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Swedish University of Agricultural Sciences
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- A study of growth performance in the Mekong Delta in Vietnam

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Lokalt tillgängliga proteinkällor i foder till Nile tilapia (Oreochromis niloticus) – En tillväxtstudie i Mekong Deltat i Vietnam

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Abbreviations

BM	Blood meal
BW	Body weight
CP	Crude protein
DM	Dry matter
DO	Dissolved oxygen
DWG	Daily weight gain
EAA	Essential amino acids
EFA	Essential fatty acids
FA	Fatty acid
FCR	Food conversion rate
FI	Feed intake (total) per fish
FM	Fish meal
GAS	Golden apple snail
N	Nitrogen
PBM	Pangasius by-product meal
PI	Protein intake
RB	Rice bran
SBM	Soybean meal
SGR	Specific growth rate
SHM	Shrimp head meal
SR	Survival rate
TAN	Total ammonia nitrogen
WG	Weight gain

Abstract

Growth performance of male-fish Nile tilapia (*Oreochromis niloticus*) fed with locally available protein sources was evaluated in an attempt to find alternative ingredients to replace fish meal (FM) in an experimental set up at the An Giang University in the Mekong Delta in Vietnam. In the four experimental diets, 100 % of the FM was replaced with protein from golden apple snail meal (GAS), pangasius by-product meal (PBM), shrimp head meal (SHM) and blood meal (BM), respectively. A control diet contained FM as the main protein source. All diets were formulated containing a dietary crude protein level of 32 %. The experimental diets were prepared manually from dry feed at the experimental location. The ingredients were mixed before the feed were pelleted and sun-dried for two days. The fish were manually fed twice a day during the experimental period of 61 days. The growth parameters estimated in this study were: total weight gain (WG), specific growth rate (SGR), daily weight gain (DWG), feed conversion ratio (FCR), feed intake (FI), protein intake (PI) and survival rate (SR). The growth performance did not differ ($P>0.05$) between Nile tilapia fed with the control diet and the diet with PBM in any of the measured growth parameters. The experimental diets containing GAS, SHM and BM showed significant lower growth performance compared to the control and PBM diets. The results showed that pangasius by-product meal may replace fish meal by 100 % with no adverse effect on the growth performance. Total replacement of FM by GAS, SHM and BM resulted in low growth performance which could indicate that these ingredients can not totally replace FM in diet of Nile tilapia as these diets were prepared in this study.

Sammanfattning

I denna studie har lokalt tillgängliga proteinkällor utvärderats i foder till Nile tilapia (*Oreochromis niloticus*) i ett försök att ersätta fiskmjöl (FM). Studien var en tillväxtstudie och utfördes på An Giang Universitetet, lokaliserat nära Mekong Deltat i södra Vietnam. Fyra foderstater utvärderades genom att 100 % av fiskmjölet ersattes separat i vardera foderstat med protein från gyllene äppelsnigelmjöl (GÄS), pangasius biproduktsmjöl (PBM), räkmjöl (RM) och blodmjöl (BM). Kontrollfodret innehöll fiskmjöl som huvudproteinkälla och samtliga foderstater var formulerade till att innehålla totalt 32 % råprotein. Foderberedningen utfördes på plats, med manuell blandning av det torra foderingredienserna med efterföljande pelletering och fodret soltorkades sedan under två dagar. Fiskarna utfodrades dagligen, morgon och eftermiddag, under en försöksperiod på 61 dagar. Tillväxtparametrarna som studerades var: total viktökning (WG), specifik tillväxthastighet (SGR), daglig viktökning (DWG), foderomvandlingsförmåga (FCR), foderintag (FI), proteinintag (PI) och dödlighet (SR). Ingen skillnad ($P>0.05$) mellan studerade tillväxtparametrar kunde urskiljas hos fiskar utfodrade med kontrollfodret och fodret som innehöll PBM. Fiskar utfodrade med foderstaterna innehållande proteinkällorna GÄS, RM och BM, påvisade signifikant lägre tillväxt jämfört med kontroll- och PBM-fodret. Resultatet visade att 100 % av fiskmjölet kan ersättas med PBM, utan någon negativ inverkan på fiskens tillväxt. Att ersatta 100 % av FM med GÄS, RM och BM resulterade i låg tillväxt, vilket indikerar på att dessa proteinkällor inte kan ersätta FM med 100 % i foder till Nile tilapia.

1 Introduction

In 2050 the global population is estimated to be 9 billion inhabitants (FAO, 2014). The growing human population makes it a challenge to provide enough food in a sustainable way. The aquaculture sector has a potential to contribute to this challenge of food supply, and can generate both employment and economic gains especially in the developing countries (FAO, 2014; FAO, 2015a). In the past decades the aquaculture and global farming of fish has grown steadily and is one of the most fast-growing food producing sectors today (FAO, 2014; FAO, 2015a). The consumption of fish in the world has never been higher and it is constantly increasing (FAO, 2015a). In year 2013, fish represent 16 % of all animal protein consumed by humans globally. This proportion seems to increase as the demand for seafood increase and the aquaculture industry steps up to meet the demands (FAO, 2013). The growth of the fishing industry has been globally with the major part of caught wild fish and aquaculture found in Asia, with the majority in China (FAO, 2014). The expansion of the aquaculture sector generates higher demands for fish feed in fish culture systems. For most aquaculture species, diets are the major cost variables, representing up to 70 % of the operating cost (Shiau, 2002). Protein ingredients are the major nutrient in animal feed and generally the most expensive. Fish meal (FM) often represents the main protein source in aquafeeds, but due to high costs and decreasing availability it is necessary to find alternatives (NRC, 1993; El-Sayed, 1998). To ensure a sustainable development of the aquaculture industries, viable diets consisting of ingredients not derived from the marine environment and that are economically profitable must be found.

1.1 Aquaculture in Vietnam

Vietnam has a coastline of about 3 400 km and inland water bodies (lakes and rivers) of 4 200 km^2, with additional 6 000 km^2 of ponds and seasonal flooded areas (FAO, 2005), favorable conditions for a thriving fishery sector. Fish is one of the main protein intakes for Vietnamese people and represent a large part of their diets (FAO, 2005: Ne, 2015), with a per capita supply reaching 19.4 kg per year. The fishery sector is steadily increasing, the total production of fishery year 2013 reached 6 million ton, more than double the production in year 2002 (GSO, 2014; figure 1). The culture of fish and crustacean is spread over the whole country, were different culture systems are used depending on the climate conditions (FAO, 2015b). Commercial production for export began in Vietnam in the early 1980s with the farming of the giant tiger prawn (*Penaeus monodon*). The diversifying of the farming practices and adaption to the species suitable for export has contributed to the aquaculture sectors rapid growth and are one of the most important sector influencing the economy of Vietnam (figure 2; FAO, 2015b), a growth even higher than the caught fish production (figure 1). Each year the country also produces around 1 million ton of fish for animal feed (FAO, 2005). Vietnam represents one of the largest users of trash fish. The main production in the southeast areas use an average of 60 % of the total caught fish as trash fish, which are utilized as direct feed for fish and livestock or processed into fish sauce and fish meal (FAO, 2005).

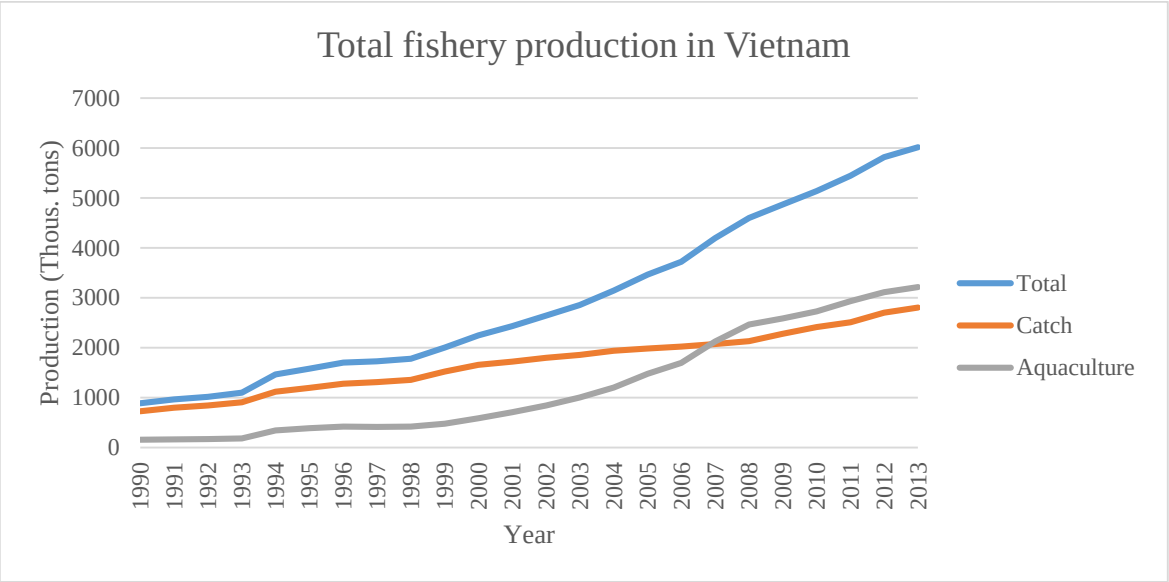

Figure 1: Total fishery production of Vietnam. *Data source:* General statistics office of Vietnam (GSO), 2014.

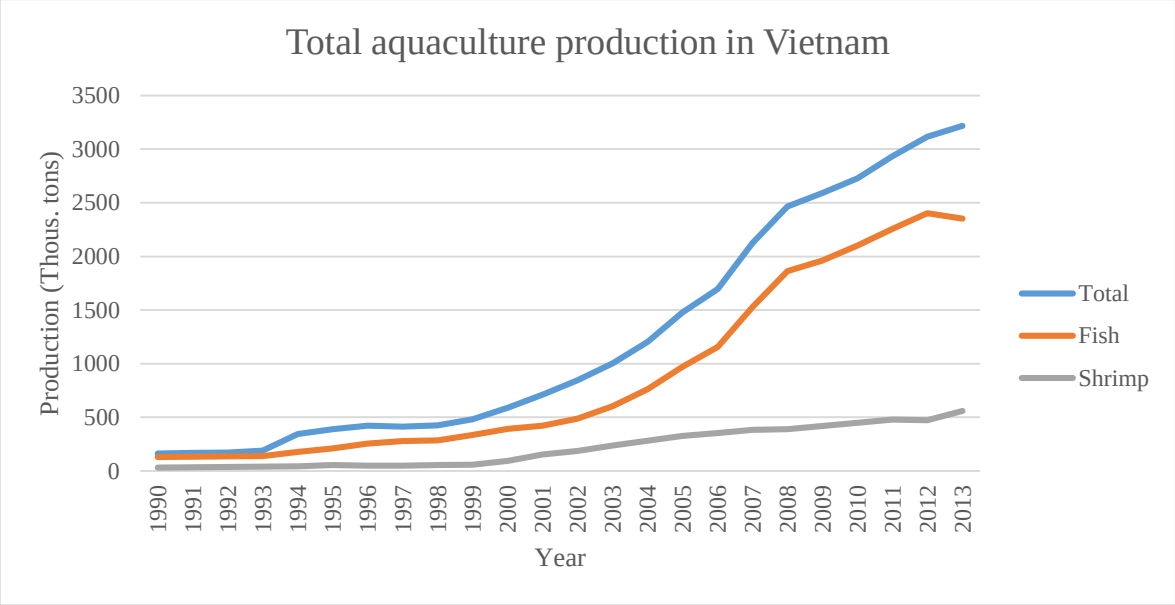

Figure 2: Total aquaculture production in Vietnam. *Data source:* General statistics office of Vietnam (GSO), 2014.

Farming of shrimp and catfish are considered to be the most developed sectors in Vietnam and today catfish accounts for most of the fish produced in the freshwater areas of Mekong River Delta (FAO, 2015b). The global demand for catfish is increasing and with new culture techniques the production is expected to be relocated to other producing countries than Vietnam. This makes room for cultivation of other species, for example Nile tilapia that has been introduced in the brackish and inland aquaculture of Vietnam (FAO, 2015b). Tilapia together with carp and catfish are among the species that are expected to globally have the fastest growth in their supply (FAO, 2014). The

trends in production are often depending on the market demands and so far the future aspect of tilapia production looks bright in Vietnam (FAO, 2015b).

1.2 Farming of tilapia species in the world

Tilapia farming is performed in 135 countries worldwide and in all continents. Asia represents about 70 % of the production, with China as the major producer, contributing to half of the global production of tilapia from year 1992 to 2003 (De Silva *et al.*, 2004; FAO, 2014; FAO, 2015c). Two of the main species in the tilapia cultivation fisheries are Nile tilapia (*Oreochromis niloticus*) and Mozambique tilapia (*O. mossambicus*), were Nile tilapia represents 90 % of the global tilapia aquaculture production (De Silva *et al.*, 2004; Tran *et al.*, 2011). The production of tilapia, worldwide, reached 3.4 million ton 2013, compared to 1.5 million ton 2004 (figure 3).

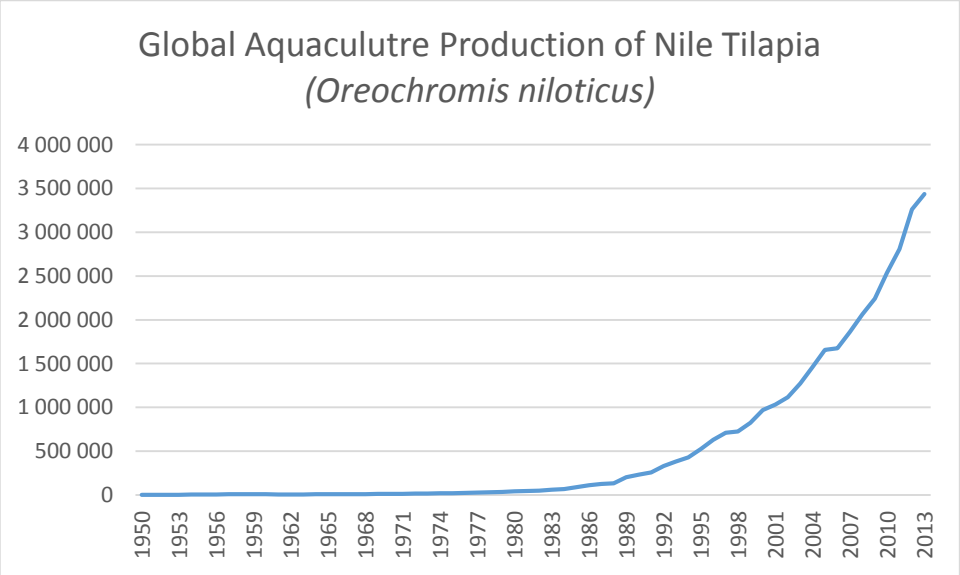

Figure 3: source of FAO, FishStat

The tilapia species are considered to be easily accessible and affordable protein source to the relatively poor people in the rural sectors, especially in Asia and in the Pacific (De Silva *et al.*, 2004). Tilapia was fist considered as a low cost alternative, but now the production has a wider range of consumers which has higher the value of tilapia species. The rapid increase of the tilapia production is due to the development of more efficient cultivation techniques with better environmental and disease management, improved feed nutrient which generate higher profits (Tran *et al.*, 2011).

All tilapia species are freshwater fish and is a fast-growing omnivore, resistant to diseases and easy to handle. The fish adapts easily to enclosed water and can utilize a wide range of feed (Shiau, 2002; De Silva *et al.*, 2004; Tran *et al.*, 2011). Most tilapia species has desirable characteristics for the fish cultivation industry (Tran *et al.*, 2011; FAO, 2015a). The production of tilapia in the tropical and subtropical areas, are the most economical beneficial due to the favorable habitat for

the tilapias growth (FAO, 2015c). Tilapia can live longer than ten years and reach a weight of about 5 kg (FAO, 2015c).

The practice of tilapia culture is diverse in many parts of the world, including both water-based systems (cages) and land-based systems (ponds, raceways and tanks). Pond cultivation practice with tilapia are often performed in poly-culturing systems were different fish species or shrimp are cultured together. (Shiau, 2002; Gupta & Acosta, 2004; Tran *et al.*, 2011). The choice of cultivation system depends on many different factors; intensity, investment cost, water access, environmental conditions (climate), and is in some way coupled to the marketing opportunities (Gupta & Acosta, 2004). Tilapia is farmed in both small- and large-scale systems. The development of technology in the industry has meant that the traditional extensive culture systems have been replaced by the semi-intensive and intensive culture system. In the extensive system the fish are only provided nutrient from natural pond organisms. With higher stock densities this may cause problems reducing the available natural food, forcing the farmers to complement with nutritional diets. The semi-intensive and intensive systems are already providing the fish with supplemental diets. In this system the diet accounts for about 30-70 % of the total production cost. To manage a successful fish production it is important to use low-cost, nutritionally balanced diets and to have a good feeding management (Shiau, 2002). Many famers choose to have mono-sex cultivation groups of tilapia, usually including only male fish. This is to avoid overpopulation, eliminate reproduction and to reduce the territorial behavior. Cultivation of mono-sex fish reduce the variation of size at harvest among the population and by using only male fish the average growth rate increase (Beardmore, Mair, & Lewis, 2001; FAO, 2015c).

1.2.1 Water quality

Water quality parameters (such as water temperature, pH, dissolved oxygen, nitrite and ammonia) are factors effecting the growth and health of the animals in aquaculture practice (El-Sayed, 2006). Water parameters for optimal growth of tilapia are shown in table 1. The water temperature is the major factor affecting the fish growth, physiology, reproduction and metabolism. Tilapia species tolerate a wide range of water temperature. The tolerance may depend on the geographically location of the tilapia fish and type of culture system. Also of the size of the fish affects the tolerance, smaller fish are more sensitive to cold water compared to larger fish. Tilapia can also be cultured in a wide range of salinity, and can have a normal growth and reproduction in brackish-water environments (El-Sayed, 2006). The handling of the fish is recommended to be minimized due to the increasing oxygen consumption of the tilapia caused by handling stress. Both ammonia and nitrite are toxic to tilapia and should be limited. By adding a chloride source such as NaCl the fish may be protected from toxicity caused by high nitrite levels (El-Sayed, 2006).

Table 1: Water quality parameters of Nile tilapia (*Oreochromis* niloticus)

Parameter	Range	Optimum for growth	Reference
Salinity (°/)	0 - 36	7	Shiau, 2002; de Azevedo *et al.*, 2015
Dissolved oxygen (mg/L)	> 2	6.0 - 6.5	Rakocy, 1989; Shiau, 2002.
Temperature, °C	20-35	28 - 32 25 - 30	El-Sayed, 2006; Azaza *et al.*, 2008; El-Sherif & El-Feky, 2009a.
pH	4 - 11	7 - 8	Rakocy, 1989; Shiau, 2002; El-Sherif & El-Feky, 2009a.
Ammonia, mg/L	< 0.8	< 0.1	El-Sayed, 2006
Nitrite, mg/L	0 – 8[1]	Not specified	Atwood *et al.*, 2001.

[1]90.7±16.43g fish, addition of sodium chloride into the water to lower the toxicity

1.2.2 Nutrient requirements

The major nutrient requirements of cultured Nile tilapia are summarized in table 2. The nutrient requirements of tilapia depend on several factors, such as fish size, age, culture system and environmental conditions (NRC, 1993; Shiau, 2002). For example, the requirements of protein may be affected by the waters salinity, with higher salinity level lowering the demands (Shiau, 2002). The maximum protein requirements of tilapia have been reported to be during larval stage, then the required levels will decrease (NRC, 1993). Nile tilapia, as well as other fish and terrestrial animals, requires ten essential amino acids; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Shiau, 2002). Fish in general do not have specific carbohydrate requirements for their diet, and it is not vital for the survival or growth of the fish to include it in the diet, regardless of fish species (NRC, 2011). Lipid requirements are difficult to define due to lipids varying chemical and functional roles (NRC, 2011).

Table 2: Nutrient requirements of Nile tilapia (*Oreochromis niloticus*)

Nutrient	Weight (g)	Requirement	Recommendations	Reference
Crude protein	< 20	40 %		
	20 – 200	34 %		
	200 – 600	30 %		
	600 - 1500	28 %		
	> 1500	26 %		NRC, 2011
Crude lipid		Not specified	10 – 15, % (min[a])	Chou & Shiau, 1996; Shiau, 2002; FAO, 2016;
Carbohydrate		Not specified[a]	35 – 40, % (max)	FAO, 2016; Chou & Shiau, 1996
Crude fiber		Not specified	8 – 10, % (max)	FAO, 2016; Chou & Shiau, 1996

The values are mostly results from studies during laboratory conditions and may be adjusted depending on culture system (NRC, 1993). [a] hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus*)

2.3 Fish meal as a protein source

Feed representing a major part of the operational costs in the fish and crustacean farming, the protein component is the single most important and the most expensive dietary component. Fish meal (FM) is one of the main protein sources in the conventional aquaculture sector due to its high protein content (30-72%), being a good source of essential amino acids (EAA), essential fatty acids (EFA) as well as it is highly digestible and palatable to most fish (NRC, 1993; El-Sayed & Tacon, 1997; NRC, 2011). In general the fish diet contents 20-60 % of FM (Leal *et al.,* 2010; Watanabe, 2002). In the fishing industry, the fish not used for direct human consumption are processed into FM and fish oil. These two products are widely used in animal feeds not only in the aquaculture but also to livestock animals, chicken and pigs etc. (FAO, 2014). Fish meal produced from waste products containing high levels of bone is most likely to have a lower percentage of high-quality protein than the meal from input material without bone or of whole fish. The waste products containing lots of bone usually contains high amounts of ash which may lead to mineral imbalance (NRC, 1993).

The global supply of FM is decreasing since world's capture fisheries have passed the peak in the amount of wild fish caught at sea. Together with an increased demand of FM it has resulted in a rise in the price of FM influencing not only the finfish culture, but also the husbandry of crustacean, pets, and livestock production (FAO, 2014). With the increasing price of the FM and fish oil it may no longer be considered as a low-values product anymore (Olsen, Toppe & Karunasagar, 2014). As a consequence, fish nutritionists have made several attempts to partially or totally replace FM (El-Sayed, 1999).

2.4 Alternative protein sources to fish meal

Approaches have been made to reduce the FM in aquaculture diets by replacing it with alternative, less expensive animal or plant protein products. The quest is to find sources that are well-utilized and has a positive effect on the fish performance comparable with those of FM (El-Sayed, 1998). Many attempts have been done to evaluate alternative protein sources that can partially or totally replace fish meal in aquafeeds. The investigations have been made of both conventional and non-conventional animal and plant protein sources (El-Sayed & Tacon, 1997; Da *et al.*, 2012, 2013a, 2013b, 2013c). The studies presented below will mainly focus on potentially alternative protein sources in diet of tilapia species. Replacement of fish meal within aquafeeds includes fishery and terrestrial animal by-products meals, linseed meals and by-products, aquatic plants, single-cell proteins, and legumes and cereal by-products (Davies & Wareham, 1988; Davies, McConnell & Bateson, 1990; El-Sayed & Tacon, 1997; El-Saidy & Gaber, 2003).

The secondary product derived from a manufactory process is often described as by-product. The by-products from the food industry are the parts from animals and plants that can be used but are not intended for human consumption. Animal protein sources include both fishery by-products (such as shrimp meal, krill meal and squid meal) and terrestrial animal by-products (such as poultry by-product meal, blood meal, feather meal and meat and bone meal) (El-Sayed, 1999). In the fish and shellfish 70 % of the total body weight may constitute for by-products. The plant by-product meals often have high protein levels and a favorable essential amino acids profile (Fontainhas-Fernandes, *et al.,* 1999). Utilizing the by-products into animal feed will contribute to a better environment and increase the production of fish food and is also economical beneficial (FAO, 2012). Animal by-product may contain high levels of ash due to the large amount of material from

bone and non-muscle tissue. Plant protein may have high fiber content which is unfavorable for the digestibility when replacing FM and may lower the quality of the fish feed (NRC, 1993).

Animal by-products often include high levels of protein. For example, poultry, meat and bone meal contains about 45-55 % of crude protein (NRC, 1993). Even if the protein content is high, it does not mean that the essential amino acid profile is such as the fish requires. Different protein sources, with a less suitable composition of the essential amino acids, may be mixed together to maintain a balanced amino acid profile for the feed for the fish (Bishop, Angus and Watts, 1995; Fontainhas-Fernandes, 1999; El-Saidy & Gaber, 2003). Some protein sources are necessary to process before they can be used to increase the digestibility, like the need to hydrolyze feather meal (80 %) (NRC, 1993). Many studies have evaluated the inclusion of animal protein sources in Nile tilapia diets with successful outcomes. For example, a study conducted by El-Sayed (1998) indicates that shrimp meal, meat and bone meal, blood meal and poultry by-product meal can totally replace FM in practical Nile tilapia diets. Plant protein includes oilseed plants such as soybean meal, cottonseed meal/cake, groundnut, sunflower, and rape seeds (El-Sayed & Tacon, 1997). Soybean, cottonseed, sunflower and linseed meals individually are commonly incorporated in practical diets of fish as replacement for fish meal (El-Sayed, 1999). Soybean meal is available worldwide and is considered to have the best amino acid profiles among the plant protein feedstuff regarding the essential amino acid requirements of fish (NRC, 1993). Sunflower, rape seed, cottonseed and leucaena (*L. leucocephala*) leaf meals may replace FM by 50 % in tilapia diets, with no adverse effect on the growth rate (Jackson, Capper & Matty, 1982). Studies have also been conducted using aquatic plants, single-cell protein and legumes and cereal by-products. Single cell protein is a group of microorganisms including unicellular algae, fungi, bacteria, cyanobacteria and yeast and are traditionally used as natural food for tilapia in semi-intensive systems (El-Sayed, 1999). The single-cells are easy accessible and effective in producing natural fish food. Studies have reported successful results when single-cell protein has been used in diets of various tilapia species. But more studies are needed to be performed regarding the natural food production, such as single-cell proteins, for pond cultivation. Especially in developing countries where the culturing of tilapia is widely practiced. Currently the single-cell protein is produced on commercial scales (El-Sayed & Tacon, 1997; El-Sayed, 1999).

The present study will focus on a replacement of FM with four different animal protein sources (golden apple snail, pangasius by-product meal, shrimp head meal and blood meal) in the diet of Nile tilapia. Therefore, these animal protein sources are discussed in more detail in the text below.

2.4.1 Golden apple snails

The golden apple snail (GAS) (*Pomacea* spp.) was originally introduced to Asia for cultivation as a food protein source for human consumption (Bombeo-Tuburan *et al.,* 1995). The snails are growing fast and have a high reproduction rate and are stated as a pest in many cultivated rice areas in Asia (Bombeo-Tuburan *et al.*, 1995). GAS is well spread in Vietnam and can be found in large amounts in most waters in the Mekong River Delta (Da *et al.*, 2012). GAS is a good source of proteins for fish feed (contains around 54 % of proteins) including the EAA and EFA (Bombeo-Tuburan *et al.,* 1995). To harvest GAS would also contribute to reduce the snail infestation in the rice fields. A study by Kaensombath & Ogle (2003) demonstrated that the nutrient value of GAS is comparable with that of FM. However, the supply of snails is irregular during the year, with a wider access during the rainy season. It would be favorably if the snails were preserved at a

temporal abundance (Bombeo-Tuburan *et al.*, 1995; Phonekhampheng, Hung & Lindberg, 2003). GAS and snail meal have been evaluated in previous studies with different levels of inclusion in both aquafeeds and in diets of broiler, pigs, tiger shrimp (*Penaeus monodon*) and pekin ducks (Creswell & Kompiang, 1981; Bombeo-Tuburan *et al.*, 1995; Ulep & Santos, 1995; Kaensombath, 2003; Diomandé *et al.*, 2008; Chimsung & Tantikitti, 2013). Results when replacing 75 % of FM by fermented GAS in diets of sex-reserved red tilapia (*Oreochromis niloticus x mosambicus*) showed even better growth performance than FM (Chimsung & Tantikitti, 2013).

2.4.2 Pangasius by-product meal

Pangasius hypophthalmus belong to the freshwater catfish family and is referred as the world's largest and most important inland fisheries (FAO, 2014). Within the Vietnamese producing and developing aquaculture sector catfish is considers as one of the most commonly cultured fish and the work within the production contributes too many peoples livelihood (Thi Thuy *et al.*, 2007; FAO, 2014; FAO, 2015b). Large production of fish results in high quantities of by-products, which if possible to utilize for fish feed. The catfish by-products accounts for about 65% of the total raw fish material and it is what remains after filleting the fish. The by-product includes the skin, bones, the head, the scarp meat and abdominal organs (Thi Thuy *et al.*, 2007). The crude protein content of the by-products may differ. It could contain 35 – 42 % in the head and bone by-product meal compared with broken meat and skin by-products containing about 45-62 % proteins (Thi Thuy *et al.*, 2007). The catfish by-product meal is utilized not only in the aquaculture sector but also in the production of livestock and pig (Thi Thuy *et al.*, 2007).The high levels of protein and fat makes catfish by-products a potentially good protein source in animal feed as well. The by-product also includes high levels of moister and must therefore be processed before incorporated in animal feed. There are different methods used when processing the by-products, but in general it involves boiling, removing the fat/oil and then drying the product (Thi Thuy, Lindberg & Ogle, 2011).

Previous studies have included pangasius by-products in various animal diets, for instance in diets of pigs and of chicken (Thi Thuy, Lindberg & Ogle, 2010; Thi Thuy, Lindberg & Ogle, 2011; Thi Thuy, 2012). It is difficult to find research performed specifically on pangasius by-product meal in diets of fish due to pangasius catfish are often referred to as fish meal. There are no published articles that have evaluated pangasius by-product meal in the diets of Nile tilapia (*O. niloticus*) specifically.

2.4.3 Shrimp head meal

Freshwater crustaceans represent the second-largest group of crustaceans used for farming and the production has had a gradient increase of growth in the past years (FAO, 2014). Production of prawn stands for more than half of the aquaculture sector in the world (FAO, 2014) and it is an important international traded fishing product. Vietnam is a significant producer and exporter of farmed shrimp and the market is expanding both international and national (FAO, 2015b). The expanding of the shrimp production, of both catch and farmed shrimp, has concomitant to the increase of shrimp waste production. Shrimp head accounting for 33 % of the total weight of the shrimp and is one of the waste products from the shrimp industry which can be used as a potential protein source in animal feed (Leal *et al.*, 2010). Utilizing the waste products into a resource is beneficial economically and it is an abundant product, providing high quality protein for fish feeds (Cavalheiro *et al.*, 2007), but also in diets of other animal species (Gernat, 2001; Khempaka, Chitsatchapong & Molee, 2011; Aladetohun & Sogbesan, 2013). The shrimp waste contains high

levels of ash and fiber which may limit the inclusion level in animal feed formulations. To make the shrimp waste easier to digest the waste can be processed by fermentations into silage or hydrolyzed before incorporated in animal feed (Plascencia-Jatomea *et al.,* 2002; Leal *et al.,* 2010). Previous studies demonstrate that 15 % silage and 20 % hydrolyzed shrimp head meal could replace FM in diets of Nile tilapia (Plascencia-Jatomea *et al.,* 2002; Leal *et al.,* 2010). The studies did not evaluate 100 % replacement of FM but Cavalheiro *et al,* (2007) indicates that it is possible to completely replace FM with shrimp head meal without negative impact on the growth performance of Nile tilapia (*Oreochromis niloticus*).

2.4.4 Blood meal

Blood meal is an animal waste product, produced from animal blood which usually is collected from locally abattoirs. The source of blood comes from various domestic animals such as cattle, pig and chicken (Weibel *et al.,* 1977). Raw blood is a perishable product with a high moister content, which makes it sensitive to deterioration and putrefaction. The raw blood is therefore processed into blood meal by drying and grounding which also is a product easier to handle and incorporated into rations (Weibel *et al.,* 1977; Donkoh *et al.,* 1999). Blood meal is considered as an alternative high quality protein source in fish feed formulations (Ogello *et al.,* 2014). It has also been evaluated as an ingredient in shrimp and broiler chicken diets (Dominy & Ako, 1988; Donkoh *et al.,* 1999). Blood meal is considered as a rather low cost and easy available product worldwide (Ogello *et al.,* 2014; Otubisin, 1987).

Blood meal contains high levels of protein (80-86 %) and may have a favorable essential amino acids profile (Otubisin, 1987; NRC 1993; Ogello *et al.,* 2014). Different methods are used for drying the raw blood (Donkoh *et al.,* 1999; Fasakin *et al.,* 2005). Solar drying is well suited in small-scale operations or when advanced technical equipment is not affordable (Donkoh *et al.,* 1999). Too much heat may affect the blood and make it less palatable and lower the digestibility (Overton, 1976). Previous studies have shown various results in fish performance when including BM in the diets of tilapia. Replacing FM by 50 % in diets of Nile tilapia and 66 % in diets of hybrid tilapia (*Oreochromis niloticus & Oreochromis mossambicus*) with BM indicated poor fish performance (Otubisin, 1987; El-Sayed, 1998). A study conducted by Aladetohun and Sogbesan (2013) demonstrated increased fish performance when replacing FM by 100 % of BM in diets of Nile tilapia fingerlings. The various results of fish performance in previous studies may depend on deficiency of amino acids content (often low in methionine and isoleucine) in the BM and also due to the product not being a very palatable ingredient (El-Sayed, 1998).

3 Aim and objective

The aim of the present study was to determine alternative protein sources for partial or total replacement of fish meal in diets to farmed fish in an attempt to create a model for sustainable fish cultivation. The objective of this study was to evaluate and examine possibilities of total replacement of fish meal in the diet of Nile tilapia (*Oreochromis niloticus*) with locally feed resources, namely golden apple snail, pangasius by-product meal, shrimp head meal and blood meal. This will be accomplished by analyzing the growth performance, feed utilization and survival rate of the Nile tilapia included in the experiment.

4 Material and Methods

4.1 Study site and experimental design

The study was carried out at the Laboratory of Aquaculture Nutrition, Faculty of Agriculture and Natural Resources, at An Giang University, in An Giang province, close to the Mekong River Delta of Vietnam during ten weeks from June to August, 2015.

The study was conducted as an open randomized design (CRD) with five experimental diets (one control diet and four test ingredient diets), fed in triplicate. In the beginning and the end of the experiment, the acclimatised fish were weighed using a digital scale. Twenty-five homogeneous fish with an average initial body weight (BW) of 36.9 ± 3.6 g/fish were distributed into each tank.

4.2 Experimental fish

In the experiment all the fish were male fish of the species Nile tilapia (*Oreochromis niloticus*) and were bought from Tien Giang hatchery, Vietnam. The fish had been stocked in a pond and reared on conventional feed. The fish were around three months old when they arrived to the Laboratory of Aquaculture Nutrition, at An Giang University. To eliminate ectoparasite infections and prevent fungal infections the fish were at the arrival washed with a solution of sodium chloride. At the Laboratory of Aquaculture Nutrition the fish were reared and quarantined in composite 500 liter tanks with a density of 100 fish per tank for one month before start of the experiment to acclimatize the fish to experimental conditions. Two weeks before the experiment was commenced the acclimatized fish were selected randomly, weighed and transferred to the experimental tanks for adaptation to the experimental conditions including feeding and handling practices. A total amount of 375 fish were included in the experiment with twenty-five fish distributed in each experimental tank.

4.3 Experimental system and facilities

The experiment was carried out in an indoor clear water system, and the Nile tilapia fish were stocked in fiberglass circular tanks with a volume of about 500 liter per tank. Aeration was provided continuous individually in each tank by an electronic low-pressure fan which distributed the air through an air stone. About 60 % of the water in the tanks was replaced with new water every other day throughout the experiment. Once a week after changing water, two handfuls of sodium chloride were added in each tank, as an attempt to remove possible algae growth. While changing water the walls and bottom of the tanks were manually cleaned by scrubbing mechanical with a cloth, without

detergent, to minimize algal growth. Faeces siphoning was performed in each tank on the days the water were not changed.

Two weeks prior to the growth study the fish were moved to the experimental tanks for acclimatisation, while being fed twice daily on a commercial pelleted feed (40 % CP, AFIEX Company in Long Xuyen city of An Giang province). During the experiment all fish were fed twice daily, *ad libitum*, with dry experimental feed throughout the experiment. Each diet was fed to triplicate groups of fish manually at 07.30-10.00 h and 16.00 h, 7 days a week, for 61 days (from 15th of June to 15th of August). The fish were weighed bi-weekly for the calculations of the amount given feed per body weight. During all handling procedure the fish were anaesthetized in a bath of 0.6 ml of ethylene glycol monophenyl ether per liter water for five minutes. During the experiment, the fish showing symptoms of disease or fish that died were eliminated.

At the initial weighing and the bi-weekly weighing occasions the weight of only five randomly selected fish from each tank were recorded. At the end of the experiment all fish from each tank were individually weighed, for documentation of their final growth.

4.4 Diet formulation

The control diet, contained fish meal as the main protein source and was formulated to meet the nutrient requirements of Nile tilapia (Tram *et al.,* 2011; Körücü & Özdemir, 2005). In the other four experimental diets, 100 % of the fish meal were replaced with protein from golden apple snail meal (GAS), pangasius by-product meal (PBM), shrimp head meal (SHM) and blood meal (BM), respectively (see table 3). All experimental diets were formulated containing a dietary crude protein level of 32 % (NRC, 1993).

Table 3: Composition of experimental diets for Nile tilapia (*Oreochromis niloticus*)

Ingredients (%)	Diets				
	Control diet	**GAS**	**PBM**	**SHM**	**BM**
Fish meal	32.0	0	0	0	0
Soybean meal	21.5	20.4	21	20.4	18.7
Rice bran	21.0	28	20.3	24	27
Wheat flour	19.8	13.3	25	22.3	28
Vitamin premix[a]	2	2	2	2	2
CMC[b]	1.5	2,5	2	2	2
Squid oil liver	2.2	3.3	0.5	3.3	3.6
Golden apple snail	-	30.5	-	-	-
Pangasius by-product meal	-	-	29.2	-	-
Shrimp head meal	-	-	-	26	-
Blood meal	-	-	-	-	18.7
Total	**100**	**100**	**100**	**100**	**100**

Control = diet including FM, GAS = golden apple snail, PBM = pangasius by-product meal, SHM = shrimp head meal, BM = blood meal. [a] Vitamin and mineral premix; BIO FISH-PREMIX; content per kg: vitamin A 300,000 UI; vitamin D_3 150,000 UI; vitamin E 2,500 mg; vitamin K_3 250 mg; vitamin B_1 500 mg; vitamin B_2 390 mg; vitamin B_5 1,500 mg; vitamin B_6 388 mg; biotin 10 mg; folic acid 150 mg; choline 5,000 mg; $FeSO_4$ 47,000-59,000 mg; $CuSO_4$

24,000-27,000 mg; ZnO 13,000-18,500 mg; $CoSO_4$ 283-960 mg; Na_2SeO_3 197-240 mg; $MnSo_4$ 5,000-6,500 mg; dicalcium phosphate 135,000-165,000 mg
[b] CMC = carboxymethyl cellulose

4.5 Fermentation trial

From the beginning the present study was supposed to include laboratory made tempeh as the test protein ingredients. The idea was to use locally available products, which were not suitable for human consumption, as a substrate. Experiments were performed using cassava root, corn and broken rice as substrates for the production of tempeh. Unfortunately the locally conditions and knowledge was not enough to make it feasible to use tempeh as fish feed. The notes documented from the performed trail are summarized and can be found in the appendix 1 of this paper.

4.6 Experimental feed ingredients, diet preparation and feeding

Soybean meal and fish meal were purchased from the AFIEX Company in Long Xuyen City of An Giang province. Rice bran, wheat flour and blood meal were purchased from the local market in Long Xuyen City of An Giang province. Blood meal was made from pig blood produced by local farmers. Premix vitamin and mineral, CMC and squid liver oil were bought from Thanh My Company, at Can Tho. Raw shrimp head was purchased from Nha Troug Seafood Company, Can Tho City, An Giang province. The raw shrimp head was sun-dried for three days and then ground to a meal before use. Golden apple snails were purchased from farmers in Tam Nong district, Dong Thap province. The meat of the golden apple snails was collected, cleaned with freshwater and oven-dried at 90 ^oC for seven hours and then milled before use. Pangasius by-product meal was purchased from the local market in Chau Thanh district, An Giang Province.

In the first batch of diets (11 kg), the feed was prepared by manually mixing all of the dry ingredients except the vitamin and mineral premix, before adding squid liver oil and distilled water. The amount of distilled water was adjusted to get the mixture into firm dough. The dough was taken through an electric meat grinder (Quoc Hung Company, Vietnam) to make pellets with diameter in the range of 1-2 mm. All pelleted diets were sun-dried for one day and then the premix of vitamin and mineral was added to each diet, by first water spraying the pellets and then adding the premix. The pellets were then dried in the shadow for 2-3 hours. In the second batch of preparing the diets, both the vitamin and mineral premix and the squid liver oil were added after making the other ingredients into pellets. The prepared diets were then sun-dried. The absorption by the pellets of vitamins, minerals and for the second batch, squid liver oil, is therefore unknown. The prepared diets were kept in plastic bags (one for each diet) in the same facility as the experimental tanks, in outdoor climate. Daily rations of each diet for each tank were portion in small plastic bags and every day after feeding the fish, the remaining feed in the bags were separately weighed and recorded. The second batch of diets was mixed with the remains form the first batch (at 22th of July), separately for each diet, after six weeks into the experiment. The second batch contained three kilo of feed for each diet, which were added into the remaining 4.8 kg of control diet, 5.1 kg of PBM diet, 5.5 kg of SHM diet and 6.5 kg of BM diet. From 40 kg of golden apple snail around 5.4 kg meat were collected, making the first batch contain 6 kg and the second batch 5 kg which were added into the reaming 0.8 kg.

4.7 Water quality monitoring

Water quality parameters for the experiment were monitored bi-weekly during the experiment. The parameters were recorded from five tanks representing each diet at the measurement occasion (tank 1-5, tank 6-10 or tank 11-15). The pH-values were recorded by a digital pH meter (OAKTON of HACH, USA) and dissolved oxygen (DO mg/L) by a digital meter (HQ30d Flexi of HACH, USA). Nitrite (mg/L) and the total ammonia (mg/L) were measured with the Sera ammonium/ammonia-test kit and Sera nitrite-test kit. Temperature (°C) was recorded daily for three weeks and then once a week with a temperature meter.

4.8 Calculations parameters

Feed intakes of the experimental diets were recorded at group level. It was assumed that all the fish fed the same experimental diet had the same intake of the ration. Calculations were made on the growth performance estimated by weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR). The total feed intake (FI), protein intake (PI) and the survival rate (SR) was also estimated. Equations used for the calculations were:

$$WG\ (\%) = ((FW - IW)/IW) \times 100$$
$$DWG = (FW - IW)/T$$
$$SGR\ (\%) = ((ln\ FW - ln\ IW)/T) \times 100$$
$$FCR = FI/WG$$
$$FI = FI/No.\ fish$$
$$PI = FI \times \%\ of\ protein\ in\ diet$$
$$SR\ (\%) = (TF - TI) \times 100$$

where FW is the final weight (g) of the fish, IW is the initial weight (g) of the fish, T is the duration of the experiment in days, FI is the total feed intake (g) and PI is the total protein intake. TF is the total number of fish at harvest and TI is the total number fish in the beginning of the experiment.

4.9 Chemical analysis

Feed analysis were carried out at SLU laboratory, determining; DM, ash, crude protein and EG-fat. DM was determined by drying 2 g of the sample in an oven at 103 °C for 16 hours. For ash determination, the same samples as for the dry matter were dried again in a heating oven at 550 °C for 3 hours. Nitrogen (N) was determined by the Kjeldahl method and crude protein (CP) was calculated as N x 6.25. EG-fat was analyzed according to the official Journal of the European Communities: Determination of crude oils and fat, Method B (1984). Using the 1047 hydrolyzing unit and a Soxtec System HT 1043 Extraction Unit (FOSS Analytical A/S Hilleröd, Denmark).

4.10 Statistical analysis

Statistical analysis was performed using Statistical Analysis System version 10.0 (SAS Institute Inc., NC, USA). The significance level was set to $P>0.05$. The effect of experimental diets on the growth performance was evaluated using the model PROC MIXED, followed by turkey's multiple comparison test to adjust for multiple comparisons. The model hade the experimental unit of tank, fix factor of diet and random factor of tank within diet.

5 Result

5.1 Chemical composition of test diets

The chemical compositions of the diets prepared in the first batch are shown in table 4. The control diet had the highest crude protein content (35.8 g kg^{-1} DM), and the diet with SHM as the main protein source had the lowest value (27.3 g kg^{-1} DM). The fat content varied from 10.2 to 7.3 g kg^{-1} DM, with the highest content in the diet with SHM and lowest in the PBM diet. The dry matter content of the diets were rather similar, with variations of 89.2 – 91 percent DM. All the diets also had similar ash content (11.2 - 9.5 g kg^{-1} DM) except in the BM diet that had a lower value (5.1 g kg^{-1} DM).

Table 4: Chemical composition (g kg^{-1} DM) of the experimental diets

	Diets				
	Control diet	**GAS**	**PBM**	**SHM**	**BM**
Crude protein	35.8	31.3	32.9	27.3	32.8
Fat (EG)	8	8,3	7.3	10.2	7.8
Dry matter	89.9	89.2	90	91	90.8
Ash	11.2	9.5	10.8	10	5.1

Control = diet including fish meal (FM), GAS = golden apple snail, PBM = pangasius by-product meal, SHM = shrimp head meal, BM = blood meal.

5.2 Growth performance

Parameters for growth performance and survival rate of Nile tilapia are presented in table 5. Growth performance did not differ ($P>0.05$) between tilapia fed with the control diet and the diet with PBM in terms of final weight, weight gain (WG), specific growth rate (SGR) and daily weight gain (DWG). However, tilapia fed with GAS, SHM and BM diets had significant lower ($P<0.05$) final weight, weight gain, specific growth rate and daily weight gain compared to the control diet. Tilapia fed with GAS diet had the lowest final weight. As for weight gain, specific growth rate and daily weight gain, no significant difference were shown among tilapia fed the diets with GAS, SHM and BM. The values of feed conversion ratio (FCR) showed no significant difference ($P>0.05$) between the dietary treatment fed to the fish. The fish in the experiment showed no feed rejection during the experiment, but the acceptability was higher in fish feed the control diet and the diets with PBM and SHM, compared to the diets including GAS and BM. The lowest total feed intake (FI) value was shown in tilapia fed the diet with BM. Tilapia fed the control diet had highest feed intake value followed by the diets including PBM, SHM and GAS. The same sequence was shown for the total protein intake (PI) among the fish with exceptions for the control diet and diet with PBM switching places. No difference in protein intake was observed between fish fed the control- diet, PBM- and SHM- diet. Health problems encountered during the mid-period of the experiment, such as several fish from the same tank died spread over three weeks. One of the fish showed symptoms with swollen intestines, the decision was made to give all experimental fish medicine (OXCIN MD 500) to treat a possible infection. In the end, the survival rate (SR) showed no significant difference between the dietary treatments expect for the lower values in the diet with PBM.

Table 5: Growth performance of Nile tilapia (*Oreochromis niloticus*) fed experimental diets

	Diets					
	Control	**GAS**	**PBM**	**SHM**	**BM**	***P*-value**
Initial weight (g)	33.7	37.1	34.0	40.5	39.2	0.1290
Final weight (g)	137.6^{a}	78.8^{b}	125.9^{a}	99.4^{c}	80.5^{bc}	< 0.0001
WG (%)	311.4^{a}	112.3^{b}	272.0^{a}	148.3^{b}	105.4^{b}	< 0.0001
SGR (% day)	2.3^{a}	1.2^{b}	2.1^{a}	1.4^{b}	1.2^{b}	< 0.0001
DWG	1.7^{a}	0.7^{b}	1.5^{a}	0.9^{b}	0.7^{b}	< 0.0001
FCR (%)	0.87	0.90	0.83	0.93	0.90	0.1466
FI (total)	124.2^{a}	76.9^{b}	115.3^{ac}	103.8^{c}	73.9^{b}	< 0.0001
PI (total)	3.5^{a}	2.5^{b}	4.0^{a}	3.8^{a}	2.3^{b}	0.0005
SR (%)	92.0^{ab}	96.0^{a}	80.0^{b}	98.7^{a}	98.7^{a}	

WG = weight gain, SGR = specific growth rate, DWG = daily weight gain, FCR = feed conversion ratio, FI = feed intake, PI = protein intake, SR = survival rate. Control = diet including FM, GAS = golden apple snail, PBM = pangasius by-product meal, SHM = shrimp head meal, BM = blood meal. Data presented are standard error of the mean. Mean values within rows with different superscript letters are significantly different (P<0.05).

5.3 Water quality monitoring

The results from the water quality monitoring are presented in table 6. The parameters dissolved oxygen (DO), pH, total ammonia nitrogen (TAN) and nitrite were measured bi-weekly throughout the experiment. The water temperature was recorded every day for three weeks, showing no vital difference in temperature between days or tanks, after the initial period the water temperature were only measured once a week only. The water parameters did not reflect any differences among the treatments during the experimental period.

Table 6: Water quality parameter recorded in the experimental tanks

Parameters	**Median**	**Maximum**	**Minimum**
Temperature (^{o}C)	27.2	30	26.5
Dissolved oxygen (ppm)	5.91	6.92	4.49
pH	7.70	8.21	7.06
TAN[2] (mg/l^{-1})	1.0	5.0	0.0
Nitrite (mg/l^{-1})	5.0	5.0	0.0

[1]TAN = total ammonia nitrogen, include both NH_3 and NH_4

6 Discussion

A growing global population increases the need of food security, where the aquaculture sector already contributes to a part of the global food supply in all continents of the world but especially in the developing countries. FM is today one of the main protein source in the diets in farming fish and crustaceans but it is also commonly used in the diets in the production of livestock, chicken and pigs (FAO, 2014). However, the availability of FM is decreasing compared to the demand, with reduced catch of wild fish due to overfishing in the sea, resulting in raised prices on FM (FAO, 2014). The catch of wild fish for fish meal production that is reported to be as high as 60 % of the landed fish affects the global environment in a negative way (FAO, 2014). Many attempts have been made to replace the FM partially or totally in the diets in the aquaculture industry with less expensive protein sources. The diet with substitute should have positive effect on the fish growth

performance or at least comparable to diets including FM (El-Sayed, 1999). The replacement of FM within aquafeeds includes meals made out of fishery and terrestrial animal by-products, linseed meals and by-products, aquatic plants, single-cell proteins, and legumes and cereal by-products (El-Sayed & Tacon, 1997; El-Sayed, 1999;). The most profitable alternative protein source to FM depends on the local conditions.

The aim of this study was to assess the growth performance of tilapia fed on alternative protein sources to FM. The study was conducted and performed at the University of An Giang, a province close to the Mekong River Delta in Vietnam. To start, an attempt was made to produce tempeh in the laboratory as the alternative protein source. As a substrate for the tempeh production it was thought to use local products such as cassava root, broken rice or yellow corn. After calculating the amount of tempeh needed for a 10 weeks feeding trial it was found not to be realistic. It was decided to only test the possibility to make tempeh using the substrates mentioned. The laboratory report on the tempeh production can be seen in the Appendix 1.

The focus of the study now changed to find other locally available protein sources. Golden apple snail, pangasius by-product, shrimp head meal and blood meal could all be purchased from the local market or local farmers and had also already shown potential in previous studies to be good protein sources in diets of fish (Kaensombath and Ogle, 2004; Chimsung and Tantikitti, 2013; Cavalheiro *et al.*, 2007; Aladetohun & Sogbesan, 2013). Although previous studies had evaluated these protein sources in tilapia species it was found interesting if the results could be performed with other environmental conditions.

Both the experimental and control diets were examined using three replicates, meaning three tanks were prepared for each diet. In the beginning, each experimental tank were prepared with 30 fish, were the total weight of all 30 fish were the same for each tank. During the two weeks of acclimatisation before the experiment had started, around 60 fish died of unknown cause. Since there were no excess of fish the dead fish could not be replaced. The remaining living fish were divided in all tanks, leaving 25 fish in each tank. One suspicion was that the fish had died due to the stress caused by the handling of the fish. To reduce the stress when handling the fish it was decided not to record the total weight of all fish in each tank but instead the weight of only five randomly selected fish from each tank. The initial weight was then extrapolated out of these figures. In the end of the experiment, at the 15th of August, all fish from each tank were weighed, representing the final weight. The mean values of the weight of the fish was used for the statistical analysis of the fish growth performance.

All experimental diets were formulated to contain 32 % of protein to meet the optimal requirements for Nile tilapia (NRC, 1993?). In the four experimental diets the FM protein was replaced to 100 % by; golden apple snail, pangasius by-product meal, shrimp head meal and blood meal, respectively. In the control diet, FM was the main protein source. The analysis of the chemical composition of the diets showed the highest content of protein in the control diet (35.8 %) and the lowest values in the diet containing shrimp head meal (27.3 %), see table 4. Higher content of protein may generate higher growth performance, but even if the protein levels are high the diet

may be deficient in one or more essential amino acids necessary for maximal growth (NRC, 2011). Even if the protein content in the control diet and the SHM diet were several percentage from the estimated value of 32 % (table 4), the fish fed these diets had similar protein intake (table 5). A more detailed analysis of the chemical composition of the individual ingredients could have determined potential absences of essential components. The fat content of the diets did not exceed the recommended values for Nile tilapia (table 2). The ash content of the feed can also affect the growth of the fish, where high levels of ash being difficult for the fish to utilize (NRC, 2011). The ash content was highest in the control diet containing FM. This indicates that the fish meal used in this diet may contained both whole and waste products. The experimental ingredients: fish meal, pangasius by-products meal, blood meal and the other including ingredients in the diets were purchased from the local market as meal or oil. Golden apple snails and shrimp head were brought in fresh form from local farmers and had to be processed into a meal at the experimental facility at the University. Both snails and shrimp head were dried in the sun prior to the grinding process. This part of the process were not carried out under strict hygienic conditions, bearing the risk of microbial growth on the products. This may explain the higher mortality in two of the three tanks where fish were fed diets containing golden apple snail.

6.1 Growth performance

The results obtained from this study indicate that pangasius by-product meal can be used to replace 100 % of fish meal without negative effects on the growth performance of Nile tilapia. Diets supplemented with golden apple snail, shrimp head meal and blood meal resulted in a lower growth performance than FM and PBM (table 5).

It was not surprising that pangasius by-product meal (PBM) came out to have comparable results to FM in this study, as PBM can be defined as a variant of FM. The difference is that fish meal are produced from fish of different species and PBM is the waste product of head and bone from only pangasius species. The little difference could be the explanation why it is difficult to find any previous studies evaluating PBM specifically as a substitute for FM.

For this study, fish fed diets with GAS had the lowest final weight. The low values were in a way unexpected as GAS had shown promising results in previous studies. Incorporating GAS at high levels in aqua feeds has shown low digestibility. A fermentation process may improve the quality and digestibility of GAS by increasing the amount of free amino acids (Chimsung & Tantikitti, 2013). Most of the studies evaluated fermented GAS but for this study minced GAS was used. This explanation is supported by the study by Chimsung & Tantikitti (2013) where they found that fermented GAS could replace FM with 100 % while minced GAS only could replace 50 % of FM with the same performance, when diets were fed to hybrid red tilapia (*Oreochromis niloticus x mosambicus*). This could also be the case with shrimp head meal. Shrimp waste included in the diets to fish was often gone through a fermentation process in previous studies. Leal *et al.*, (2010) found that hydrolyzed shrimp protein can replace 20 % of FM in diets of Nile tilapia. A study by Plascencia-Jatomea *et al.* (2002) showed improved growth rate of the fish when FM were replaced by 15 % of shrimp protein silage. Cavalheiro *et al*. (2007) conducted a study that showed silage shrimp head meal completely replaced FM in tilapia diets, with no adverse effect on the fish growth

performance. Both drying and fermenting are a way of preserving the nutrient content of the products. Based on the discussion above, both GAS and shrimp head meal could be fermented to increase their nutritional value and the ability for the fish to utilize these products.

As for the last test protein source, blood meal (BM), previous studies have shown varying results. BM replacing FM by 50 % in Nile tilapia diets, showed reduced effects in fish performance in the study conducted by Otubisin (1987). Also El-Sayeds (1998) study showed poor fish performance when FM was replaced by 66 % BM in hybrid tilapia diets. The opposite results were shown by Aladetohun and Sogbesan (2013) were total replacement of FM had improved effects on the growth performance of Nile tilapia fingerlings. The varying results of previous studies in the performance of tilapia indicate that the inclusion of BM in the feed should be limited. The unsuccessful results of inclusion of BM in our study may indicate a deficiency in some essential amino acids. El-Sayed (1998) argues that BM has low levels of methionine and isoleucine and that the product is not palatable.

The Feed Conversion Ratio (FCR) is a measurement of how efficient the fish can convert the feed into body mass. The FCR values in table 5, are about twice as good as reported in most previous studies. Feeding Nile tilapia with 30-36 % CP previous studies shows values around 1.3 - 2.0, which are more reasonable (Leal *et al.*, 2010; Al-hafedh, 1999). It is possible that feeding the fish manually in clear water generates less feed waste.

6.2 Water quality monitoring

The temperature, pH-value, dissolved oxygen, ammonia and nitrite levels were monitored in the tanks just before the water was changed every second week. The water was measured in five different tanks, each one representing one diet. The values found for water temperature, dissolved oxygen and pH regarding all the tanks were in the range of the requirements of Nile tilapia culture (table 1). The test for ammonia and nitrite levels range between 0.0 to 5.0 mg/liter. This means that if the test instrument showed levels of 5.0 mg/liter, the water may have contained higher amounts than 5.0 mg/liter. The upper limit of the nitrite level for Nile tilapia is not specified in the literature. The nitrite levels in the water showed 5.0 mg/liter on four of the five measuring occasions which most likely are toxic levels for the fish. The same values were measured for all the tanks except for one occasion when one tank showed values of 2.0 mg/liter (tank with fish fed the diet with GAS). The recommended ammoniac levels for Nile tilapia is below 0.1 mg/liter. The test for ammonia and ammoniac levels in this study showed at one occasion 5.0 mg/liter in the water of all tanks, otherwise were the levels mostly around 0.0 to 1.0 mg/liter. High levels of ammonia and nitrite in the water are toxic for the fish (Shiau, 2002; El-Sayed, 2006). The water was change after the monitoring of the water quality parameters, this may reduce the toxic levels of ammoniac and nitrite. The water in the tanks were changed every other day and once a week a handful of salt were added to each tank. The high levels of ammoniac and nitrite may have caused the high mortality appeared during the experimental period. With tank culture systems the environmental conditions are easy to control and with continuous monitoring toxic levels are easy to detect and reduce.

6.3 Parameters effecting the results

The conditions to produce the diets used in this study was not completely controlled. The ingredients in the diets were bought on the local market or from local farmers. The actual nutrient

content was not tested. The vitamin and mineral premix was added with sprayed water after pelleting the feed. There was no control that the fat-soluble vitamins (A, D, E and K) actually was absorbed by the pellets. By preparing two batches of feed a little different, the nutrient content may have differed. No chemical analysis were done of the second batch. The sun-drying process of the feed may have resulted in bacterial contamination that was not controlled. The fed was stored in outdoor climate, that is on average 30 degrees Celsius. How this could have affected the quality of the feed over time was not controlled.

There were large variations in size among the fish included in the study. The fish from the same tank, weighed between 21 and 58 grams already at the initial weighing. It is not unrealistic to think that this variation in size could have had an impact on the final growth rate. At the end of the study it was found that the number of fish in the individual tanks varied from the original 25 reduced by the number of dead fish. However, two tanks contained 26 fish at the end and since one fish was found on the ground it could be speculated in that the fish could actually jump from one tank to another.

6.4 Future studies

It would be interesting to conduct the same study under more controlled conditions both regarding the ingredients in the feed and the environment for the fish. A better control of the commercially available feed ingredients and the diet preparation at the study site, could have detected possible deficiencies in the diets. The preparation of the diets could be done differently, mostly regarding the inclusion of the vitamin and mineral premix to ensure an even distribution in the feed. Processing of GAS and SHM by fermentation prior to the feed production, may have increased their nutritional value. Maybe it is possible to find a way to make the diet containing blood meal more palatable. A mixture of the protein sources in the feed could possibly give an even better growth performance than using just one protein source to replace the fish meal.

To be able for the others to repeat the study or to make a study in larger scale, it would be optimal if the environmental conditions for the fish could be monitored more thoroughly and more continuously. Preferable the initial size and weight of the fish should be within certain limits, maybe ± 10 % of the weight. The fish has to be weighted individually or batchwise to increase the liability of the results of a growth study.

7 Conclusion

Pangasius by-product meal may replace fish meal by 100 % in diet of Nile tilapia (*Oreochromis niloticus*), with no adverse effect on the growth performance. When fish meal was replaced, to 100 %, with golden apple snail, shrimp head meal and blood meal, as prepared in this study, this resulted in low growth performance. This could indicate that these ingredients can not replace fish meal at such a high inclusion level or these ingredients have to be processed in another way.

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9 Appendix 1

Laboratory report: for production of tempeh - with Corn, Cassava root and Broken rice as a substrate

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May, 2015

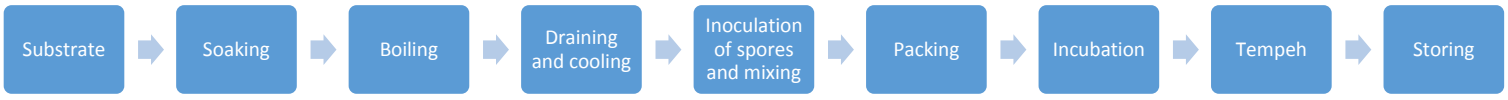
Introduction:

Different methods are used in the production of food to enhance the taste, texture, shelf life, and nutritional value. One commonly used process is fermentation, by definition an anaerobic process. However, in food technology the word is used for all processes where microorganisms are used. In Indonesia, tempeh (or tempe), soybeans fermented with one or several fungi, is widely consumed as a meat substitute. It forms an important part of the diet of many Indonesians, and may supply much of the total dietary protein. Tempeh made from soybeans is the most popular type of tempeh. However, tempeh can also be made from other legumes, such as peas, and from different cereals, such as barley and wheat.

Tempeh produced from soybeans is soaked and boiled and then inoculated with a culture starter containing spores of Rhizopus species. Sometimes, especially when tempeh is produced traditionally, other molds, and also yeasts and bacteria can be present in the starter. In industrial production of tempeh, usually a pure culture starter is used with only R. oligosporus. This fungus has been used in food production for probably thousands of years to improve the nutritional and quality of soybeans. Fortunately, *Rh. oligosporus* forms no known toxins.

In tempeh production, the soybeans are knitted together by the fungal mycelia and a firm 'cake' is produced, which can be prepared in different ways. The production process includes two major steps. First, the soybeans undergo a pre-treatment where they are soaked, boiled/steamed, drained and cooled. During this step the amount of water in the soybeans increase, making them tender and more accessible for growth of R. oligosporus. In addition, the microbial load on the soybeans is reduced. In the second step, the pre-treated soybeans are inoculated with spores of R. oligosporus, followed by mixing, packing, and incubation for 1-2 days. Thereafter, the fresh tempeh either can be stored, in dried or freeze form, or cooked directly, usually to a stewing or just fried.

As far as known, there has been no report of traditional soybean tempeh containing toxin or bacteria with adverse effects on humans. Reasons to the latter relate to the rapid growth of *R. oligosporus* which quickly depletes all fermentable carbon sources, the presence of lactic acid bacteria, the incubation under micro-aerobic conditions, and the customary heating of the tempeh product prior to consumption.



This present study was designed to evaluate the suitability of producing tempeh using the substrates: corn, cassava root and broken rice. The method were partly based on previous studies using the same or similar substrates (Cuevas-Rodrıgueza E.O., *et al.,* 2004; Muambi Shambuyi *et al.,* 1992; Rusmini, Simon and Djien Swan, 1974). The culture starter contained spores of three different strains of *Rhizopus oligosporus*; J104, J189 and J190. This study will evaluate which of the three strains of *Rh. oligosporus* that would be the most suitable one for tempeh production made of the three different substrates. The substrates were prepared in different ways and inoculated with different concentrations of *Rh. oligosporus* spores.

Hypothesis

One of the three strains of *Rh. oligosporus* will be more suitable for mycelium growth on each of the substrates, and the kind of strain may differ between the substrates.

Material

- Corn
- Cassava root
- Broken rice
- *Rhizopus oligosporus* strains: J401, J189 and J190
- MEA, malt extract agar; Malt extract (500 gram, Merck, 1.05391.0500), peptone, glucose, distillated water, agar (food store, agar agar, Cao Cap) (picture 3, in the appendix)
- Sterile bottles or sterile tubes to collect spore suspension
- Sterile loop
- Tubes for serial dilution
- Pipettes for serial dilution
- Sterile 0.9% NaCl (picture 1, in the appendix)
- Lactic acid, 90 % (picture 2, in the appendix)
- Bürker counting chamber, (0, 0025 mm^2) or hemozytometer (0, 0025 mm^2).
- Microscope (minimum 10x zoom)
- Sterile plastic bags
- Sterile petridishes (9×1.5 cm)

- Equipment for autoclaving
- Equipment to boil the products
- Incubation room, 37 degrees
- Incubation room, 35 degrees
- Incubation room, 2 degrees
- Freezer, around – 18 ^{o} C – Storage of produced tempeh

Procedure

1. Spore production

1.1 MEA (Malt Extract Agar) - slopes

- Malt extract agar were prepared as followed:

1 liter Malt extract agar:
Glucose (20g) + peptone (1g) + malt extract (20g) + agar (20g) + 1 liter distillated water

- One liter of MEA were prepared in a one liter sterile bottle. First the suspension were mixed until no lumps were visible and then the whole bottles were autoclaved in 120 ^{o}C for 20 min.
- The suspension were divided in nine 200 ml bottles with 100 ml MEA in each bottle.
- The bottles were then cooled down, with an angle to make a "slope", until the MEA were solidified.
- To reduce the time making the MEA solidified, the bottles were incubated at 2 ^{o}C, for approximately one hour.

1.2 Inoculate spores

Many different strains of *Rhizopus oligosporus* can be used in tempeh production. J401, J189 and J190 are common strains and therefore used as start cultures in this experiment.

- Three bottles containing MEA were prepared for each of the three strains of *Rh. oligosporus*.
- A loop of spores were inoculated in the middle surface of the MEA slopes in each bottle.
- All bottles were incubated at 37 ^{o}C for 5 days.
- The lids of the bottles were kept loose to allow an even flow of sufficient oxygen.
- When the mycelial growth was middle-to-dark grey, the spores were ready to be harvested.

1.3 Harvesting spores

- The bottle containing the best growth of the fungi from one of each strain of *Rh. oligosporus* were collected.
- 0, 9 % NaCl were poured into each bottle, up to the 100 ml mark.
- By tilting the liquid over the surface, the mycelium were soaked. With the sterile/sterilized loop, the surface of mycelium were gently rubbed. The liquid turned grey, as the spores were released into the suspension.

1.4 Quantifying spores

- Spore suspension (~ 9 microliter) were dropped on the haemocytometer and the number of spores on approximately 20 squares were calculate with the help from a microscope, starting with 10x zoom and then adjusted.
- The concentration of the spores were calculated for each strain of *Rh. oligosporus*. See calculations below.

Calculation
J401
Final concentration: 5×10^4

300 gram
5 x 10^4 final concentration
5 x 10^4 x 300 gram = 15 x 10^6 spores needed
Counting the spores in the haemocytometer gave:
329 spores/ (20 squares x 2, 5 x 10-7 x 1 dilution) = 6, 58 x 10^7
15 x 10^6/6, 58 x 10^7 = 0, 228 ml = 228 µl of spore suspension

J189
Final concentration: 5 × 10^4

300 gram
5 x 10^4 final concentration
5 x 10^4 x 300 gram = 15 x 10^6 spores needed
Counting the spores in the haemocytometer gave:
187 spores/ (20 squares x 2, 5 x 10-7 x 1 dilution) = 3, 74 x 10^7
15 x 10^6/3, 74 x 10^7 = 0, 40 ml = 400 µl of spore suspension

J190
Final concentration: 5 × 10^4

300 gram
5 x 10^4 final concentration
5 x 10^4 x 300 gram = 15 x 10^6 spores needed
Counting the spores in the haemocytometer gave:
49 spores/ (20 squares x 2, 5 x 10-7 x 1 dilution) = 0, 98 x 10^7
15 x 10^6/0, 98 x 10^7 = 1, 53 ml = 1530 µl of spore suspension

2. Production of tempeh

2.1 Soaking

By soaking the substrates in acidified water, growth of spoilage bacteria may be inhibited. In this experiment lactic acid was added in the soaking water, but other acids such as acetic acid can also be used.

→ A certain portion of each substrate were soaked in 500 ml of tap water containing 0.12 M lactic acid. The volume of the solution was adjusted depending on the amount of the substrate. The vessel were covered and the substrate were soaked for approximately 3 hours in room temperature.

- **Corn:** 100 gram of corn were soaked in 275 ml acidified tap water.
- **Cassava rot:** The cassava root was washed and the outer of approximately 2 mm was removed. After removing the outer, the root were cut in 8-mm cubes. 100 gram of the cubes of cassava root were soaked in 275 ml acidified tap water.
- **Broken rice:** 300 gram of broken rice were soaked in 500 ml acidified tap water.

2.2 Boiling or steaming

The boiling or steaming contributes to leaching out of substances into the water that can inhibit or disturb the fermentation. In addition, contaminating bacteria are destroyed that might interfere with subsequent fermentation, and some nutrients required for growth of *Rh. oligosporus* are released.

→ The substrates were drained from the soaking water and boiled in tap water until the surface were slightly soften.

- **Corn:** The corn were boiled in tap water for 25-30 minutes.
- **Cassava root:** The cassava root were boiled for approximately 20 minutes.
- **Broken rice:** The broken rice were boiled for 7 minutes, just until the rice seeds were soften, but still had a firm structure.

2.3 Draining and cooling

Before inoculation, the boiling water is discarded and the substrates are cooled and dried. The drying is important to avoid an initially rapid growth of microorganisms in the available water on the boiled substrate. After boiling, the substrates may contain bacteria-spores. This may not be a problem if the substrate are dried, followed by inoculation with a big amount of *Rh. oligosporus* spores and incubation at a temperature favourable for mycelium growth. The fermentation is normally complete when the bacteria start to multiply. The bacteria will die during cooking of the ready-fermented tempeh. If the bacterial growth in some way is facilitated, the bacteria will grow over *Rh. oligosporus*, which are seen easy.

→The hot boiling water were removed and the substrates were drained on towels until desirable temperature was reached. The water activity for the drained cassava root should not be lower than 0, 98-0, 99.

- **Corn, cassava root and broken rice:** All substrates were cooled down to room temperature.

2.4 inoculation and mixing

In Indonesia, inoculum is taken from pieces of a previous fermentation cake or from the wrapper in which the cake was made. Industrially, different strains of *Rhizopus* are used. *Rh. oligosporus* is the principal species used, and has been found to be the best choice. A suitable amount of *Rh. oligosporus* spores to inoculate depends on substrate. Too many spores can give such a considerable growth that so much heat is produced that the fermentation is deteriorated. In order to get a homogenous growth, it is of major importance that the spore suspension is mixed with the substrate to a homogenous mixture.

Rh. oligosporus produces many different enzymes that break down among other substances like proteins, carbohydrates, and lipids. *Rh. oligosporus* has a very high proteolytic activity, which is of importance especially in for example soybean tempeh production, since the substrate has a high protein content. Because of protein metabolism, deamination following hydrolysis releases free ammonia, causing the pH to gradually increase during the tempeh production. The proteolytic activity is important for the tempeh quality. A substrate containing noticeable

amounts of starch, makes it of minor importance that *Rh. oligosporus* has low amylase activity and no pectinase activity. *Rh. oligosporus* NRRL 2710 (J189) has high lipolytic activity and probably use lipids, above all lipid acids, as primary carbon/energy source. Sugars present in a substrate cannot be utilized as carbon source by *Rh. oligosporus*.

→ Calculations were made of how many milliliter of spore suspension that was needed. A guideline were to add a small volume of very concentrated spore suspension to the substrate, and in that way avoid making it too wet and slimy. The substrate were inoculated by adding spore suspension drop-wise, followed by thoroughly mixing.

- **Corn, Cassava root and Broken rice:** Each substrate were divided in portions and inoculated with all three strains of *Rh. oligosporus* separately containing different concentrations of the spore suspension, see table 1. Due to earlier experiments with rice-tempeh, was the broken rice only inoculated with spores from the strain J401.

2.5 Packing

To get a desirable result, it is important to pack the inoculated substrate in a way that a white mycelium develops that bind the substrate together to a compact cake. The balance between keeping the substrate moisture and at the same time exclude air is very important. Too much available air results in rapid mycelium growth but strong unfavorable sporulation. On the other hand, too little air generally results in inhibition of mycelium growth and favoring of thermophilic spoilage bacteria and growth of yeasts.

The cake should not be too thick, since air then is hindered to reach the center of the cake, resulting in uneven mycelium growth. The substrate should be packed well so that air pockets do not appear that can disturb the fermentation.

→The inoculated substrates were packed tightly into plastic bags and petri dishes. Half of the volume of the bag were filled with the substrate and then flatten out forming a cake, about 1 cm thick. The bags were then carefully seal with tape and as much air as possible were squeeze out. Then air holes were made over the surface of the bags with 1 cm intervals using a sterile needle.

- **Corn, Cassava root and Broken rice:** Portions of each substrate were packed into petri dishes and/or plastic bags, see table 1.

2.6 Incubation

The temperature is very important for a successful fermentation. Different temperatures give different incubation times, e.g. 80 h at 25 ºC, 26 h at 28 ºC and 22-24 h at 37 ºC. At high temperatures, some substrate may easily get dry. In addition, spores of thermophilic bacteria that survived the boiling grow more than *Rh. oligosporus*, resulting in a product with unsatisfied quality. Because of fungal germination and growth, considerable heat is developed which can only slowly dissipate into the surrounding environment. Unless diverted, strong growth of *Rh. oligosporus* can increase the temperature in the cake with 10 ºC above incubation temperature. Therefore, adequate airing during incubation is essential.

A successful fermentation should result in a compact, white cake composed of the substrate completely bound together by pure white mycelium, which has permeated the entire mass of the substrate. It is not known, however, whether the mycelium penetrates some of the substrate or only grows outside them.

→The substrates were incubated in the time and temperature suitable for that specific substrate. The tempeh was ready when it held together as a cake and had a well-developed mycelium that covered the particles of the substrates. All the substrates were incubates at 37 ºC and monitored regularly for up to 5 days.

2.7 Storing

When the fermentation is ready, a white tempeh cake will be presented. If the cake incubates for too long, the mycelium may sporulate and the cake will turn grey and inedible. The storing of tempeh can be done differently. Tempeh can be dried or stored in the fridge (around 8 ºC), up to 3-4 days. For longer storing of tempeh, freezing (around – 18 ºC) is a good preservation method. When putting the tempeh in the freezer the fungal growth will stop.

→Depending on the use of tempeh, the tempeh cake can be stores in room temperature, in the fridge or freezer, or dried.

- **Corn, Cassava root and Broken rice:** When the substrates were covered in mycelium or the sporulation had started, the products of tempeh were stored in the freezer. The products that had no growth of mycelium after 5 days or was strongly covered in spores, were thrown away.

3. Summarizing table of preparations of substrates

Table 1: Preparations with corn, cassava root and Broken rice

Substrate	Amount (gram)	Soaked in lactic acid	Strain of *Rh. oligosporus*	Concentration of spores	Packed
Corn	50	X	J401	$5 * 10^5$	Plastic bag
	50	X	J189	$5 * 10^5$	Plastic bag
	50		J190	$5 * 10^4$	Plastic bag
	50		J190	$5 * 10^4$	Petri dish
	50		J401	$5 * 10^4$	Plastic bag
	50		J189	$5 * 10^4$	Plastic bag
Cassava root	50	X	J189	$5 * 10^5$	Plastic bag
	50	X	J190	$5 * 10^5$	Plastic bag
	50		J401	$5 * 10^4$	Plastic bag
	50		J189	$5 * 10^4$	Plastic bag
	50		J190	$5 * 10^4$	Plastic bag
Broken rice	100	X	J401	$5 * 10^5$	Plastic bag
	50	X	J401	$5 * 10^5$	Petri dish

Choice of preparation were partly based on previous studies but also on earlier experiences.

Results

Substrate	*Rh. Oligosporus*	Soaking in lactic acid	Results
Corn	J401	Yes	Almost no mycelium growth, only sporulation, no use.
		No	Good growth of mycelium, saved in the freezer. 18 h of incubation.
	J189	Yes	Lot of sporulation, no use.
		No	Small growth of mycelium and sporulation, no use.
	J190	No	Good mycelium growth, minimum sporulation, both packed in petri dish and plastic bag. After 18 h incubation. Saved in the freezer.
Cassava root	J401	No	Mycelium growth and small sporulation, were put in the freezer after 18 h of incubation. Not whole covered.
	J189	Yes	No growth
		No	No growth
	J190	Yes	Small mycelium growth and sporulation, not whole covered (18 h), put in the freezer.
		No	No growth
Broken rice	J401	Yes	After 48 h of incubation, small growth of mycelium only in the corners and some sporulation were shown. No further growth after 5 days of incubation.
		No	Small growth of mycelium in the corners, (48 h incubation). Small sporulation. No further growth after 5 days.

Picture of the resulting products can be seen in the appendix on the last page

Discussion

A challenge with this experiment was to get the suitable incubation temperature at the right time for each production step; the fungi, MEA-bottles and the fermenting products. With limited incubation opportunities good planning was necessary. For optimal mycelium growth of the tempeh cake is it important to incubate the products in the right temperature and monitor often.

The optimal storage temperature for the *Rh. oligosporus* when it is not used is 2 ^{o}C, with higher temperatures the risk of the fungi getting bad is increased. Incubation in room temperature may be possible if it is fairly regular and over 30 ^{o}C, depending on what incubation temperature that is needed.

The tap waters quality and content of chlorine may differ depending on which country the experiment are performed in. Which may affect the results of this experiment.

Rh. oligosporus

Based on the results, these strains are most suitable to be used for the different substrates in tempeh production:

Substrate	**Strains of *Rh. oligosporus***
Corn	J190
Cassava root	J401/J190
Broken rice	J401

Corn

Strains from J401 gave in this experiment the best mycelium growth on the corn. But using whole particles of corn, the cakes was not as compact as wanted. Suggestion for continues studies using corn as the substrate in tempeh production, the seeds should be smash before any other preparations are done. By making smaller particles the mycelium may grow the particles tighter together and form a more compact tempeh cake. Mycelium growth with corn packed in plastic bags were as good as corn packed in petri dish.

Cassava root

The results from making cassava-tempeh may not be reliable due to the substrate not being properly dried before inoculation with the spore suspension. The mycelium growth was almost non-existent on all of the trails with cassava root. J401 and J190 were the strains that may have potential to make tempeh out of cassava root. Also the particles of cassava root, 8-mm cubes, may be too big for the mycelium to create a compacted cake.

Broken rice

The experiment conducted at SLU with Jasmine rice tempeh was successful when using the strains J140 as starter culture. Therefore only strains of J401 was used in the experiment conducted at AGU, when broken rice was used as substrate. The mycelium growth was visible but a more careful monitoring of the incubated products would be necessary get to a better result. The mycelium growth was as good when the broken rice were packed in plastic bags as when packed in petri dishes.

Future study

- Temperature, drying after boiling and monitoring of products in incubation are essential parts of the production.
- Using combinations of J140, 189, and J190 may give a better result and are something that can be included in continues experiments on all substrates.

Reference

Corn

Cuevas-Rodrıgueza E.O., , MiIan-Carrillo J. Mora-Escobedoc R., Cardenas-Valenzuela O.G., Reyes-Morenoa C., 2004. Quality protein maize (Zea mays L.) tempeh flour through solid state fermentation process. Lebensm.-Wiss. u.-Technol. 37 (2004) page 59–67. http://ac.els-cdn.com/S0023643803001348/1-s2.0-S0023643803001348-main.pdf?_tid=24a0fcd6-0233-11e5-8bde-00000aab0f02&acdnat=1432485618_d2183b21d55a19fde64c1cfbe0bffd28

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Rice

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Tempeh production

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Appendix

Analysis at SLU – rice tempeh

Tempeh production performed at SLU with jasmine rice as substrate were fermented in a petri dish and analysed for DM and CP at the SLU- laboratory:

Prov från Torbjörn L		Proven kom 12.5-2015					
Hedvig till Vietnam							
Sample		DM %		Cp (N x 6,25) % of the sample			Cp % av DM
		103 C 16h		1	2	Means	
Uncooked rice		86,5		6,94	6,68	6,81	7,9
Rice tempeh		32,4		2,66	2,76	2,71	8,4

Pictures

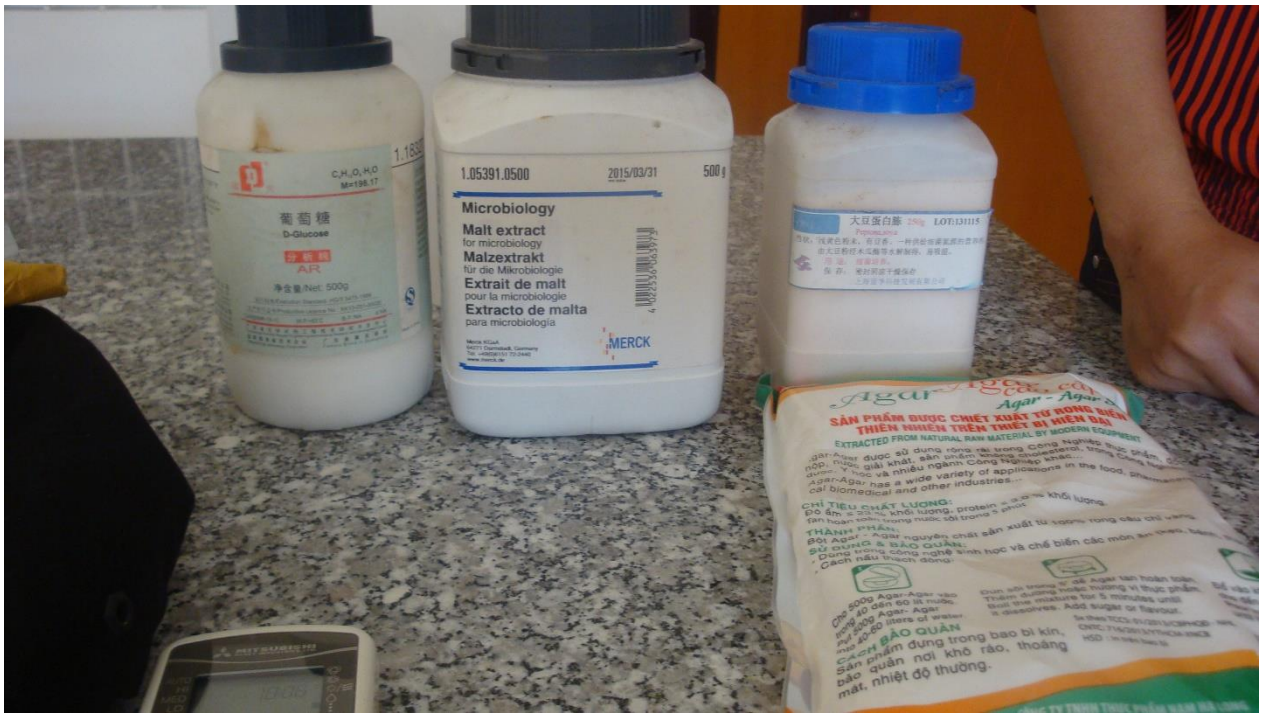
Products used as substrates in the experiment.

In the upper left corner you can see a picture of yellow corn. Below there is a picture of broken rice and to the roght it the cassava root.

Chemical products

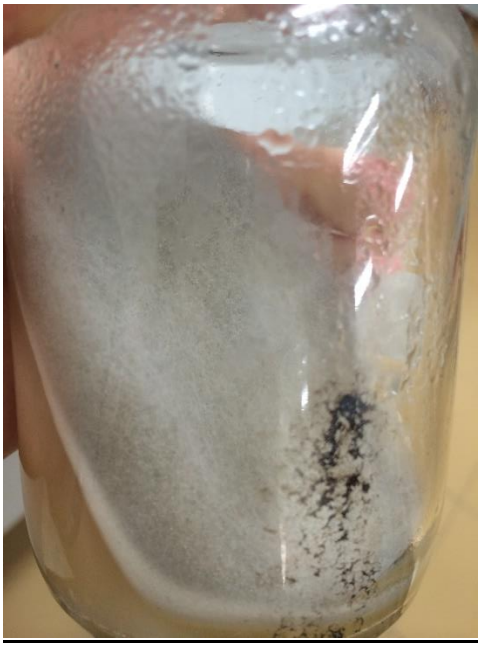
Picture 1: Natriclorid 0,9 % *Picture 2: Lactic acid*

Products used for MEA



Picture 3:From the left: D-Glucose, Malt extract, Peptone and agar

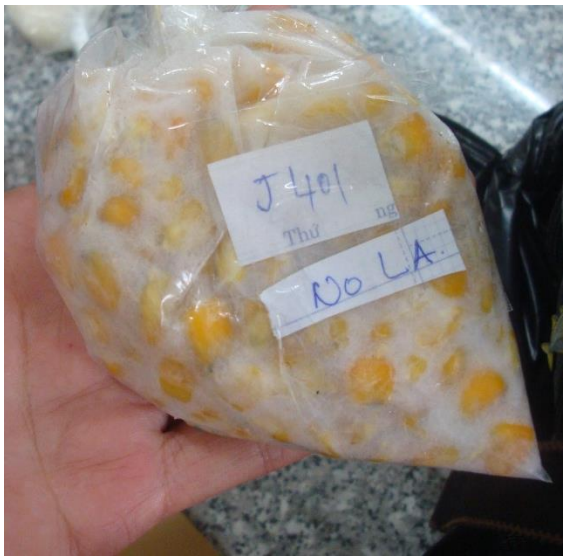
Rh. oligosporus



Growth of Rh. oligosporus incubated at 37 degrees for 6 days.

Results of tempeh production

Corn



J401, Not soaked in lactic acid

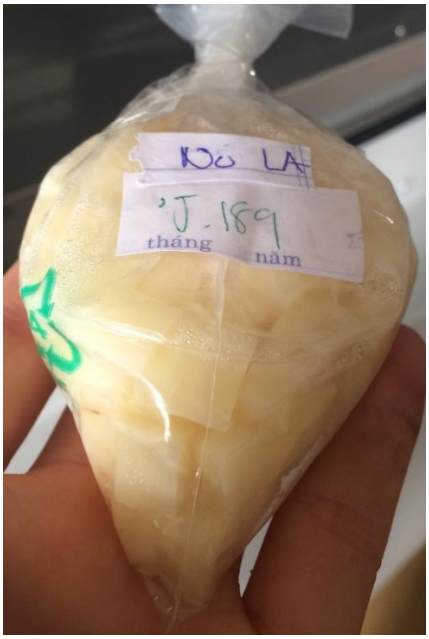
J190, Not soaked in lactic acid

J190 petri dish and J190 plastic bag, both not soaked in lactic acid

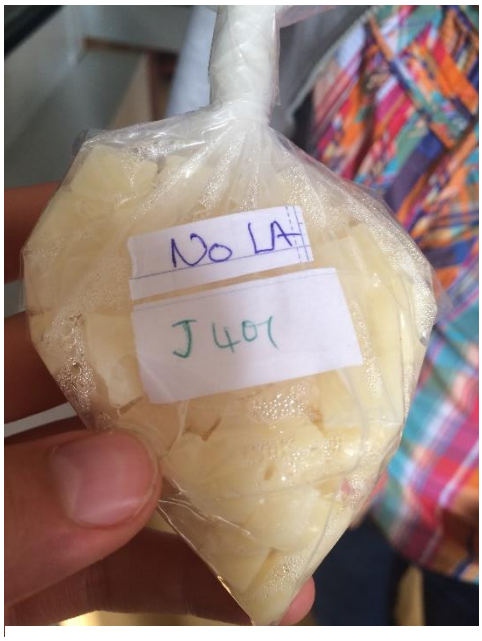
J189, Soaked in lactic acid

J189, Soaked in lactic acid

Cassava root



J189, Not soaked in lactic acid



J401, Not soaked in lactic acid

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