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**Studies on the nutritional quality of plant materials used as fish feed
in Northern Vietnam**

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Eidesstattliche Erklärung

Hiermit versichere ich, diese Arbeit selbstständig angefertigt und keine anderen, als die angegebenen Hilfsmittel verwendet zu haben. Zitate sind im Text kenntlich gemacht. Diese Arbeit ist noch nicht in dieser oder anderer Form einer Prüfungsbehörde vorgelegt worden.

Stuttgart den 08. 06. 2009.

Euloge brice Dongmeza

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1. Summary

Aquaculture output has been growing at a high pace for decades. According to recent data published by the FAO, in 2007, 49% of fish and fishery products (excluding fish meal) consumed as food were farmed. This share is expected to reach 50% in 2008. Fish demand has risen worldwide as populations have grown and incomes have increased; thus fish are highly likely to continue becoming more expensive over the next two decades. However, this expansion might also cause a significant rise in the price of fishmeal which could endanger the availability of fish to the lower income groups and poor people in developing countries. Consequently the use of supplementary feeds in fish culture is therefore expected to increase. Fisheries and aquatic products are an important source of protein in Vietnamese diets. Nevertheless, in the upland areas, fish is scarce and expensive, and signs of protein malnutrition such as discoloration of hair and skin could be frequently observed among the poor inhabitants of Son La province, Northern Vietnam where the average price for fish on the local market was approximately 1.4 US\$ kg⁻¹ in 2005 which can be considered high, particularly when compared to the monthly per capita income in Son La of approximately 13.4 US\$. In some villages nearly all households have at least one pond. The major inputs to the ponds system are crop leaves and residues and occasionally grasses and weeds. Fish are cultured in tilapia and cyprinid-based polyculture, the main species being grass carp which is the only species capable of efficiently ingesting and digesting the soluble cell contents of the leaf material used as input to the pond system. Despite these constraints, fish farming contributes enormously to food security in the region and generates incomes. Fish fed exclusively from macrophyte diets grow slow, and thus the annual fish production is low in the region. Up to now, none of the feeds currently used in Son La Province have been tested to assess the nutritional potential of the plant feed ingredients currently used as feed for fish in northern Vietnam. Neither their use as feed for grass carp, nor the seasonality of the chemical composition has been studied; furthermore, the digestibility and the antinutrient content of these plant residues and materials have not been investigated. It has widely been recognized that encouraging the further development of aquaculture production can contribute in a sustainable manner to food security and poverty alleviation in developing countries.

Therefore, it is the objective of this study to present a quantitative and qualitative evaluation of the gross chemical composition, energy and antinutrient content of the plant residues used as fish feed in the course of the year in Son La Province, Northern Vietnam. Subsequently feeding experiments were conducted and the apparent digestibility and metabolisability of the nutrients and energy of some of these plant residues in grass carp were determined.

All the experiments were conducted simultaneously in a computer controlled respirometric system, which allowed feeding and continuous measurement of oxygen consumption and in a water recirculation aquaria system where the faeces collection was more viable.

During the analysis, the plant materials were divided into two groups:

the first group contained plant material available all year round like bamboo, banana and cassava leaves. Banana leaves had a constant chemical composition and antinutrient content all over the year. Significant temporal changes were detected in the crude protein, acid detergent fibre, ash and energy as well as in the phenolic and phytic acid (PA) content of cassava leaves. Significant changes were also observed in the lipid and neutral detergent fibre content of the bamboo leaves whereas their antinutrient content remained constant during the course of the year. Only the saponin (SAP) from cassava leaves had a pronounced haemolytic activity against cattle erythrocytes.

The second group contained feed items occasionally fed to fish by farmers. The results of proximate analysis showed a great variability in the crude protein, crude fibre, ash and energy content of the plant feedstuffs expressed as % dry matter (DM) which ranged respectively between 1.4 and 27.4%, 2.4 to 30.5%, 2.9 to 30.4% and 8.5 to 22.1 kJ g⁻¹. Analysis of antinutrients showed that with the exception of napier grass and cassava tubercle tannic acid was detected in all other feedstuffs with concentration varying from 0.1 to 4.9% DM; SAP concentration ranged from 0.9 to 9.9% DM, however it was not detected in cassava tubercle and shingle flatsedge. The cyanide content of the different cassava products ranged between 323 ppm (in peels) to 102 ppm in tubercle. The amount of PA content was high (8.8% DM) for napier grass, but it was generally within the tolerable limits of fish for the other feedstuffs analysed in this study.

During the feeding experiment fish were fed six different plant feedstuffs (additionally to the same amount of a reference diet fed also to a control group). Significant additional body weight increase was obtained with grass carp fed additional amount of dried banana or fresh maize leaves. Whereas for grass carp fed additional dried maize or bamboo leaves, weed mixture or barnyard grass we had a significant diminution of the expected body weight gain (BWG). The apparent digestibility coefficient for crude protein, lipid and energy of the supplemented banana leaves were 92.9 ± 2.5 , 62.4 ± 7.0 and $25.2 \pm 0.7\%$ respectively and of fresh maize leaves 70.6 ± 5.5 , 35.9 ± 12.0 and $39.5 \pm 6.8\%$ respectively.

The O₂ consumption of the fish fed the different experimental diets was measured and it was found that the metabolic cost (amount of O₂ per unit of BWG) of the diets containing plant leaves was higher than those of the control groups and was significantly higher for the diets containing bamboo leaves or weed mixture.

In this study, the analysis of the different feedstuffs fed to fish in northern Vietnam showed that some of them had relatively high protein and low fibre content (banana and cassava leaves); however, for some feedstuff the antinutrient content was high.

The results of the present study indicate that the protein and other nutrients of banana and fresh maize leaves are valuable supplements in fishmeal-based diets for grass carp as they meet the nutritional demands and excel a fast growth of these fish. The findings clearly show that fresh and dry maize leaves have a contrary effect on the growth of grass carp. The role of dietary plant fibres and some antinutrients on nutrient assimilation in grass carp could be elucidated in this work.

The information provided in this study is a good base for scientists and extension workers to develop improved feeding strategies based on plant material available locally for grass carp. These findings should be further tested under pond conditions as they could lead to an increase of fish productivity with plant based feeds and enhance the livelihood of the small-scale farmers in the rural areas in Vietnam.

2. Zusammenfassung

Die Aquakulturproduktion ist in den letzten zehn Jahren stetig und signifikant gestiegen. Nach neuesten Berechnungen der FAO stammen 49% der in 2007 weltweit konsumierten Fische und Fischprodukte aus Aquakultur. Für 2008 wird erwartet dass dieser Anteil sogar noch auf 50% steigen wird. Die Gründe für diesen Zuwachs liegen in der weltweiten Zunahme der Bevölkerung und deren Einkommen. Es wird auch erwartet, dass die Preise für Fischprodukte wie z.B. Fischmehl steigen werden. Dieser preisliche Anstieg könnte für einkommensschwache Haushalte in Entwicklungsländern eine erhebliche Bedrohung ihrer Ernährungssicherung darstellen. Einhergehend mit einer erhöhten Fischproduktion, wird sich auch der Bedarf an Futterzusätzen erhöhen.

Fisch und andere Aquakulturprodukte sind eine wichtige Proteinquelle in der vietnamesischen Küche. Jedoch ist Fisch in den Hochlandgebieten, wie z.B. der nordvietnamesischen Provinz Son La, selten und teuer. Häufig wurden bei der ärmeren Bevölkerung von Son La Proteinmangel beobachtet, z.B. in Form von Pigmentstörungen von Haaren und Haut. Die Durchschnittspreise für Fisch lagen dort auf dem lokalen Markt bei ca. 1.4 US\$ kg⁻¹ in 2005, was im Vergleich zu dem monatlichen Pro-Kopf-Einkommen von 13.4 US\$ einen beträchtlichen Anteil darstellt.

In Son La wird in den Tälern Aquakultur betrieben. Fische werden in Polykulturteichen gehalten und im Wesentlichen mit Blättern und Nebenprodukten der pflanzlichen Produktion sowie mit Unkräutern und Gräsern gefüttert. Die produzierten Fische bestehen überwiegend aus Karpfen und Tilapien. Dabei ist nur die am meisten verwendete Art, der Graskarpfen, anatomisch und physiologisch daran angepasst, pflanzliches Material mechanisch und enzymatisch aufzubrechen und dadurch die Zellinhalte aufzunehmen und zu verwerten.

Obwohl die Aquakultur in der Region fast ausschließlich auf der Fütterung von minderwertigem Pflanzmaterial besteht, trägt die Fischzucht einen wesentlichen Beitrag zur Ernährungssicherung und Einkommensgenerierung der lokalen Bevölkerung bei. Es wurde weitgehend anerkannt, dass die weitere Entwicklung der Aquakultur nachhaltig die

Ernährungssicherung und die Reduktion der Armut fördern könnte. Bis heute wurden jedoch keine der Futtermittel, die in Son La eingesetzt werden getestet um ihr nutritives Potential als Fischfutter zu bestimmen. Weder das Futter für die Graskarpfen, noch deren jahreszeitlich bedingten Variationen in der chemischen Zusammensetzung wurden bisher analysiert. Des Weiteren wurden auch nicht die Verdaulichkeit und die antinutritiven Inhaltsstoffe der genutzten Pflanzen untersucht.

Folglich ist es die Zielsetzung dieser Arbeit, eine quantitative und qualitative Auswertung der in Son La eingesetzten Futtermittel für Fische zu präsentieren. Dabei wurde die grobe chemische Zusammensetzung, der Energiegehalt und die Art und Menge der antinutritiven Inhaltsstoffe analysiert.

Anschließend wurden Fütterungsexperimente mit Graskarpfen durchgeführt und die scheinbare Verdaulichkeit und Metabolisierbarkeit der in einiger dieser Pflanzenrückstände enthaltenen Nährstoffe und Energie ermittelt. Alle Experimente wurden parallel in zwei Systemen durchgeführt, a) in einem computergesteuerten Respirometrischen System, das eine kontinuierliche Messung des Sauerstoffverbrauchs ermöglichte und b) in einem geschlossenen Wasserkreislaufsystem, in dem der Fischkot gesammelt werden konnte.

Während der Analyse wurden die Pflanzenrückstände in zwei Gruppen geteilt:

die erste Gruppe enthielt das pflanzliche Material, welches ganzjährig verfügbar war, wozu Bambus-, Bananen- und Maniokblätter zählten. Bananenblätter, die in verschiedenen Jahreszeiten gesammelt wurden, wiesen durchgehend eine konstante chemische Zusammensetzung und antinutritiven Stoffgehalt auf. Bedeutende jahreszeitliche Änderungen in Maniokblättern wurden im Rohprotein, Fett, in neutrallöslichem Fasergehalt, der Asche und dem Energiegehalt, sowie der Menge an phenolischen Substanzen und Phytinsäure festgestellt. Im Jahresverlauf zeigten auch Bambusblätter signifikante Unterschiede in ihren Fett- und neutrallöslichen Fasergehalten, während die Menge an antinutritiven Substanzen konstant blieb. Die Saponine der Maniokblätter hatten eine ausgeprägte hämolytische Aktivität gegen Vieherythrozyten.

Die zweite Gruppe enthielt Futtermittel, die den Fischen nur gelegentlich von den Bauern gefüttert wurden. Die Ergebnisse der chemischen Analyse zeigten eine große Variabilität in Rohprotein (1.4 - 27.4% der Trockenmasse (TM)), Rohfaser (2.9 - 30.4% TM), Energie (8.5 - 22.1 kJ g⁻¹ TM) und im Aschegehalt (2.4 - 30.5% TM) der pflanzlichen Futterstoffe.

Die Analyse der antinutritiven Inhaltsstoffe zeigte, dass mit Ausnahme von Napiergras und Maniokwurzeln, in allen weiteren Futtermitteln Tannine (Gerbsäuren) mit Konzentrationen zwischen 0.1 bis 4.9% der TM vorhanden waren. Der Saponingehalt reichte von 0.9 bis 9.9% der TM, wobei sie nicht in Maniokwurzeln und Zyperngras festgestellt wurden. Der Zyanidgehalt der unterschiedlichen Maniokprodukte erstreckte sich zwischen 102 ppm in den Wurzeln bis zu 323 ppm in den Schalen. Mit Ausnahme von Napiergras, in dem der Phytinsäuregehalt mit 8.8% der TM sehr hoch war, lagen die Phytinsäurewerte der anderen in dieser Studie untersuchten Futtermittel deutlich unter dem für Fische tolerierbaren Richtwert.

Während der Fütterungsversuche wurden den Fischen zusätzlich zu einem Fischmehl-basierten Grundfutter sechs verschiedenen Pflanzenmaterialien (Maniok-, Bananen-, Bambus- und frische und trockene Maisblätter, Hühnerhirse und ein Mix aus Unkräutern) gefüttert. Die Kontrollgruppe bekam nur das Grundfutter in der gleichen Menge, wie die Fische, die zugefüttert wurden. Die zugefütterten Fische bekamen also insgesamt eine größere Menge Futter und mehr Nährstoffe als die Fische der Kontrollgruppe. Graskarpfen, die zusätzlich zum Grundfutter auch noch frische Mais- oder getrocknete Bananenblätter erhielten, zeigten einen signifikant gesteigerten Körpergewichtszuwachs im Vergleich zur Kontrollgruppe. Im Gegensatz dazu zeigten Graskarpfen die zusätzlich mit getrockneten Mais- oder Bambusblättern, Hühnerhirse oder dem Mix aus Unkräutern gefüttert wurden ein deutlich geringeres Wachstum als die Fische aus der Kontrollgruppe.

Die scheinbaren Verdaulichkeitskoeffizienten lagen bei Bananenblättern bei $92.9 \pm 2.5\%$ für Rohprotein, $62.4 \pm 7.0\%$ für Fett und $25.2 \pm 0.7\%$ für Energie. Bei frischen Maisblättern lagen die Koeffizienten bei $70.6 \pm 5.5\%$ für Rohprotein, $35.9 \pm 12.0\%$ für Fett und $39.5 \pm 6.8\%$ für Energie.

Die durchgeführten Sauerstoffverbrauchsmessungen ergaben, dass die metabolischen Kosten (verbrauchte Menge O₂ pro Einheit Massenzunahme) für die Fischgruppen, die mit Blättern

oder Gräsern zugefüttert wurden, allgemein höher waren, als die der Kontrollgruppen; für die Fische deren Futter zusätzlich Bambusblätter, Unkräuter oder Gras enthielten war der O₂ Verbrauch pro Einheit Massenzunahme sogar signifikant höher als die der Kontrollgruppen.

Die in dieser Studie enthaltenen Analysen der in Nordvietnam eingesetzten Fisch-Futtermittel zeigten, dass einige einen relativ hohen Anteil an Proteinen und einen geringen Anteil an Fasern hatten, wie z.B. Bananen und Maniokblätter. Jedoch zeigten einige Futtermittel ebenfalls einen hohen Gehalt an antinutritiven Inhaltsstoffen auf.

Die Ergebnisse dieser Studie deuten an, dass das Protein und die anderen Nährstoffe von Bananen- und frischen Maisblättern eine wertvolle Ergänzung für fischmehlbasierendes Graskarpfenfutter sind, da sie den Bedarf der Fische decken und das Wachstum beschleunigen. Zusätzlich zeigen die Ergebnisse deutlich, dass getrocknete und frische Maisblätter einen gegensätzlichen Effekt auf das Wachstum der Fische haben.

Die Rolle der pflanzlichen Fasern und einiger antinutritiver Nährstoffe auf die Nährstoffassimilation in Graskarpfen konnte in dieser Studie beleuchtet werden. Die hier präsentierten Informationen stellen eine gute Basis für Wissenschaftler und staatliche Berater dar, um auf lokal verfügbaren, pflanzlichen Futtermitteln basierende Fütterungsstrategien zu entwickeln. Diese Ergebnisse sollten jedoch noch unter lokalen Bedingungen getestet werden, um eine gesteigerte Produktivität, erreicht durch pflanzenbasierte Futtermittel, nachzuweisen. Eine solche gesteigerte Produktivität würde letztendlich zu einer verbesserten Lebensgrundlage für die Kleinbauern in den ländlichen Gegenden Vietnams führen.

3. General Introduction

3.1 Introduction

Aquaculture output has been growing at a high pace for decades. The total world aquaculture production in the year 2006 was 51.7 mmt or 36% of total world fisheries landings of 143.6 mmt (FAO 2007).

In 2007, 49 % of fish and fishery products (excluding fish meal) consumed as food were farmed, a share expected to reach 50 % in 2008. This will be an important milestone in the history of aquaculture and in world fisheries supply.

The transition to an increasing reliance on culturing fish and other aquatic organisms has been the result of a combination of several factors, the most important being the inability of natural supplies to keep pace with rising demand (Williams, 1996). Fish demand has risen worldwide as populations have grown, incomes have increased, the nutritional benefits of fish have become better known, and luxury products from aquatic organisms have become status symbols.

Globally, production from capture fisheries has leveled off and most of the main fishing areas have reached their maximum potential. Future increases in demand for fish cannot be met by increasing catches from most natural fishery resource systems (FAO 2006). Thus fish are highly likely to continue becoming more expensive to consumers compared with other food products over the next two decades. Prices for food fish are likely to rise under nearly all scenarios. Therefore rapid aquaculture expansion is the only scenario which can lead to a drop in the projected real prices of low-value food fish. However, this expansion might also cause a significant rise in the price of fishmeal (Delgado *et al.*, 2003); which could endanger the availability of fish to the lower income groups and poor people in developing countries (Williams, 1999). The scenario that could better lead to slightly lower real fish prices is the one that improves efficiency in fishmeal and fish oil conversion through rapid technological progress.

There are some apprehensions that aquaculture will not be able to supply the growing demand for fish, especially the requirements of the lower income groups in the developing countries, unless deliberate interventions are made to either involve these groups in aquaculture production and/or improve aquaculture efficiency and productivity to make fish affordable for these consumers.

3.2 General aspects of the aquaculture system in the study region: the district Yen Chau in the province Son La in Vietnam

The consumption of fish is a tradition of Vietnamese people and aquatic products will remain a primary source for animal protein for them for the foreseeable future. Fisheries and aquatic products provide more than 30 to 40% of the protein in Vietnamese diets (Oxfam, 2000). MoFI estimates that between 60 to 70% of the cultured and caught fisheries products are consumed domestically (DANIDA, 2002). Home consumption of fish in Viet Nam was estimated by FAO at 17.4 kg per capita per year in 1997. Of this 17.4 kg about 5.7 kg was freshwater and diadromous fish (FAO, 2001). A later survey undertaken jointly by Food and Agriculture Organization (FAO), MoFI and Danish International Development Agency (DANIDA) indicates that the actual domestic consumption could be much higher than the above-mentioned estimates (DANIDA, 2002). A continuous increase in the total domestic and per capita consumption of fish was observed in all three surveys.

Freshwater aquaculture is the largest contributor to total aquaculture production (57.3%) (FAO, 2006). Since 1990 aquaculture production has been increasing continuously over the years (see Figure 1) and Viet Nam is currently acknowledged as having the fastest aquaculture growth rate in Asia (FAO, 2007). The target of the Vietnamese national fisheries development plan is to produce 2.0 million tonnes of aquaculture products in 2010 (MoFI, 2004).

Both fisheries and aquaculture activities in Vietnam are concentrated along the coast and in the river deltas.

The period between 1999 and 2001 witnessed a peak in growth of aquaculture in Vietnam, aquaculture now employs about 670 000 workers out of the total of 4 millions in fisheries.

The area under aquaculture has now reached nearly one million hectares (902 900 ha) with a total output of over one million tones which provides about 80 percent of the materials required for the processing and export sectors. The total revenue obtained in 2004 was over 6 000 billion VND (1 US\$ = 15 870 VND) with an export turnover of US\$ 2.397 billion (MoFI, 2005). The aquaculture sector has been considered as one of the key economic sectors of the nation, according to MoFI (2005).

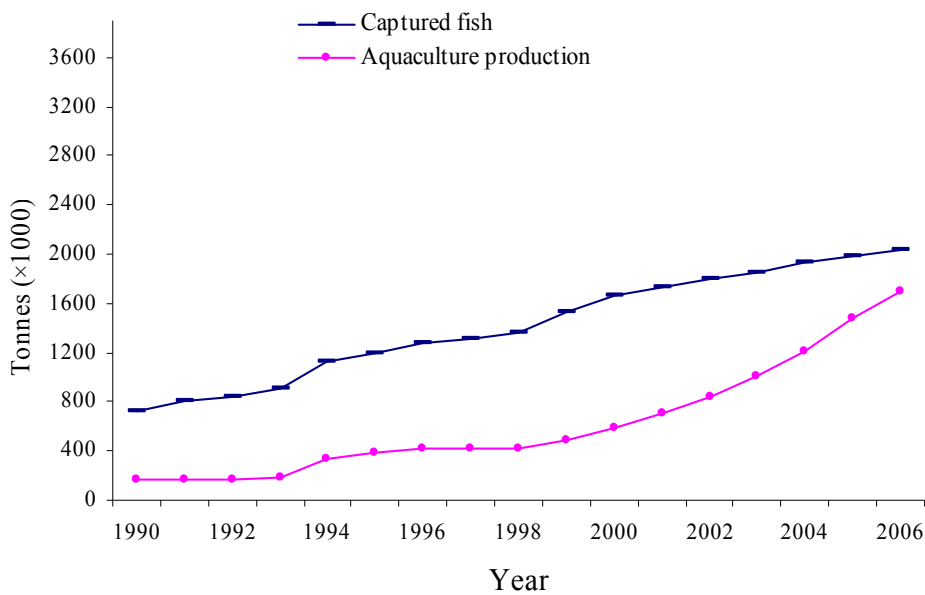


Figure 1: Reported aquaculture production and capture fish in Viet Nam
 (from 1990 to 2006)
 (GSO (General Statistic Office) of Vietnam, 2007)

Son La province is located in the mountainous region of North-Western Vietnam on an altitude of approximately 600 m above sea level (Tuan, 2003) and is considered to be one of the poorest provinces in the country (Minot *et al.*, 2003). The climate is characterized by low temperatures, hoarfrost and little rainfall in winter and high precipitation accompanied with hot temperatures in summer. The poorest people in Vietnam are often found among members

of the country's ethnic minorities and the population of remote areas such as the Northern mountainous areas (DFID, 2000; ADB, 2002).

In the upland areas, fish is scarce and expensive, and signs of protein malnutrition such as discoloration of hair and skin could be frequently observed among the inhabitants of Son La province, Northern Vietnam.

In the district Yen Chau of the Son La province, settlements of the ethnic Black Thai minority are located on the valley bottoms with paddy rice as major crop. In addition, maize, cassava and occasionally cotton are produced as cash crops on the hillsides and vegetables and fruits are grown in home gardens. Common animals reared comprise poultry, ruminants and pigs, but fish farming is also a typical activity in this region. Fish are cultured in tilapia and cyprinid-based polyculture, the main species being grass carp. The ponds have a continuous water-flow and the fish are fed mainly grass and leaf material. Depending on the village, 40-70% of all households have at least one pond. The pond size varies between 250 m² and 12 000 m². However, most ponds are smaller than 1000 m². The ponds are integrated with other farming activities through manifold linkages (Steinbronn *et al.*, 2005). Out of the five fish species commonly used in the project region (grass carp (*Ctenopharyngodon idella*); bighead carp (*Hypophthalmichthys molitrix*); mud carp (*Cirrhinus mrigala*); common carp (*Cyprinus carpio*); tilapia (*Oreochromis niloticus*)), only grass carp are capable of efficiently ingesting and digesting the soluble cell contents of the leaf material used as input to the pond system. Major inputs to the ponds system are cassava, banana and bamboo leaves and occasionally grasses and weeds, as well as manure from large ruminants and pigs (Steinbronn *et al.*, 2006)

Only slow growth can be expected from fish fed exclusively macrophyte based diets (Wiley and Wilke, 1986). Up to now, none of the feeds currently used in Son La Province has been tested as feed for grass carp, nor have the seasonality of the chemical composition and the digestibility and the antinutritional content of these plant residue materials been investigated. It has been reported in several studies that the use of plant materials (including leaves) is usually restricted because of the presence of one or more endogenous antinutritional factors or antinutrients (Jackson *et al.*, 1982; Wing Keong *et al.*, 1989; Makkar and Becker, 1997; Makkar, 2007).

For the pond system, grass carp plays an important role as they shred the leaf material and can fertilize the pond. In the example of grass carp fed duck weed, 45% of the gross energy of the plant feed were still contained in the faeces (Cui et al. 1992). For fish consuming leaves with higher fiber content, this figure is normal and can even be higher. Thus fecal material of the fish (grass carp) can contribute to the detritus pool in the pond.

Although the aquaculture system in Son La shows features which are usually associated with intensive systems such as a frequent water exchange and apparently being supplementary feed-based (Edwards et al., 1988), annual fish yields are relatively low, varying from 300kg/ha (van Anrooy et Evans, 2001) to an average of 1,6t ha⁻¹, according to the General Statistic Office of Vietnam (GSO 2008). This annual production is rather lower in comparison with other integrated carp polyculture systems in Vietnam, which are reported to yield over 6 tons ha⁻¹ during a 9-month production season (Luu *et al.*, 2002).

The low productivity can partly be explained by the low quality and limited availability of water as well as a food base that is apparently adequate for grass carp, but not for other fish species.

Despite these constraints, fish farming contributes enormously to food security in that region, generates income and plays a significant role in farmers' lives. Low-income people in developing countries, who usually consume comparatively low amounts of animal protein, often depend on fish as a major source of protein in their diets (Kent, 1997).

The average price for fish on the local market was approximately 1.4 US\$ kg⁻¹ in 2005 (1 US\$ = 15 870 VND), which can be considered high, particularly when compared to the monthly per capita income in Son La of approximately 13.4 US\$ (GSO, 2004), indicating that local fish demand is in excess of supply leaving farmers a potential scope for increasing their fish production. Small improvements in the system might lead to big increases in fish yields (Steinbronn et al., 2006) and ameliorate the income of the local poor smallholder. It has widely been recognized that encouraging the further development of aquaculture production can contribute in a sustainable manner to food security and poverty alleviation in developing countries (Tacon, 1997; Edwards, 2000; Prein and Ahmed, 2000).

3.3 Grass carp (*Ctenopharyngodon idella* Val.)

The Grass carp is a member of the Cyprinid family (Cyprinidae). It is the only member of the genus, and there are no subspecies (Lin, 1935). Sometimes it is referred to as the white amur. It is a native Chinese freshwater fish with a broad distribution. Grass carp has a long history in aquaculture and is one of the most important species cultured in inland water bodies in China and in many other countries in the world. In 2002, 39 countries and regions reported cultured production of grass carp to FAO. The fact that it is easily cultured and hardy (Dupree and Huner, 1984), has a fast growth rate (Blackburn and Sutton, 1971; Shireman *et al.*, 1977, 1978; Shireman *et al.*, 1980), large size (Prowse, 1966), lack of fine inter-muscular bones and most importantly, its feeding habits (Tang, 1970; Lopinot, 1972) make the fish an ideal species for culture in different areas. Rapid expansion of its culture outside China implies that this great potential is being realized.

Grass carp exhibit strong preferences for different macrophyte feeds depending on the aquatic system. It is basically a herbivorous fish (He and Xie, 1966; Opuszynski, 1972; Wen, 1990) that naturally feeds on certain aquatic weeds. Grass carp daily consumption rates are highly variable, depending on the interplay of different factors including temperature, oxygen, food availability, and preference (Chilton II and Muoneke, 1992). Jensen (1986) reported consumption rates of up to 300% body weight/day, but according to Opuszynski (1972), daily consumption at 20°C is about 50% body weight, and maximum daily consumption at 22-33°C is 100-120% body weight. It has been pointed out that fish fed hydrilla (*Hydrilla* spp.) consumed $127 \pm 17\%$ of their body weight /day (Wattendoff and Anderson, 1987). However, Wiley and Wike (1986), using experimental fish of roughly the same size, never found daily consumption rates exceeding 50% body weight. An inverse linear relationship between grass carp size and consumption was reported by Osborne and Sassic (1981), thus macrophyte consumption by large grass carp, weighing more than 6 kg, may be as low as 25-28% body weight /day (Shireman and Maceina, 1981).

Food habits of grass carp change as they grow and develop (Chilton II and Muoneke, 1992). The fry/larvae feed on zooplankton. Opuszynski (1968) reported that fry feed on animal food until they reach lengths of 3-4 cm and the proportion of macrophytes consumed increases

with grass carp growth (Opuszynski, 1972). Watkins *et al.* (1981) examined gut contents of fingerlings ranging from 17 to 117 mm total body length (TL). Fish of 17- 31 mm TL consumed primarily benthic invertebrates. In fish of 32-86 mm TL, periphyton comprised the major portion of the diet. Hydrilla, *Hydrilla* spp., along with bank vegetation comprised 86% of the diet of larger fish: larger fish could naturally tolerate higher amount of macrophytes in their diet and probably made good use of it.

Food habits of grass carp may also be affected by stocking site and situation (Chilton II and Muoneke, 1992). For example, Kilgen and Smitherman (1971) reported that when stocked alone in ponds, grass carp (more than one year old) ate mainly macrophytes (75-95% by volume), but a maximum of 18% of their gut contents were insects. When they were stocked in combination with other species, their diet consisted of 84% macrophytes and only 9% insects.

Consumption by young fish, below 60 g of body weight, tends to be greater when they are fed mixed plant/animal food than when they are fed plant food exclusively. Fischer (1973) suggested that plant food should optimally only comprise about 25% of the grass carp diet; a result suggesting that young grass carp should be omnivorous rather than exclusive herbivores for normal growth to occur (Fischer, 1972a and b). Data from a number of studies suggest that grass carp need cellulose and protein in the diet for optimal growth and health (Chilton II and Muoneke, 1992). In older fish, consumption is twice as high on a pure plant food diet as it is on either an animal food diet or a mixed regime (Chilton II and Muoneke, 1992).

Growth in grass carp is also influenced by which plant species are consumed (Cai and Curtis, 1989).

Although a major part of the food ingested by grass carp is also excreted, a relatively high percentage of the retained material is assimilated. As a result, feed conversion efficiencies (ratio of the weight gain of the fish to the dry weight of food consumed) may be supposed to be very high (Chilton II and Muoneke, 1992). Sutton and Blackburn (1972) reported conversions ranging from 22 to 79%, which are fairly high for herbivorous animals; However, Stott and Orr (1970) reported values as low as 7.5% (for lettuce). In the pond system, they can

play an important role as they shred the leaf material, and the high amount of faeces excreted by grass carp can fertilize the pond and increase the zooplankton number and the biomass in the pond (Mitchell *et al.*, 1984, Maceina *et al.*, 1992).

Under culture conditions, grass carp can well accept artificial feed such as the by-products from grain processing and vegetable oil extraction meals, and pelleted feeds, in addition to aquatic weeds and terrestrial grasses; the culture of grass carp can be well integrated into crop farming and animal husbandry, to maximize the utilization of natural resources. Thus grass carp can be produced at low cost. On the other hand, it is acceptable to consumers in many countries and it very likely has good potential for development.

There have been great efforts devoted to research on this species. The most important achievement has been success in the development of induced breeding technology which ensures a constant supply of seed for large-scale farming (Konradt, (1968); Bailey and Boyd (1971); Dupree and Huner (1984)). Another important aspect of research was the study of nutritional requirements (Fischer and Lyakhnovich, 1973; Stanley, 1974; Dabrowski, 1977; Liao *et al.*, 1980; Lin *et al.*, 1980; Huisman and Valentijn, 1981; NRC, 1983; Mao *et al.* 1985; Liao and Wang, 1987; Huang and Liu, 1989) on one side, and the access of knowledge on the bioenergetics of grass carp (Carter and Brafield, 1991, Cui *et al.*, 1992) on the other side.

As this species is easily susceptible to diseases, a lot of studies on disease control under culture conditions have also been conducted (Luo and Liu, 1993, Qiu *et al.* 2001, Zhong *et al.*, 2002, Mao *et al.*, 2004). All these efforts over the years promoted the culture of grass carp and boosted its production: with only about 10 000 tonnes year⁻¹ in 1950, the global production of farmed grass carp had reached over 3.9 million tonnes year⁻¹ by 2006, occupying the third place in the ranking of production for individual species, just in front of the common carp (*Cyprinus carpio*) (with 3.4 million tones in 2006) (Fao, 2007).

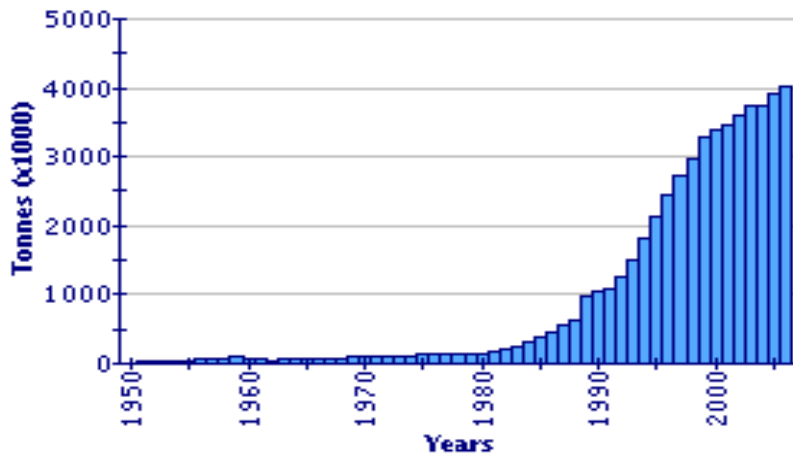


Figure 2: Evolution of the global aquaculture production of grass carp (*Ctenopharyngodon idella* Val.). (FAO Fishery Statistic, 2007)

3.4 Objectives

The main objectives of this study were:

- to make a quantitative evaluation of the gross chemical composition, energy and antinutrient content of the plant materials used as fish feed in the course of the year in Son La Province, Northern Vietnam;
- to assess the apparent digestibility and metabolisability of the nutrients and energy contained in the different plant leaf materials and grasses mainly used as fish feed for grass carp in Northern Vietnam.

The study was conducted in the framework of the Special Research Program “Sonderforschungsbereich 564” titled “Research for Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia”

3.5 Hypothesis

The primary hypothesis was that the digestibilities of nutrients in the reference diet are constant and might not be affected by the addition of test ingredients. (Thus the digestibility coefficient of the tested ingredients might all be positive).

In the second hypothesis we assumed that supplementing known amount of plant leaves to grass carp fed a known amount of reference diet might lead to an additional body weight gain as compared to those fish fed only the reference diet.

3.6 Experimental approach

The feeding experiments to test the digestibility and metabolisability of the different plant residues in grass carp were conducted simultaneously in a computer controlled respirometric system (Focken *et al.*, 1994) which allowed feeding and continuous measurement of oxygen consumption of individual fish, and in aquaria (recirculation system) where faeces collection could be done during the feeding experiment.

Data on oxygen consumption from feeding experiments, together with initial and final carcass chemical composition values were used to set up complete energy budgets for fish, showing proportions for indigestible, metabolized and retained energy. These provided comprehensive information on growth physiology as well as the energy partitioning in the fish fed different experimental diets (as affected by different plant material supplements tested with the reference diet).

We generally assumed in this study that there are no interferences (interactions) between the different components of the diets, i.e. between the reference diet and the tested ingredient. The experimental diets consisted of a basal reference diet for the fish of all the groups and a supplement of test ingredient for the fish of the groups other than the control (or reference group).

4. Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the mountainous regions of Northern Vietnam

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4.1 Abstract

In the upland of Northern Vietnam the aquaculture system of the Black Thai farmers mostly depends on green leaves which they use as major feed input to the ponds. A study was conducted to assess the quality of two groups of plant residues used as fish feed (principally for grass carp) in Northern Vietnam. The first group was constituted of residues commonly fed to fish, such as cassava (*Manihot esculenta*), banana (*Musa nana*), and bamboo (*Bambusa vulgaris*) leaves, and the second group included residues occasionally fed to fish by farmers, such as barnyard grass (*Echinochloa crusgalli*), mixed weeds from paddy fields, Elephant grass (*Pennisetum purpureum*), mulberry (*Morus*), maize (*Zea mays*), sweet potato (*Ipomoea batatas*), peanut (*Arachis hypogaea*); cassava tubercles and crop residues such as rice bran, cassava peels. In the first group the possible temporal changes in their nutrient, energy and antinutrient contents during the course of the year were evaluated. In the second group the nutrient, energy and antinutrient content were determined. During this study significant temporal changes were observed in the proximate composition, energy and antinutrient contents of banana leaves during the course of the year. Significant ($P < 0.05$) temporal changes were observed in the proximate composition of cassava and bamboo leaves as well as in the content of some antinutrient of cassava leaves. The saponin from cassava leaves had a pronounced haemolytic activity against cattle erythrocytes. Results of proximate analysis indicated the high potential of some of these plant materials such as cassava and mulberry leaves as fish feed because of their higher protein and energy content. However, the protein and energy content of these leaves were generally very low when compared to that of the common standard fish feed. Thus, these plant feedstuffs alone may probably not be sufficient to cover the requirements for rapid growth in cultured grass carp. The data presented here could be used for formulating cost-effective and balanced animal feeds for the use of small-scale farmers in rural areas in Northern Vietnam.

**5. Digestibility and metabolisability of the major nutrients and energy of
Banana and bamboo leaves in grass carp
(*Ctenopharyngodon idella*)**

Submitted to Aquaculture Research

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5.1 Abstract

In the uplands of Northern Vietnam, culture of grass carp contributes significantly to income and household food security of farmer's ethnic minority Black Thai. Generally Banana and bamboo are important perennial upland plants and, thus their leaves are frequently used by small scale fish farmer as fish feed. A 13 weeks feeding trial was conducted simultaneously in a recirculation and in a respirometric system to determine the apparent digestibility coefficients (ADC) and metabolisability of the nutrients of banana and bamboo leaves, assess crude protein, lipid and gross energy conversion and estimate the energy repartition in grass carp. For this purpose three diets were used: diet "A" (reference diet); diet "B", and "C" contained the same amount of reference diet as in diet A and were supplemented with banana and bamboo leaves respectively. Each treatment in each experimental system had five replicates. Reference and test diets were fed to fish and faecal samples were collected and the oxygen consumption was measured in order to setup an energy budget of fish over the whole experimental period. The body weight gain of the fish fed diet B was significantly higher in aquaria unit. The ADC of the supplemented banana leaves were 92.9 ± 2.5 , 52.3 ± 11.4 and $26.2 \pm 0.7\%$ for crude protein, lipid and gross energy and the ADC of bamboo leaves were 19.2 ± 15.8 , -83.0 ± 26.6 and $8.7 \pm 11.9\%$ for crude protein, lipid and gross energy respectively. The fish fed the diet C consumed a significantly higher amount of O_2 per unit of BWG when compared to the fish of the reference group. Generally, the energy losses were high in the case of fish fed plant leaf containing diets. The results of the present study indicated that banana leaf material has good potential for use as supplement in fishmeal-based diet for grass carp.

6. Investigations on the digestibility and metabolisability of the major nutrients and energy of maize leaves and barnyard grass in grass carp (*Ctenopharyngodon idella*)

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6.1 Abstract

In the uplands of Northern Vietnam, culture of grass carp contributes significantly to income and household food security of farmer's ethnic minority Black Thai. Indeed maize is one of the most important upland crops and barnyard grass the most important weed in the paddy rice fields, thus these are frequently used by small scale fish farmer as fish feed. An 8-week feeding experiment was conducted simultaneously in a recirculation and in a respirometric system to determine the digestibility and metabolisability of the nutrients of maize leaves and barnyard grass, to assess their crude protein, lipid and energy conversion and to estimate the energy allocation in grass carp.

The following experimental diets were used: diet "A" (reference diet) containing 39 % crude protein with 19.8 MJ.kg^{-1} gross energy; diets "B", "C", and "D" which contained the same amount of the reference diet as the control diet, supplemented with a known amount of dried barnyard grass, dried maize leaves or fresh maize leaves respectively. Each treatment in each experimental system had five replicates. Reference or test diets were fed to fish and faecal samples were collected and the oxygen consumption was measured in order to setup an energy budget of fish over the whole experimental period. The body weight gain (BWG) of the fish fed diet D was found to be significantly higher than that of the group fed diet A which also in turn was significantly higher than that of fish fed diet B or C. The apparent digestibility coefficients (ADC) of nutrients and gross energy for the different experimental diets in fish kept in the aquaria were, for diets A, B, C and D respectively, 94.1, 60.9, 70.5 and 84.7% for protein, 91.3, 60.7, 76.8 and 71.8% for lipid; 95.9, 44.5, 6, 60.6 and 69.1% for gross energy. The partial ADC of plant leaf ingredients were determined and barnyard grass and dried maize leaves were found to be not only poorly digestible but also having negative impact on the digestibility of the reference diet, while fresh maize leaves were well digested. The results of the present study indicated that fresh maize leaves have a good potential to be used as supplement in diets for grass carp.

7. Digestibility and metabolisability of the major nutrients and energy of cassava leaves and a mixture of weeds in grass carp (*Ctenopharyngodon idella*)

Submitted to Animal feed Sciences and Technology

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7.1 Abstract

In the uplands of Northern Vietnam, culture of grass carp contributes significantly to income and household food security of farmer's ethnic minority Black Thai. Indeed cassava is one of the most important upland crops and weeds from paddy fields are commonly available in that study region; therefore these are frequently used by small scale fish farmers as fish feed. A 12 weeks feeding trial was conducted simultaneously in a recirculation and in a respiration system to determine the apparent digestibility coefficients (ADC) and metabolisability of the nutrients of cassava leaves and a mixture of weeds, assess crude protein, lipid and energy conversion and estimate the energy allocation/utilization in grass carp. Three diets were used: diet "A" (reference diet); diet "B", and "C" contained the same amount of reference diet as in diet "A" and were supplemented with cassava leaves and a mixture of weeds respectively. All the diets contained 1% of a marker (TiO_2). The fish (initial body weight of 13.9 – 15.5 g) were kept individually in the both different experimental units. Each treatment in each experimental system had five replicates. They were fed the experimental diets at the rate of $7 \text{ g/kg}^{-0.8} \text{ day}^{-1}$ for the groups "A" (control); $14 \text{ g/kg}^{-0.8} \text{ day}^{-1}$ for groups "B" and "C".

No significant difference was found in the body weight gain (BWG) between the fish fed diet B and those fed the reference diet only (A) which were however significantly higher to the BWG of the fish fed diet C. The apparent digestibility coefficients (ADC) of the nutrients and energy of the reference diet in grass carp were 82.3 ± 3.5 , 82.3 ± 11.4 and $92.8 \pm 1.0\%$; the ADC of the diet supplemented with cassava leaves were 40.5 ± 10.3 , 48.1 ± 9.1 and $60.1 \pm 7.0\%$ and the ADC of the diet supplemented with Weed mixture were 49.3 ± 9.7 , 47.3 ± 3.8 and $36.4 \pm 6.4\%$ for crude protein, lipid and energy respectively. Generally the nutrients and energy contents of the cassava leaves and weed mixture were poorly digestible or even not at all digestible. The fish fed the diet C consumed a significantly higher amount of O_2 per unit of BWG when compared to the fish of other groups. The results of the present study indicated the limitation of the potential of cassava leaves and weeds from northern Vietnam as supplement in fishmeal-based diet for grass carp.

8. General discussion

In the preceding chapters the nutritional potential of plant feed ingredients currently used as feed for stomach-less fish in northern Vietnam has been assessed and discussed. In the first part of this study reported in Chapter 1, the nutrient, energy and antinutrient contents of the different plant materials commonly used as fish feed in Son La (Northern Vietnam) during the course of one year were evaluated. The main plant ingredients used as fish feed in the study region were evaluated in subsequent studies for their suitability as feed for the major fish species cultured in the mountainous regions of Northern Vietnam, namely grass carp. The digestibility and metabolisability of the major nutrients and energy in grass carp of the following leaf material were studied: banana and bamboo leaves in Chapter 2, maize leaves and barnyard grass in Chapter 3, cassava leaves and weed mixture in Chapter 4.

8.1 Potential of the plant leaf materials used as fish feed in Son La

In this study, the analysis of the different feeds fed to fish in Son La was done and grasses, bamboo and rice bran had higher fibre content. Generally, lower digestible energy has been found in feedstuff containing high levels of fibre (Anderson *et al*, 1991). Some of the different feedstuffs analysed in the present work had a relatively high protein and low fibre content. This was the case for banana, cassava, mulberry, peanut and sweet potato leaves. However, for some of them, antinutrient contents were high. The antinutrient contained in the different plant materials analysed in this work were presented and discussed in Chapter 1. It was concluded that the use of these feedstuffs as feed for fish by the small scale fish farmer should be done with caution.

8.2 Reasons for selecting the six plant ingredients tested as feed ingredients in this work

The selected six plant leaf materials tested during the present work were the fish feeds mostly used by the farmers in the project region. The most important criterion of selection

was the fact that they constituted the major protein suppliers in the ponds in Son La as listed by Steinbronn *et al.* (2006). All the six selected plant leaf materials tested as feed ingredient for grass carp accounted for a cumulative value of 85.2% of the total nitrogen (protein) input to the ponds in the North-West Vietnam region (Steinbronn *et al.*, 2006). Another criterion of selection was the availability of most of them during most times of the year, though the application of feed in the ponds was often based on the availability of these plant materials on the farm (Steinbronn *et al.*, 2005).

8.3 Plant leaf utilization and fish growth

In the present work, the best partial apparent digestibility coefficients (ADC) of nutrients and gross energy were obtained for banana and fresh maize leaves in grass carp. These ADC values were positive and the fish fed these plant leaves as supplement to the reference diet grew significantly better than those fed only the reference diet. In those two cases our second hypothesis was justified.

However, the ADC of nutrients and energy of some of the plant materials (bamboo and cassava leaves, barnyard grass and mixed weeds) tested in grass carp were found to be all or partially negative (Table 1).

Table 1: Partial digestibility coefficients of nutrients and energy of plant leaf feed ingredients in grass carp (% DM total nutrient or energy of leaf or grass)

| Nutrient | Banana leaves | Bamboo leaves | Cassava leaves | Weed mixture | Barnyard grass | Maize leaves (dried) | Maize leaves (fresh) |
|---------------|---------------|---------------|----------------|--------------|----------------|----------------------|----------------------|
| Crude protein | 92.9 ± 2.5 | 19.2 ± 15.8 | -36.1 ± 28.5 | -51.8 ± 38.8 | -71.3 ± 24.0 | 28.4 ± 25.4 | 70.6 ± 5.5 |
| Lipid | 52.3 ± 11.4 | -83.0 ± 26.6 | 19.1 ± 17.5 | -56.1 ± 15.2 | -42.1 ± 24.0 | 40.3 ± 24.2 | 35.9 ± 12.0 |
| Gross energy | 26.2 ± 0.7 | 8.7 ± 11.9 | 19.5 ± 15.9 | -46.6 ± 15.8 | -21.8 ± 22.5 | 24.6 ± 17.1 | 39.5 ± 6.8 |

Value are mean (n = 5) ± standard deviation.

Such negative values are scarce in the literature; most of them might not have been published up to now, as it has often been recommended to round these value to 0 (Glencross *et al.*, 2007) and the nutrient or the ingredient has only been considered as not digested or not

digestible. Zhou *et al.* (2007) also obtained and reported negative apparent digestibility values of protein for feather meal (-5.7%), and of energy (-22.9%) for silkworm manure in bluntnose black bream. If the values obtained for the ADC values are lower than 0 (or higher than 100), the implicit assumption that the digestibility (absorption) of nutrients and energy from the reference (or basal) diet is constant or unaffected by the test ingredients, cannot be justified, and the alternative hypothesis “interaction is present” has to be assumed (Sugiura *et al.*, 1998). In these particular practical cases an important part of the nutrients of the reference feed which was a component of the experiment diet might have been washed out by the plant leaves or grass present in the diet and this was reflected in the poor growth of fish groups fed those diets. Thus the negative values obtained for the ADC of these plant materials do not only mean that the nutrients of these materials were not digestible (or digested), but, moreover they also hampered or inhibited the utilization of the nutrients of the reference diet. Glencross *et al.* (2007) already pointed out that the determination of nutrient utilization or interference with nutrient utilization because of incorporation of any one ingredient in a basal diet is perhaps the most complex step in the ingredient evaluation process. The same authors also argued that this complexity is largely related to the wide variety of factors that may impact on nutrient or energy utilization. Thus knowledge of this interactive property of the different feed ingredients can be of high importance in practical feed formulations.

Some of the plant materials tested in this study could be better utilized by grass carp. However, we used, for the experimental purposes, only small fish, due to experimental equipment constrains. A better utilization could be expected in larger fish with a longer adaptation to the consumption and digestion of plant based feed in practical culture ponds systems in which they grow rapidly (Shireman, 1980). It has also been found that in older fish, consumption is twice as high on a pure plant food diet as it is on either an animal food diet or a mixed regime (Chilton II and Muoneke, 1992).

The ADC for crude protein, lipid and gross energy obtained for reference (control) diet in grass carp in the three different feeding experiments conducted in this study were similar and comparable to those reported by Law (1986). Our results, as well as the methods used to determine them were thus sound and reliable. However, the siphon method used in the present

study for the faeces collection was found to be inefficient by some authors, like mentioned by Wu *et al.*, 1995. The possible errors which could be due to the inefficiency of the faecal collection method have been discussed in the previous chapters (2, 3 and 4). Nutrients content of tested ingredient (such as protein and lipid) seemed to play an important role in the utilization of the tested feedstuffs. Banana and Maize leaves which were better utilized by the fish had not only a relatively higher protein but also higher lipid content which were also relatively good digestible for grass carp (Table 1). This was of major importance for covering the different needs of the experimental fish as Fischer (1973) found out that for grass carp consuming both plant and animal feeds, growth might be covered mainly by animal proteins and by lipids of both animal and plant origin whereas it is most likely that carbohydrates in the diet (mostly in plants) and proteins of plant origin are chiefly used in metabolism processes. It could be observed that not only the protein and lipid content was essential for a good utilization of the leaf material but also other important characteristics such as the fibre and mineral content. In some cases also the antinutrients might have played a detrimental role in the utilization of the leaf material. This was the case for the cassava leaves, which, despite a better nutrient profile with less fibre content, could not be well utilized by the fish.

Barnyard grass, Bamboo leaves and the weed mixture were poorly utilized by the fish and were detrimental to the utilization of the mixed reference diet. It was observed that the higher NDF content of these plant materials negatively influenced their utilization by the grass carp. Previous studies have indicated that grasses were poorly digested in grass carp (Law, 1978; Law and Syed Razlan, 1981), however Venkatesh and Shetty, 1978 reported that grass carp fed with hybrid napier grass achieved interesting weight gain. It has already been settled that the quality of a feed is reflected by its digestibility and the balance of nutrients (Cho and Kaushik, 1985). Low concentrations of dietary fibre can have beneficial effects on fish growth (National Research Council, 1993) and high concentrations of fibre decrease digestibility of dry matter and gross energy in the diet and can reduce the availability of other nutrients in some species (Falge *et al.*, 1978; Spannhof and Plantikow, 1983; Hilton *et al.*, 1983; Steffens, 1989; de Silva *et al.*, 1990).

8.4 Why plant leaf material was mixed to a reference feed for the digestibility experiment

Some authors (Hickling (1966), Stanley (1974), Cai and Curtis (1989)) suggested that grass carp, even small fingerlings, could maintain growth when consuming only plant food. Thus according to these results we could test the plant materials alone without mixing them with reference diet. However, these preceding results were in contradiction with that published by Fisher (1972) who found in many cases that plant protein alone without small amounts of animal protein will retard the growth of grass carp. Thus Fisher and Lyakhnovich (1973) suggested that diets (for grass carp) should contain 75% animal food and 25% plant food for optimum growth. Stanley (1974) found that immature grass carp fed an exclusive diet of plant food (*Elodea*) exhibited a negative nitrogen balance. Plant material consumed contained less than half the nitrogen excreted, resulting in a net loss of about $140 \text{ mg N day}^{-1} \text{ kg}^{-1}$ body weight. Besides suggesting a reason why grass carp have been known to consume animal matter, these data indicated the need for sources of protein (nitrogen) additionally to plant material. Stroband (1977) also found that animal food tends to stimulate growth in young grass carp. Later, Law (1986) also suggested that a faster growth rate and a shorter time to harvest may be expected if the grass carp were fed with a nutrient-balanced pelleted diet instead of grass and vegetables only. This affirmation of Law (1986) was already confirmed in the previous finding of Shireman et al., 1978 who showed that the growth rate of grass carp fed only ryegrass was significantly lower than that of grass carp fed a mixture of catfish chow- ryegrass pellets. Thus, we choose to mix these plant leaf materials to be tested with a reference diet according to all these previous works in order to evaluate the nutritive value and the nutrient digestibility of the tested plant leaf materials in grass carp.

The fish of the control group (Group A) received $7 \text{ g kg}^{-0.8} \text{ day}^{-1}$ (which was almost equivalent to 2.5 times maintenance ration for grass carp) reference diet, which was the minimum allowing an acceptable growth of our fish over a short experimental period. The fish of the other two diet groups (B and C) also received $7 \text{ g kg}^{-0.8} \text{ day}^{-1}$ (~2.5 times maintenance) reference diet. From this point of view all the fish groups were comparable. Thus we fed the same amount of reference diet to the fish of all the different experimental groups. Additional to the reference diet, the fish of the groups B and C also received a known

supplemental amount ($7 \text{ g kg}^{-0.8} \text{ day}^{-1}$) of plant leaf material, an amount which could be totally consumed by the fish (to avoid feed losses) and which was supposed to result in an additional growth (body weight gain) of the fish, if the supplemented plant leaf material could be well digested and utilized by the fish.

It is also necessary to mention at this point that the protein contribution of the plant leaf material to the total diet protein content was lower than 40% in each case (Table 2).

Table 2: Protein contribution from plant leaf or grass in the mixed feed
(in % of total diet protein DM)

| Plant leaf ingredient | protein contribution |
|-----------------------|----------------------|
| Bamboo leaves | 32.6 |
| Banana leaves | 26.8 |
| Cassava leaves | 38.1 |
| Weed | 26.1 |
| Maize leaves | 39.9 |
| Barnyard grass | 23.5 |

We assumed that very low supplementation levels of plant leaf material might not produce any significant additional growth during the short experimental period. Thus we supplemented a rather high amount of plant leaf material that could however be totally consumed by the experimental fish. It has been stated that grass carp should consume an acceptable amount of plant leaf material to sustain a remarkable growth, plant material is generally of low digestibility and herbivorous fish have to consume large quantities to obtain sufficient nutrients for growth (Wiley and Wike, 1986). Thus the diets were formulated to more closely mimic the natural feeding habit of grass carp.

8.5 Energy cost of plant leaf utilization

It was generally observed that the amount of oxygen consumed per g of body weight gained (BWG) by the fish fed only the reference diet (control group “A”) in the three digestibility experiments (Chapter 2, 3 and 4) were almost similar, varying from 0.7 ± 0.3 to 1.0 ± 0.4 g. This could be considered as a relative constant parameter to characterize the feed quality. The different experimental diets containing plant leaf materials which were poorly digested and utilized by the grass carp also induced higher oxygen consumption per g of BWG: the metabolic cost of the feed utilization was higher for fish fed poorly digestible diets. Francis et al., (2002) already pointed out that common carp fed diets containing relative high level of saponin had higher oxygen consumption (and metabolic rate) throughout the experiment, indicating continuous higher metabolic activity when compared to fish fed the control diet. This could possibly be a result of physiological stress induced by the higher dietary saponin content and probably also by other antinutrients as in the present work, all the experimental diets supplemented with plant leaf materials contained a relative high amount of saponin (between 0.8 to 4.0% DM of feed). Additionally to saponin, it was also observed in the different chapters of this work that the oxygen uptake per unit of fish weight produced was also related to the NDF content of the different experimental diets and was higher for the fish fed diets containing higher levels of fibre.

8.6 Economical aspects of the use of plant residues as feed for Grass carp in Son La (Northern Vietnam)

Taking into account the fact that grass carp can also consume and successfully utilize some crop residues and terrestrial plants leaves with no economical value, the culture of grass carp can be well integrated into crop farming and animal husbandry, to maximize the utilization of natural resources. Thus there is a possibility, with appropriate feeding management to produce grass carp at low cost. In the present work the protein source of our reference diet consisted basically of fish meal, which is however getting more expensive and scarce (Josupeit, 2007a, b) and difficult to afford for poor farmers. Under culture conditions, grass carp can, however, well accept feeds based on other protein sources, such as the by-products from grain

processing and vegetable oil extraction meals which represent cheaper protein sources. Combinations of such by-products with other crop residues, with caution being given to the total feed fibre content, might represent a better way of reducing feed cost in rural aquaculture farming, thus significantly enhancing the economic profitability of this activity and generate an even higher income for the farm households in this poor rural area in Northern Vietnam. Relying on locally available feed ingredients might also ensure the sustainability of the fish farming activity in the area and significantly facilitate the management of the local fish production and therefore boost this sector of activity. Additionally to the increased household economic security, the additional production from aquaculture might also imply an increased availability of protein for household consumption; this could ameliorate the nutritional and health status of the local population. Generally, in situations where aquaculture has been targeted to increase on-farm production of resource-poor households in certain agro-ecological regions, the positive income changes were reportedly more significant (Gupta *et al.*, 1999).

For the pond, in case of polyculture system, grass carp fed plant residues can play an important role as they shred the leaf material, and the high amount of faeces rejected by grass carp can fertilize the pond and increase the biomass production in the pond (Mitchell *et al.*, 1984, Maceina *et al.*, 1992) and thus increase the total fish production.

8.7 Conclusion

The key conclusions of the present work can be outlined as follow:

1- The results of the present study indicated that the nutrients of banana (chapter 2) and fresh maize leaves (chapter 3) could meet the growth requirements of grass carp and support a fast growth of these fish. Thus banana and fresh maize leaves have good potential for use as a supplement in fishmeal-based diet for grass carp.

2- The findings (chapter 3) clearly show that grass carp could differently use fresh and dried maize leaf materials. Fresh leaf material based feed and also selected plant ingredients of high nutrient and low fibre content are better suited for supplementing grass carp diets.

3- The study clearly shows the role of dietary plant fibres and some antinutrients on nutrient assimilation in grass carp. Accordingly, caution should be exercised in selecting plant feeds of high nutrient make-up. A low fibre and antinutrient content of the feed could also be an equally important criterion.

4- For the other remaining plant materials analysed in the present study and not tested in feeding experiment in grass carp, some of them might be able to sustain an acceptable growth of fish (grass carp) according to the existing literature. This can be made particularly possible if, they were either treated to reduce their antinutrient content or they were mixed during the feeding, according to their respective antinutrient content, which could help reduce the overall content of the detrimental factors in the so formulated diets. If the fibre content of the plant residues could be reduced by physical or chemical treatments, the utilization of nutrients could potentially be increased. Various chemicals such as sodium hydroxide (Sawar *et al.*, 1992), urea (Sawar *et al.*, 1994), ammonia (Williams *et al.*, 1984), and alkaline hydrogen peroxide (Sultan *et al.*, 1992) have been used for upgrading poor quality straws.

It was pointed out that urea, a cheap source of NH_3 after hydrolysis, has given satisfactory results for nutritional enhancement of wheat straw (Ali *et al.*, 1993). Upgrading of straw by urea treatment is caused by swelling cellulose micro fibrils and disrupting of the cell wall structures to a point where enzymes can hydrolyze intracellular components (nutrients) otherwise not accessible (Sarwar *et al.*, 1994). The treatment of plant residues on this way for their use as feed for herbivorous fish has so far not been evaluated. This possibility should be followed, though the fibre content was found to influence the feed digestibility in the present work (Chapter 2, 3 and 4). However all this treatments should be conducted with consideration of the fact that fresh leaf materials might be better utilized than dried leaf materials as it has been found in the Chapter 3 of this study.

5- The information provided in this thesis could be a good base for further work for the development of feeding strategies using locally available feedstuffs. This could lead to an increase of productivity of fish as well as of other animal species with plant based feeds and

enhance the livelihood of the small-scale farmers in the rural areas in Vietnam. Thus, this study has a strong focus on resource-poor households.

Further studies are needed to test the efficiency and elucidate the profitability of using banana and fresh maize leaves as feeds for grass carp under practical production conditions for small-scale farmers in Vietnam in association with different amounts of fish meal based diets or diets based on by-products from grain processing and vegetable oil extraction.

9. References

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