0.2	0.0
	2	0.1	0.2	0.2	0.2	0.3	0.9	0.2	0.0
	3	0.2	0.2	0.2	0.2	0.3	1.1	0.2	0.1
F3.3	1	0.2	0.3	0.1	0.2	0.3	1.0	0.2	0.1
	2	0.1	0.1	0.2	0.2	0.3	1.0	0.2	0.1
	3	0.1	0.2	0.2	0.2	0.2	0.9	0.2	0.0
F3.4	1	0.2	0.2	0.2	0.2	0.2	1.0	0.2	0.0
	2	0.2	0.1	0.2	0.2	0.4	1.2	0.2	0.1
	3	0.1	0.2	0.2	0.3	0.5	1.2	0.2	0.1
F3.5	1	0.1	0.2	0.1	0.1	0.1	0.5	0.1	0.0
	2	0.1	0.1	0.1	0.1	0.1	0.5	0.1	0.0
	3	0.1	0.1	0.1	0.1	0.1	0.5	0.1	0.0
F3.6	1	0.1	0.1	0.1	0.1	0.1	0.4	0.1	0.0
	2	0.1	0.1	0.1	0.1	0.1	0.4	0.1	0.0
	3	0.1	0.1	0.1	0.1	0.1	0.4	0.1	0.0
F3.7	1	0.1	0.2	0.1	0.1	0.1	0.5	0.1	0.0
	2	0.1	0.1	0.1	0.1	0.1	0.5	0.1	0.0
	3	0.2	0.1	0.1	0.1	0.1	0.5	0.1	0.0

Trial 4

14/10/2008

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	6.1	6.2	9.0	8.6	10.2	40.0	8.0	1.8
	2	9.0	6.6	8.0	8.5	9.3	41.3	8.3	1.1
	3	7.1	7.9	9.2	9.6	6.2	40.1	8.0	1.4
F4.1	1	6.7	8.4	7.2	8.5	9.7	40.5	8.1	1.2
	2	7.8	6.7	7.7	8.9	9.4	40.5	8.1	1.1
	3	6.3	7.8	8.5	8.3	9.6	40.5	8.1	1.2
F4.2	1	6.4	7.2	8.2	9.2	8.5	39.5	7.9	1.1
	2	6.6	6.3	8.0	9.4	9.9	40.1	8.0	1.6
	3	8.8	6.8	7.8	8.1	9.7	41.2	8.2	1.1
F4.3	1	6.2	7.6	8.0	9.1	8.9	39.7	7.9	1.1
	2	5.8	7.6	10.1	10.0	7.3	40.7	8.1	1.8
	3	8.0	7.6	10.0	6.8	7.8	40.1	8.0	1.2

21/10/2008

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	11.8	10.0	7.1	7.0	10.1	46.0	9.2	2.1
	2	9.6	10.6	9.4	7.6	9.4	46.6	9.3	1.1
	3	8.3	7.1	10.5	9.0	10.7	45.7	9.1	1.5
F4.1	1	8.5	11.3	9.1	10.0	7.9	46.8	9.4	1.3
	2	10.9	8.1	10.3	8.0	9.7	47.0	9.4	1.3
	3	6.9	9.7	9.4	11.0	9.0	46.0	9.2	1.5
F4.2	1	9.5	9.5	8.4	7.7	7.1	42.2	8.4	1.1
	2	7.3	8.8	6.8	10.8	11.0	44.8	9.0	1.9
	3	11.1	7.5	11.1	9.2	9.6	48.6	9.7	1.5
F4.3	1	9.7	7.2	8.8	9.4	8.6	43.7	8.7	0.9
	2	8.5	8.4	10.8	10.8	6.4	44.8	9.0	1.8
	3	8.4	11.5	9.3	8.0	10.2	47.3	9.5	1.4

28/10/2008

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	8.9	7.7	11.8	12.7	13.9	55.0	11.0	2.6
	2	11.4	9.1	11.3	10.9	13.1	55.9	11.2	1.4
	3	8.6	12.3	10.1	10.5	13.1	54.6	10.9	1.8
F4.1	1	9.6	10.6	9.3	11.8	13.8	55.2	11.0	1.8
	2	9.9	9.6	11.9	12.0	13.3	56.7	11.3	1.5
	3	8.9	10.9	12.6	11.0	10.8	54.2	10.8	1.3
F4.2	1	9.7	9.4	11.5	9.7	12.5	52.8	10.6	1.4
	2	7.2	10.3	9.1	13.3	13.2	53.1	10.6	2.6
	3	9.3	11.2	11.7	13.1	13.0	58.3	11.7	1.6
F4.3	1	9.2	10.7	12.0	9.9	11.0	52.7	10.5	1.1
	2	9.8	12.8	7.8	10.2	12.8	53.5	10.7	2.2
	3	10.2	9.5	9.4	11.8	14.2	55.2	11.0	2.0

4/11/2008

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	15.8	13.0	11.4	9.3	15.8	65.3	13.1	2.8
	2	13.0	10.7	15.7	12.8	13.8	66.0	13.2	1.8
	3	10.5	11.1	14.3	11.8	15.9	63.6	12.7	2.3
F4.1	1	12.9	11.3	13.8	16.9	11.8	66.6	13.3	2.2
	2	14.7	12.4	11.6	14.3	16.0	69.0	13.8	1.8
	3	14.6	13.0	12.9	12.9	9.3	62.7	12.5	1.9
F4.2	1	14.9	11.5	10.8	13.7	12.1	63.1	12.6	1.7
	2	10.1	16.0	12.7	16.1	11.8	66.6	13.3	2.7
	3	11.4	14.3	15.0	14.6	14.5	69.8	14.0	1.5
F4.3	1	14.2	13.1	13.0	11.8	11.8	63.8	12.8	1.0
	2	11.4	9.3	15.9	11.8	16.0	64.4	12.9	3.0
	3	11.2	11.3	14.2	11.9	17.5	66.0	13.2	2.7

11/11/2008

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	19.2	10.4	13.4	18.3	15.4	76.7	15.3	3.6
	2	18.0	12.4	16.6	14.5	14.8	76.4	15.3	2.2
	3	13.4	14.0	12.1	18.2	16.7	74.4	14.9	2.5
F4.1	1	11.6	15.5	15.0	15.1	18.5	75.7	15.1	2.4
	2	16.5	14.8	13.7	16.2	18.9	80.2	16.0	2.0
	3	15.3	16.3	14.9	17.2	15.1	78.8	15.8	1.0
F4.2	1	14.2	16.1	12.2	15.3	17.1	74.9	15.0	1.9
	2	18.9	14.2	13.2	15.0	18.2	79.5	15.9	2.5
	3	16.6	17.3	12.8	17.8	17.5	82.0	16.4	2.1
F4.3	1	16.0	15.9	13.6	14.2	14.8	74.5	14.9	1.0
	2	12.8	18.2	10.5	19.6	13.7	74.8	15.0	3.8
	3	13.2	13.3	13.8	16.9	20.8	77.9	15.6	3.3

18/11/2008

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	12.5	17.0	16.3	20.3	22.8	88.9	17.8	3.9
	2	14.1	16.2	18.7	21.3	17.5	87.8	17.6	2.7
	3	13.8	18.6	14.4	16.1	20.9	83.8	16.8	3.0
F4.1	1	17.1	12.8	17.8	17.6	20.8	86.1	17.2	2.9
	2	16.5	18.1	21.9	17.6	18.9	92.9	18.6	2.0
	3	17.0	19.0	19.0	17.1	17.1	89.2	17.8	1.1
F4.2	1	14.0	18.8	16.8	18.6	20.3	88.4	17.7	2.4
	2	17.4	14.8	21.2	17.8	21.1	92.3	18.5	2.7
	3	14.8	20.5	21.7	19.3	17.4	93.7	18.7	2.7
F4.3	1	15.7	18.6	15.7	18.1	17.4	85.4	17.1	1.3
	2	14.5	12.3	22.2	16.1	21.1	86.0	17.2	4.2
	3	15.4	16.0	16.1	19.9	24.0	91.4	18.3	3.7

25/11/2008

		Number of fish							
	Rep.	1	2	3	4	5	Sum	Mean	Std
Control	1	19.9	22.4	27.2	19.6	14.5	103.6	20.7	4.6
	2	20.0	16.6	18.4	21.7	25.0	101.6	20.3	3.2
	3	16.3	16.4	19.3	21.7	24.7	98.4	19.7	3.6
F4.1	1	15.0	20.4	19.6	20.8	24.3	100.1	20.0	3.3
	2	19.7	18.6	19.9	21.4	25.0	104.5	20.9	2.5
	3	20.0	21.4	21.8	19.5	19.6	102.3	20.5	1.1
F4.2	1	16.5	15.9	20.3	24.3	22.3	99.3	19.9	3.6
	2	17.1	19.8	21.4	25.2	24.6	108.1	21.6	3.4
	3	17.3	21.3	22.9	23.6	25.4	110.5	22.1	3.1
F4.3	1	18.8	18.3	22.4	19.7	21.0	100.2	20.0	1.7
	2	14.1	16.3	18.5	25.3	25.2	99.4	19.9	5.1
	3	18.0	18.4	27.8	18.0	23.7	105.9	21.2	4.4

2/12/2008

		Number of fish							
	Rep.	1	2	3	4	5	Sum	Mean	Std
Control	1	16.6	22.8	24.5	22.4	31.5	117.9	23.6	5.3
	2	20.8	19.9	24.0	23.4	23.9	112.0	22.4	1.9
	3	18.6	18.9	22.5	23.9	28.0	111.8	22.4	3.9
F4.1	1	17.7	21.6	24.5	23.0	27.3	114.0	22.8	3.6
	2	23.7	25.5	21.3	23.0	28.8	122.3	24.5	2.9
	3	22.1	26.3	22.7	22.0	24.0	117.1	23.4	1.8
F4.2	1	18.1	24.3	25.9	23.4	20.0	111.6	22.3	3.2
	2	22.5	19.2	27.3	25.5	28.0	122.6	24.5	3.7
	3	20.7	23.8	28.5	27.4	27.6	128.0	25.6	3.3
F4.3	1	21.4	20.9	21.6	24.4	26.3	114.5	22.9	2.3
	2	17.0	30.0	18.7	19.6	28.8	114.1	22.8	6.1
	3	21.2	20.7	27.8	20.7	31.9	122.2	24.4	5.1

9/12/2008

		Number of fish							
	Rep.	1	2	3	4	5	Sum	Mean	Std
Control	1	25.6	19.8	25.9	28.0	36.2	135.5	27.1	5.9
	2	23.9	28.2	23.7	28.5	33.2	137.4	27.5	3.9
	3	20.5	25.8	21.8	25.9	32.7	126.7	25.3	4.8
F4.1	1	23.8	24.5	27.8	26.7	31.6	134.5	26.9	3.1
	2	32.6	25.4	26.2	26.3	27.2	137.8	27.6	2.9
	3	25.1	25.2	31.2	26.4	25.8	133.7	26.7	2.6
F4.2	1	20.8	29.5	28.5	32.6	32.0	143.2	28.6	4.7
	2	26.2	31.0	30.0	26.6	30.9	144.7	28.9	2.4
	3	32.0	27.2	30.7	23.9	33.8	147.5	29.5	4.0
F4.3	1	25.8	24.4	25.7	27.6	30.3	133.7	26.7	2.3
	2	21.6	22.7	23.5	34.4	33.7	135.8	27.2	6.3
	3	23.8	23.7	23.7	31.8	36.2	139.1	27.8	5.8

Standard length

		Number of fish							
	Rep.	1	2	3	4	5	Sum	Mean	Std
Control	1	9.3	8.7	8.9	9.2	10.3	46.4	9.3	0.6
	2	8.7	9.3	8.6	9.4	9.8	45.8	9.2	0.5
	3	8.5	9.2	8.7	9.1	10.1	45.6	9.1	0.6
F4.1	1	8.3	9.0	10.0	9.5	10.0	46.8	9.4	0.7
	2	10.0	9.2	9.4	9.1	9.1	46.8	9.4	0.4
	3	8.9	9.1	9.7	9.5	9.2	46.4	9.3	0.3
F4.2	1	8.7	9.6	9.4	10.1	10.0	47.8	9.6	0.6
	2	8.6	10.0	9.7	9.4	10.1	47.8	9.6	0.6
	3	10.1	9.7	10.1	9.2	10.1	49.2	9.8	0.4
F4.3	1	9.2	9.1	9.5	9.4	9.9	47.1	9.4	0.3
	2	8.4	8.9	9.1	10.2	10.5	47.1	9.4	0.9
	3	9.1	9.3	9.0	10.0	10.5	47.9	9.6	0.6

Intestine length

	Rep.	Number of fish					Sum	Mean	Std
		1	2	3	4	5			
Control	1	13.0	13.8	13.9	12.3	17.6	70.6	14.1	2.1
	2	13.9	14.5	13.6	14.5	14.5	71.0	14.2	0.4
	3	12.5	13.0	14.0	13.0	15.0	67.5	13.5	1.0
F4.1	1	12.7	14.0	13.0	13.1	14.2	67.0	13.4	0.7
	2	15.7	12.2	16.0	15.5	13.3	72.7	14.5	1.7
	3	14.1	11.0	16.2	15.5	13.5	70.3	14.1	2.0
F4.2	1	14.3	14.3	13.9	14.2	14.0	70.7	14.1	0.2
	2	12.5	13.4	13.0	14.1	13.5	66.5	13.3	0.6
	3	14.4	14.3	17.0	12.7	15.0	73.4	14.7	1.6
F4.3	1	14.9	11.6	13.5	12.4	14.2	66.6	13.3	1.3
	2	12.8	12.3	11.7	16.3	15.6	68.7	13.7	2.1
	3	16.5	14.7	14.5	14.0	14.5	74.2	14.8	1.0

Intestine weight

	Rep.	Number of fish					Sum	Mean	Std
		1	2	3	4	5			
Control	1	1.31	1.32	1.48	1.55	2.28	7.94	1.59	0.40
	2	1.73	2.01	1.82	1.90	2.00	9.46	1.89	0.12
	3	1.26	1.26	1.65	1.55	2.22	7.94	1.59	0.39
F4.1	1	1.24	1.46	1.74	1.62	2.17	8.23	1.65	0.35
	2	1.89	1.40	1.82	1.78	1.72	8.61	1.72	0.19
	3	1.57	1.53	2.38	1.58	1.48	8.54	1.71	0.38
F4.2	1	1.34	2.07	1.83	1.97	1.86	9.07	1.81	0.28
	2	1.46	1.85	1.95	2.09	1.89	9.24	1.85	0.24
	3	1.86	1.71	1.99	1.44	2.15	9.15	1.83	0.27
F4.3	1	2.36	1.56	1.86	1.74	2.19	9.71	1.94	0.33
	2	1.53	1.39	0.62	2.63	2.19	8.36	1.67	0.77
	3	1.62	1.79	1.68	2.07	2.38	9.54	1.91	0.32

Weight of fish liver in Trial 4 (g)

	Rep.	Number of fish							
		1	2	3	4	5	Sum	Mean	Std
Control	1	0.44	0.45	0.53	0.50	0.79	2.71	0.54	0.14
	2	0.63	0.61	0.48	0.72	0.82	3.26	0.65	0.13
	3	0.38	0.47	0.47	0.44	0.62	2.38	0.48	0.09
F4.1	1	0.49	0.45	0.51	0.51	0.60	2.56	0.51	0.05
	2	0.64	0.47	0.60	0.45	0.45	2.61	0.52	0.09
	3	0.54	0.41	0.59	0.42	0.47	2.43	0.49	0.08
F4.2	1	0.41	0.59	0.43	0.58	0.58	2.59	0.52	0.09
	2	0.31	0.52	0.50	0.61	0.43	2.37	0.47	0.11
	3	0.50	0.67	0.59	0.54	0.65	2.95	0.59	0.07
F4.3	1	0.60	0.58	0.39	0.52	0.72	2.81	0.56	0.12
	2	0.46	0.30	0.41	0.67	0.79	2.63	0.53	0.20
	3	0.48	0.46	0.31	0.55	0.69	2.49	0.50	0.14

Declaration of Originality

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